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Research Article

Antimicrobial Activity of *Mentha arvensis* L. (Lamiaceae)

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Abstract: The present study has been designed with the objective to examine the ethanol extract of *Mentha arvensis* L. leaves (family Lamiaceae). In order to investigate its *in-vitro* antimicrobial potential against strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. The ethanolic extract was found to be the most effective and showed antibacterial activity against the organisms tested. The zone of inhibition (mm) at various concentrations of ethanolic extract of *Mentha arvensis* was found in the range 0.3µg/ml – 10µg/ml on tested all the test organisms. The antibacterial activity was more significant against *Staphylococcus aureus* i.e. 20mm zonetion. The study revealed the ethanol extract of *M. arvensis* leaves against microbes.

Keywords: Antibacterial activity, *Mentha arvensis*, Ethanolic extract.

1. Introduction

Lamiaceae is one of the major sources of antimicrobial compounds. *M. arvensis* is one of the members of Lamiaceae which is commonly called Methanol mint, Corn mint and Japanese mint an essential oil bearing crop is cultivated for natural menthol, which is widely used in pharmaceutical, cosmetic and flavoring industries. Mints have been known to man for a long time and are used in all continents of the world, and was introduced into India in 1952 from Japan. Corn mint plants consist of shoots, having over ground main stems with big leaves, small flowers and stolon with crawling succulent stems and underground rhizomes.

The leaves of *M. arvensis* L. the common edible aromatic herb has been described to possess various medicinal properties including antimicrobial properties.

The search for compounds with antimicrobial activity has gained increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic resistant microorganisms (Davis, 1987). However, there has also been a rising interest in the research for natural

products from plants for the discovery of new antimicrobial and antioxidant agents in the last three decades and in recent times [Dapkevicius *et al.*, (1998); Wang *et al.*, (1998); Nascimento *et al.*, (2000); R'ios *et al.*, (2005)]. More so, many of these plants have been known to synthesize active secondary metabolites such as phenolic compound found in essential oils with established potent insecticidal (Kambu *et al.*, 1982) and antimicrobial activities, which indeed has formed the basis for their applications in some pharmaceuticals, alternative medicines and natural therapies.

Reynolds *et al.*, (1996); Lis-Balchin *et al.*, (1997); Santos *et al.*, (1995); Oloke *et al.*, (1988) remarked that the world health organization has needed to be recognized medicinal plants as the best source for obtaining a variety of synthetic drugs. No doubt, some studies have identified and isolated the main active ingredients in the plants responsible for this antimicrobial activity [Carson *et al.*, (1995); Fabricant *et al.*, (2001)].

Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of microbes. The objective of this research is to evaluate the potential of plant

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extracts and phytochemicals on standard Microbial bacterial strains which are isolated from hospital. Moreover, we investigated the synergistic effects of *M. arvensis* L. with antimicrobial activity against bacteria. The purpose of this work is to evaluate the chemical composition and antimicrobial activities of ethanolic extract of *M. arvensis* L. leaves on the selected five bacteria.

2. Materials and Methods

2.1 Plant Materials

Fresh leaves of *Mentha arvensis* L. were collected from the normal fields of the Kurnool District, Andhra Pradesh Fresh plant material was washed thoroughly with running tap water, air dried and then homogenized to fine powder and stored in an airtight bottle.

2.2 Microorganisms Used

The microorganism is obtained from the National Chemical Laboratory (NCL), Pune, India. Amongst five microorganisms investigated, *Staphylococcus aureus* is the only gram-positive bacteria while four gram-negative bacteria are *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Shigella flexneri*. All the microorganisms were maintained at 4°C on nutrient agar slants.

2.3 Preparation of Bacterial Suspension

A loop full of bacterial culture which has been taken from pure slant cultures with the help of an inoculation needle and mixes it with sterile distilled water in a test tube under sterilized condition. The content is mixed thoroughly until a suspension is formed.

2.4 Extraction of Plant Material

60 grams of plant material were taken in a Soxhlet apparatus and extracted with 250ml of ethanol for 6 hours. The extract was filtered and concentrated under reduced pressure on a water bath at below 60°C. 25-50 milligrams of each crude drug have been taken in a test tube and dissolved in 1ml of the same solvent. For the sample was applied 6mm (or) 4 mm sterile paper disc and allowed to dry at laminar airflow chambers.

2.5 Phytochemical Screening

Phytochemical analysis of the *M. arvensis* L. extract was performed and the Phytoconstituents reported in Table 1.

Table 1. Preliminary phytochemical screening of the plant *M. arvensis* L.

