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Annals of Biological Research, 2025, 16(1):1-5
(https://www.scholarsresearchlibrary.com/)

ISSN 0976-1233
CODEN (USA): ABRNBW

Antimicrobial Activities of *Mentha arvensis* (L.) against Some Pathogenic Bacteria

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Received: 07 Jun, 2023, Manuscript no. ABR-23-101674; **Editor assigned:** 09 Jun, 2023, Pre QC no. ABR-23-101674 (PQ); **Reviewed:** 22 Jun, 2023, QC no. ABR-23-101674 (Q); **Revised:** 03 Jan, 2025, Manuscript no. ABR-23-101674 (R); **Published:** 10 Jan, 2025

ABSTRACT

Mentha arvensis (L.), commonly known as menthol mint is an erect, branched perennial herb that is used to treat liver and spleen diseases, asthma, and jaundice. The ethanol and aqueous extracts of the leaf and stem of Mentha arvensis (L.) were subjected to tests to determine their effect against two species of pathogenic bacteria, viz., Citrobacter freundii and Micrococcus luteus. The antibacterial activity was analysed using the disc diffusion method at different inhibitory concentrations. The results revealed that ethanol was the best extractive solvent as compared to aqueous for testing the antibacterial properties of leaf and stem extracts. Mentha arvensis (L.) extract shows strong inhibitory activity against both bacterial strains. The maximum activity was recorded against Citrobacter freundii at 12.5 mg/ml concentration with a 19.00 mm zone of inhibition, while maximum inhibition by 12.5 mg/ml ethanolic stem extract was 11 mm against Citrobacter freundii while maximum inhibition of Micrococcus luteus was 9 mm due to an ethanolic leaf extract of Mentha arvensis (L.), and 7.3 mm by 12.5 mg/ml ethanolic stem extract. The results also revealed that ethanolic leaf extract of Mentha arvensis (L.) inhibited the growth of both bacterial strains significantly as compared to stem extract. The obtained results provide support for the use of these plants in traditional medicine and suggest their further advancement in investigation.

Keywords: *Mentha arvensis* (L.), Ethanolic extract, Aqueous extract, Antimicrobial activity, Disc diffusion

INTRODUCTION

In tropical and subtropical areas, infectious illnesses brought on by bacterial and fungal infections are the main causes of death. India is one of the wealthiest nations in the world when it comes to the genetic resources of medicinal plants. It is a varietal emporium of plants. Its topography and climate vary greatly, which has an impact on the vegetation and floristic makeup of the region. Furthermore, the agro-climatic conditions are favourable for bringing in and domesticating new exotic plant types.

Medical plants are living, irreversible resources that may be depleted if overused but can be sustained if utilised carefully and wisely. In the past, people have neglected the value of medicinal herbs. Nonetheless, nowadays, medicinal plants are valued as both a source of economical health treatment and a source of money. Secondary metabolites are responsible for the majority of a plant's therapeutic effects. These metabolites provide plants used in the food and pharmaceutical sectors with colour, taste, and scen.

There are 6990 species in the Lamiaceae family (Labiatae), which is made up of 264 genera. In India, there are close to 350 species and 64 genera. *Salvia, Ocimum, Mentha, Coleus, Leucas, Nepeta*, and other common examples may be found across the nation. The family's main genera include *Stachys* (300 species), *Thymus* (340 species), and *Salvia* (700 species).

Mentha arvensis (L.), often called menthol mint, is a perennial herb with upright branches that can reach a height of 75 cm. It has flowing rootstocks and inflexible branching stems. Asthma, jaundice, and illnesses of the liver and spleen are all treated with this herb. Natural menthol is produced from menthol mint, an essential oil-bearing crop, and is utilised extensively in the pharmaceutical, cosmetic, and flavouring sectors. Consequently, the purpose of the current study was to examine the possible antibacterial activity of *Mentha arvensis* (L.) against two harmful microorganisms, *Citrobacter freundii* and *Micrococcus luteus*.

MATERIALS AND METHODS

Collection of *Mentha arvensis* (L.): *Mentha arvensis* (L.) aerial parts were collected in the Agra area of Uttar Pradesh. The plants' individual sections were meticulously chopped with a cutter to remove any contaminating bits. The harvested plant components were then placed in plastic bags and sealed. A refrigerator was used to retain the specimens that were collected and delivered to the lab. In accordance with Srinivasan et al., the stored specimens were carefully washed in tap water; surface sterilised with 0.1% $HgCl_2$, dried, pulverised, and packaged (Figure 1).

