

Antibacterial activity of some medicinal plants of family Lamiaceae from Braj region

Uma Sharma, Rajneesh K. Agnihotri, Showkat Ahmad, Surabhi Mahajan, and Rajendra Sharma

Department of Botany, School of Life Sciences, Khandari Campus, Dr. B.R. Ambedkar University, Agra.

ABSTRACT

The ethanol and aqueous extracts of leaf and stem of three species of *Ocimum* viz. *Ocimum sanctum*, *Ocimum basilicum* and *Mentha arvensis* were subjected to see their effect against two species of pathogenic microorganisms: *Citrobacter freundii* and *Micrococcus luteus*. The antibacterial activity was analyzed using disc diffusion method at different inhibitory concentration. The results revealed that ethanol was the best extractive solvent for antibacterial properties of leaf and stem extracts. Out of the three test plants under study, *Ocimum sanctum* demonstrated strong inhibitory activity against both bacterial strains. The maximum activity was recorded against *Citrobacter freundii* at 12.5mg/ml concentration with 20.00 mm zone of inhibition. However, the moderate activities were exhibited by *Ocimum basilicum* and *Mentha arvensis* with 16.00 mm and 19.00 mm zone of inhibition respectively. The results also indicated that leaf extract of all the test plants inhibit the growth of both bacterial strains significantly as compared to stem extract. The obtained results provide a support for the use of these plants in traditional medicine and suggest their further advance investigation.

Key words: Antibacterial activity, aqueous extract, disc diffusion, *Mentha arvensis*, *Ocimum basilicum*, *Ocimum sanctum*, traditional medicine.

Introduction

Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern (Westh *et al.*, 2004). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens (Bandow *et al.*, 2003). Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. There is continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious disease (Rojas *et al.*, 2003). Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Saxena and Sharma, 1999; Ahmad and Beg, 2001). Higher and aromatic plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts (Hulin *et al.*, 1998).

The members of the family Lamiaceae are of great importance as the source of volatile aromatic oils, medicines and ornamentals (Pandey and Mishra, 2008). Therefore, the present investigation was aimed to screen potential antibacterial activities of *Ocimum sanctum*, *O. basilicum* and *Mentha arvensis* against *Citrobacter freundii* and *Micrococcus luteus*.

Materials And Methods

1. Plant material:

Aerial parts (leaf and stem) of *Ocimum sanctum*, *O. basilicum* and *Mentha arvensis* were collected from Agra region of U.P. Each plant parts devoid of contaminant parts of the plants were carefully cut with cutter. Plant parts, thus collected, were kept in polythene bags which were subsequently sealed. Specimens collected were brought to the laboratory and were stored in a refrigerator. The stored specimens were thoroughly washed with tap water and surface sterilized with 0.1% HgCl₂ following Srinivasan *et al.* (2004) and then dried, powdered and packed.

2. Extraction of active constituents:

2.1. Aqueous extract:

Different parts of the each plant i.e., leaf and stem were separately homogenized with sterile distilled water at 1:8 w/v ratio in a pestle and mortar and filtered through muslin cloth. The filtrate thus obtained was further

strained through Whatman No. 1 filter paper (Zore *et al.*, 2004). The extraction was carried out at room temperature.

2.2. Organic Extract (Soxhlet extraction):

About 100 gm of powdered material was uniformly packed into a thimble and placed in an extraction chamber that was suspended above the flask containing the solvent ethanol and below a condenser. The flask was heated up to 65°C and the ethanol evaporated and moved into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the plant material. The extraction chamber was designed so that when the solvent surrounding the sample exceeded a certain level it overflowed and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the methanol extract was removed and ethanol was evaporated by using rotary evaporator. Then extract kept in refrigerator at 4°C to detect antibacterial properties (Okeke *et al.*, 2001).

3. Test Organism:

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. The typed culture of bacteria were maintained on Nutrient agar slants and stored at 4°C prior to use.

4. Screening for antibacterial activity:

In vitro antibacterial activity of selected plant extract was tested by disc diffusion method (Mukherjee *et al.*, 1995).

For susceptibility testing, Plant extract was dissolved in suitable solvent, plant extract solution with different concentration (12.5mg/ml, 6.25mg/ml, 3.12mg/ml and 1.56mg/ml) were prepared by serial dilution. Sterile discs having a diameter of 6 mm were impregnated with 25 µl of each serial dilution of extract solution. Some colonies from the pure culture were mixed in nutrient broth. This broth was inoculated on entire surface of nutrient agar plate with the culture moistened cotton swab. Plant extracts containing disc were placed on inoculated surface of agar plate with the help of sterile forceps. These plates were incubated for 24 hours at 37°C. The diameter of the zones of inhibition around each of the disc was taken as measure of the antibacterial activity. Each experiment was carried out in triplicate and mean diameter of the inhibition zone was measured in millimeter.