Phytoconstituents	<i>M. arvensis</i> L.
Tannins	+
Alkaloids	-
Anthraquinones	-
Catecholic Compounds	-
Phenols	+
Saponins	-
Steroids	+
Flavonoids	+
Triterpenoids	-
Volatile Oils	+

[(+ : present) (- : absent)]

2.6 Antimicrobial Activity

Antimicrobial activity of extract from *M. arvensis* L. was tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Klebsiella pneumoniae* and *Staphylococcus aureus* by using the agar diffusion method. The bacterial lines were cultivated in Brain Heart Infusion medium (BHI) and incubated at 37°C for 24 hours. After this period, they were replicated on a Petri dish containing Muller Hilton (MH) agar. The plates containing the microorganisms were then perforated and the cavities were filled with 25µl of the extract solutions at 10, 5, 2.5, 1.25, 0.6 and 0.3% concentrations. Microbial growth was determined by measuring the diameter of the zone of inhibition. The results were expressed in terms of the diameter of the inhibition zone: < 9mm inactive, 9-12mm partially active, 13-18mm active, >18mm very active (Rios *et al.*, 1998; Alves *et al.*, 2000).

3. Result and Discussion

The Phytochemical analysis of the *M. arvensis* plant extract was performed the Phytoconstituent reported are tannin, phenols, steroids, flavonoids and volatile oils. Where, alkaloids, catecholic compound, saponins and triterpenoids are not found.

Table 2. Antimicrobial activity of the ethanolic leaf extract of *M. arvensis* L.

Micro Organisms	Concentration of the extract %						
	10	5	2.5	1.25	0.6	0.3	0.15
<i>E. coli</i>	13 ± 0.1	12 ± 0.3	10 ± 0.7	9 ± 0.8	8 ± 0.2	7 ± 0.1	NZ
<i>S. aureus</i>	20 ± 0.7	19 ± 0.8	18 ± 0.5	13 ± 0.4	12 ± 0.7	9 ± 0.7	NZ
<i>P. aeruginosa</i>	12 ± 0.1	10 ± 0.7	9 ± 0.4	8 ± 0.2	7 ± 0.2	NZ	NZ
<i>K. pneumoniae</i>	13 ± 0.8	12 ± 0.7	10 ± 0.7	9 ± 0.6	8 ± 0.7	7 ± 0.3	NZ
<i>S. flexneri</i>	14 ± 0.7	12 ± 0.6	10 ± 0.1	9 ± 0.1	8 ± 0.6	7 ± 0.7	NZ

Inhibition zone in mm; NZ – No Zone

The antibacterial activity of the ethanolic extract of leaf of *M. arvensis* L. has studied against gram-positive & gram-negative bacteria organism at various concentrations of ethanolic extract. The ethanolic extract of *M. arvensis* L. at µg concentration exhibited a significant antibacterial activity.

The antibacterial activity of *Staphylococcus aureus* was higher than the other bacteria. The inhibition zone diameter of *S. aureus* was 20mm at 10% concentration and it was 7mm at 0.3% concentration.

The antibacterial activity of *P. aeruginosa* was the lowest and the 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 ranging from 7mm to 14mm at 0.3% to 10% of concentration. The plant extract was found to have a moderate antibacterial activity against these three bacteria. According to the parameters, *M. arvensis* L. extract was classified as “very active” against *Staphylococcus aureus*, “active” against *E. Coli*, *K. pneumoniae* and *Shigella flexneri*, and “partially active” against *P. aeruginosa*.

4. Conclusion

The use of plants and their extracts in the treatment of diseases back to 460-370 BC when Hippocrates practiced the art of healing by use of plant based drugs (Sofowora, 1982). *M. arvensis* have been used for thousands of years to enhance the flavor and aroma of food. In addition, the plant is rich in a wide variety of secondary metabolites such as tannins, phenols, steroids, flavonoids and volatile oils, which were found *in vitro* to have antimicrobial properties (Blanc *et al.*, 1972; Baslas *et al.*, 1980; Yoshida *et al.*, 1990).

In this connection, the present study was conducted to evaluate the antibacterial activity of ethanol extracts of *M. arvensis* L. In this study, the results obtained showed that the ethanol extract of *M. arvensis* inhibited the growth of *E. coli*, *P. aeruginosa*, *S. flexneri*, *K. pneumoniae* and *S. aureus*.

The antibacterial activity of *M. arvensis* L. has not studied previously. The antibacterial evaluation revealed that *M. arvensis* L. extract was active against all five bacterial strains, being classified as very active, active and partially active.

This study is a preliminary evaluation of antimicrobial activity of *M. arvensis* L. It indicates that *M. arvensis* L. has the potential to generate novel metabolites. The plant extracts demonstrated antibacterial activity could result in the discovery of novel antibacterial agents. Besides, the same way also is used for self medication in domestic settings.

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