Figure 1: *Mentha arvensis* (L.)

Extraction of active constituents

Aqueous extract: Using a pestle and mortar and sterile distilled water at a 1:8 w/v ratio, different plant components, such as the leaf and stem, were individually homogenised before being filtered through muslin fabric. Further straining was done using Whattman No.1 filter paper to produce the filtrate. At room temperature, the extraction was done [1].

Organic extract (Soxhlet extraction): The powdered substance was uniformly packed into a thimble and put in an extraction chamber that was suspended above the flask holding the solvent ethanol and below a condenser. This extraction chamber held around 100 gm of the powdered substance. The ethanol evaporated when the flask was heated to 65°C, proceeded to the condenser, where it was transformed into a liquid, and trickled into the extraction chamber holding the plant material. The extraction chamber was made to have an overflow and trickle back into the boiling flask whenever the solvent level around the sample went over a particular threshold. The methanol extract flask was removed at the conclusion of the extraction operation, and ethanol was evaporated using a rotary evaporator. Then, extract was stored at 4°C in the refrigerator to assess its antibacterial qualities [2].

Test micro-organisms: Two different strains used for testing antibacterial activity were *Citrobacter freundii* (MTCC- 1658) and *Micrococcus luteus* (MTCC-1538). These were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh. A nutrient agar slant was used to maintain the typed bacterium culture, which was then kept at 40^{o}C until it was needed.

Antibacterial activity screening: The disc diffusion technique was used to assess the *in vitro* antibacterial activity of a chosen plant extract [3-4]. Plant extract solution with varied concentrations (12.5 mg/ml, 6.25 mg/ml, 3.12 mg/ml, and 1.56 mg/ml) was generated by serial dilution for susceptibility testing. Plant extract was diluted in a suitable solvent. 6mm-diameter sterile discs were impregnated with 25 µl of each successively diluted extract solution [5-7]. Nutrient broth was combined with a few colonies from the pure culture. A cotton swab dipped in culture was used to inoculate this broth throughout the whole surface of the nutrient agar plate. With the use of sterile forceps, plant extracts containing discs were applied to the agar plate's infected surface. 24 hours at 37°C were spent incubating these plates. Measurement of the

antibacterial activity was done by measuring the diameter of the zones of inhibition surrounding each disc. The inhibitory zone's mean diameter was determined in millimetres for each experiment in triplicate (mm) [8-10].

RESULTS AND DISCUSSION

The disc diffusion technique was used to evaluate the antibacterial effects of *Mentha arvensis* (L.) at various concentrations [11]. Tables 1-3 and Figures 2-5, which show the effects of various doses of crude leaf and stem extracts, demonstrate that both aqueous and ethanol extracts of leaves and stems were effective at preventing bacterial growth.

Table 1: Antibacterial activity of the aqueous extracts of *Mentha arvensis* (L.) against *Citrobacter freundii* and *Micrococcus luteus*

Plant name	**Aqueous extract against *Citrobacter freundii***	
Mentha arvensis (L.)	Leaf (zone of inhibition)	Stem (zone of inhibition)
	7.6 mm	6.3 mm
	Aqueous extract against *Micrococcus luteus*	
	Leaf (zone of inhibition)	Stem (zone of inhibition)
	7.5 mm	6.4 mm

Table 2: Antibacterial activity of the ethanolic extracts of *Mentha arvensis* (L.) against *Citrobacter freundii*

Plant name	**Different concentrations of Ethanolic extracts**				
***Mentha arvensis* (L.)**	**Leaf extract against *Citrobacter freundii***				
	12.5 mg/ml	6.25 mg/ml		3.12 mg/ml	1.56 mg/ml
	19 .3	9		7	6.5
	Stem extract against *Citrobacter freundii*				
	12.5 mg/ml		6.25 mg/ml	3.12 mg/ml	1.56 mg/ml
	11		9	8	6

Table 3: Antibacterial activity of the ethanolic extracts of *Mentha arvensis* (L.) against *Micrococcus luteus*

Plant name	**Different concentrations of Ethanolic extracts**				
***Mentha arvensis* (L.)**	**Leaf extract against *Micrococcus luteus***				
	12.5 mg/ml	6.25 mg/ml		3.12 mg/ml	1.56 mg/ml
	9	8		7	6.5
	Stem extract against *Micrococcus luteus*				
	12.5 mg		6.25 mg/ml	3.12 mg/ml	1.56 mg/ml
	7.3		6.8	6.2	6

As compared to aqueous extracts, *Mentha arvensis* (L.) shown considerable inhibitory action in ethanol extracts (Figures 2-5) [12]. Because (1) certain active chemicals were found in water extracts, albeit in little amounts, and (2) active substances were soluble in organic solvents and hence absent from water extract.