Results And Discussion

The antibacterial activity of the *Ocimum sanctum*, *O. basilicum*, *Mentha arvensis* were assessed using the disc diffusion method at different concentration. The effect of different concentrations of the crude leaf and stem extracts are presented in Table 1- 2 and Fig. 1-4 reveals that both aqueous as well as ethanol extract of leaves and stem were successful in inhibiting the bacterial growth.

All the test plants showed significant inhibitory activity in ethanol extracts (Fig. 3 & 4) when compared to aqueous extracts (Fig. 1 & 2). Possibly because (1) the some active substances were present in water extracts but in low concentrations (2) active substances were soluble in organic solvents and therefore not present in water extract (De Boer *et al.*, 2005).

In the present study it was found that all the plant extracts worked in dose dependent manner i.e. showed maximum activity at highest concentration (12.5mg/ml). This is in accordance with the results reported by Ahmad *et al.* (2012) who found the inhibitory effects of *Holoptelea integrifolia* leaf extract against all the four bacterial strains increased with an increase in inhibitory concentration, however, degree of toxicity of different concentrations of plant extract may differ from one microorganism to another.

The results in our study indicated that leaf extract of all the test plants inhibit growth of both the test bacteria in high extends as compared to stem extract. It may be because the leaves are rich in bioactive molecules which are known to show medicinal activities as exhibiting physiological and antimicrobial activities (Vlctinck and Pieter, 2005).

It is also evident from the above findings that out of the three test plants studied viz., *Ocimum sanctum*, *O. basilicum* and *Mentha arvensis*, the extracts of different parts of *O. sanctum* showed high degree of inhibition as compared to *O. basilicum* and *M. arvensis* against both the bacterial strains viz., *Citrobacter freundii* and *Micrococcus luteus*. However, *O. basilicum* and *Mentha arvensis* also inhibit the growth of both tested pathogens in great extends.

It can be concluded that plant extracts under study have great potential as antimicrobial agents against micro-organisms and they can be used in the treatment of infectious disease caused by resistant micro-organisms. Such screening of various natural organic compounds and identification of active agents is the need of the hour because successful prediction of lead molecule and drug discovery will pay off late in drug development.

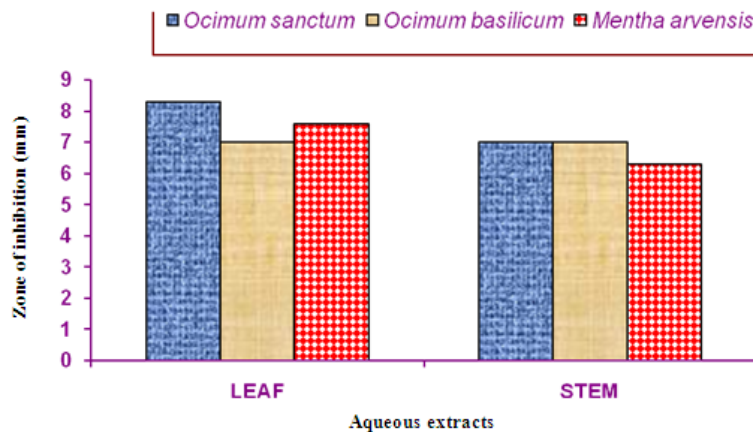


Fig. 1: Showing Inhibition zone diameter of aqueous leaf and stem extracts of plants against *Citrobacter freundii*.

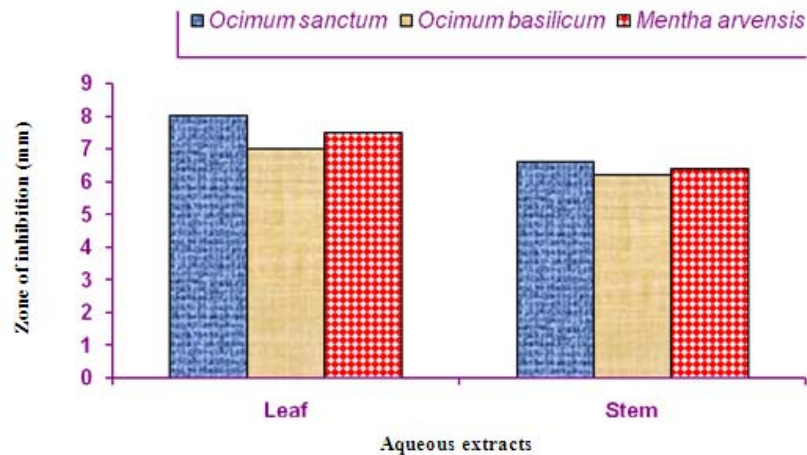


Fig. 2: Showing Inhibition zone diameter of aqueous leaf and stem extracts of plants against *Micrococcus luteus*.

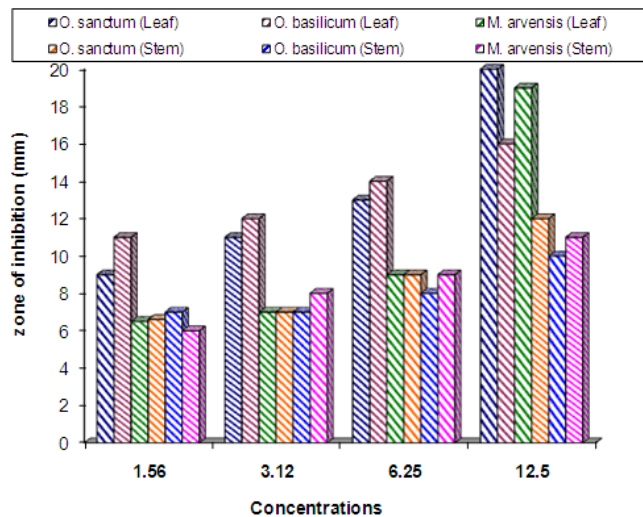


Fig. 3: Inhibition zone diameter of ethanolic leaf and stem extracts of plants against *Citrobacter freundii*.

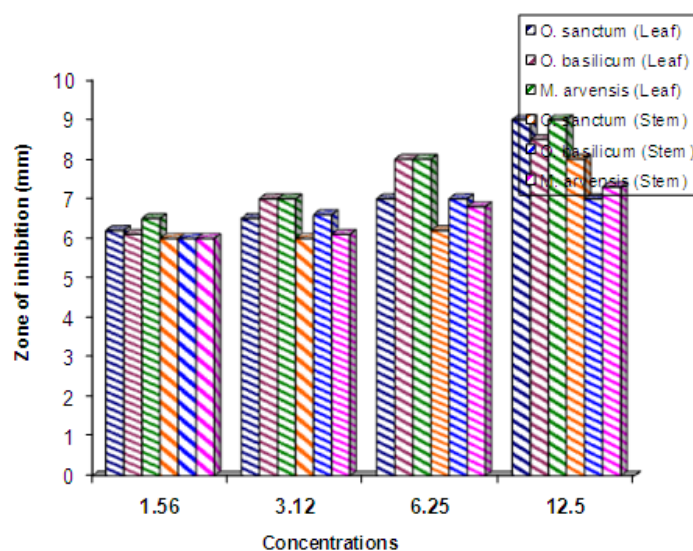


Fig. 4: Inhibition zone diameter of ethanolic leaf and stem extracts of plants against *Micrococcus luteus* at different concentration

Table 1: Antibacterial activity of aqueous and ethanol leaf and stem plant extracts against *Citrobacter freundii*.

Plant	Plant part used	Zone of inhibition (mm)				
		Aqueous	Ethanolic Concentration (mg/ml)			
			12.5	6.25	3.12	1.56
<i>Ocimum sanctum</i>	Leaf	8.3	20	13	11	9.0
	Stem	7.0	12	9.0	7.0	6.6
<i>Ocimum basilicum</i>	Leaf	7.0	16	14	12	11
	Stem	7.0	10	8.0	7.0	7.0
<i>Mentha arvensis</i>	Leaf	7.6	19	9.0	7.0	6.5
	Stem	6.3	11	9.0	8.0	6.0

Table 2: Antibacterial activity of aqueous and ethanol leaf and stem plant extracts against *Micrococcus luteus*

Plant	Plant part used	Zone of inhibition (mm)				
		Aqueous	Ethanolic Concentration (mg/ml)			
			12.5 mg	6.25mg	3.12mg	1.56 mg
<i>Ocimum sanctum</i>	Leaf	8.0	9.0	7.0	6.5	6.2
	Stem	6.6	8.0	6.2	6.0	6.0
<i>Ocimum basilicum</i>	Leaf	7.0	8.5	8.0	7.0	6.1
	Stem	6.2	7.0	7.0	6.6	6.0
<i>Mentha arvensis</i>	Leaf	7.5	9.0	8.0	7.0	6.5
	Stem	6.4	7.3	6.8	6.1	6.0

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