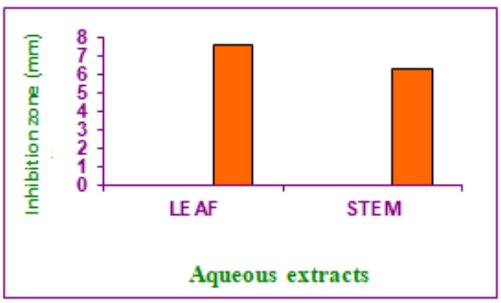

Figure 2 : Inhibition zone (mm) diameter of *Cirtobacter freundii* by aqueous leaf and stem extracts of *Mentha arvensis* (L.)

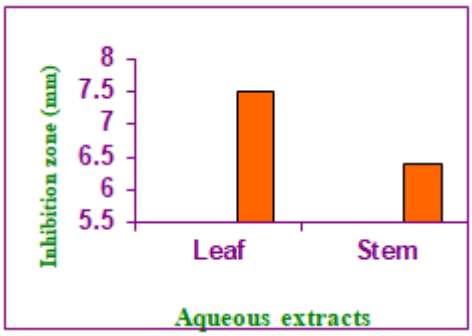

Figure 3: Inhibition zone (mm) diameter of *Micrococcus luteus* by aqueous leaf and stem extracts of *Mentha arvensis* (L)

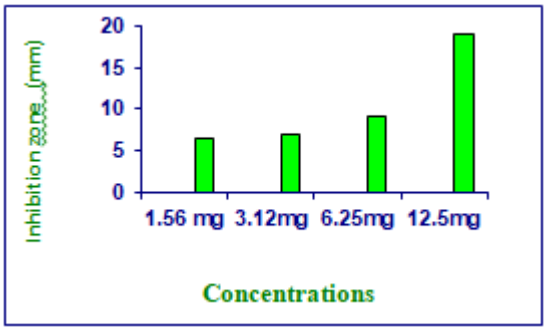

Figure 4: Inhibition zone (mm) diameter of *Citrobacter freundii* by different concentrations of ethanolic leaf extracts of *Mentha arvensis* (L.)

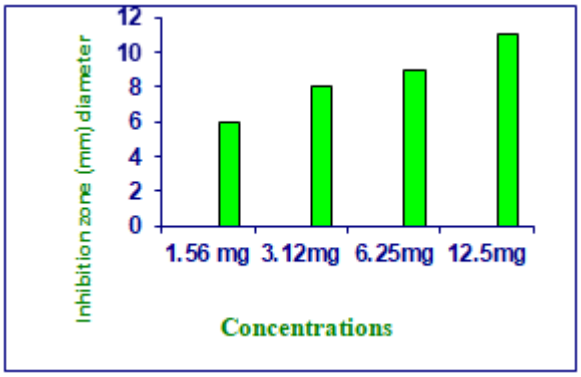

Figure 5: Inhibition zone (mm) diameter of *Citrobacter freundii* by different concentrations of ethanolic stem extracts of *Mentha arvensis* (L.)

The plant extracts in the current investigation were shown to function in a dose-dependent way, exhibiting their highest level of activity at a dosage of 12.5 mg/ml [13]. The findings of our investigation showed that leaf extract from the test plant significantly inhibits the development of both test bacteria when compared to stem extract. It could be because the leaves are abundant in bioactive compounds that have been shown to have therapeutic effects as well as physiological and antibacterial properties.

It is also evident from the above findings that the aqueous and ethanolic extracts of different parts of *Mentha arvensis* (L.) inhibit the growth of both tested pathogens to great extents.

CONCLUSION

According to the above study's findings, plant extracts have a lot of promise as antimicrobial agents against bacteria and can be utilised to treat infectious diseases brought on by hard-to-treat germs. It is imperative that different natural organic compounds be screened in order to identify active agents since good lead molecule prediction and drug discovery will pay dividends later in the drug development process.

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