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RESEARCH ARTICLE

In Vitro Evaluation of Antimicrobial Potential of *Ocimum tenuiflorum* Leaf Extracts against Waterborne Pathogens

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ABSTRACT

The *in vitro* antimicrobial activity of leaves of *Ocimum tenuiflorum* was investigated against water isolates of *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Shigella dysenteriae* using disc diffusion method. The aqueous extracts did not show significant activity, but the organic extracts had significant activity with the ethanol extracts demonstrating the highest activity against all the test bacteria. The ethanol extract of *Ocimum tenuiflorum* had a great potential of antibacterial activities (inhibition diameters ranged from 12–28 mm) against all the tested organisms. The Minimum Inhibitory Concentration (MIC) of the ethanol extract ranged between 4 and 8 mg/ml and the Minimum Bactericidal Concentration (MBC) ranged between 16 to 32 mg/ml for all organisms. In conclusion, these extracts could be promising natural antimicrobial agents with potential applications in controlling bacterial contaminants of water.

KEYWORDS

Ocimum tenuiflorum, Antimicrobial Activity, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC)

INTRODUCTION

Medicinal herbs have a long history of use in Asia and are being increasingly studied by pharmacological researchers[1]. These herbs have many potential clinical and therapeutic applications in the modern medical setting, as numerous studies have revealed that they contain bioactive components, and have resulted in a better understanding of their physiological, therapeutic and clinical actions.[2,3] Antimicrobial agents can also be derived from herbs, and over 1000 plants exhibit antimicrobial effects[4]. Traditionally, these herbs are said to provide safe and effective treatments against many diseases.

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During the last two decades, WHO's health Assembly has passed a number of resolutions in response to this resurgence of interest in the study and use of traditional medicines and in recognition of the importance of medicinal plants to health care of people in many developing countries.

Among the plants known for medicinal value, the plants of genus Ocimum belonging to family Lamiaceae are very important for their therapeutic potentials. *Ocimum tenuiflorum* has two varieties i.e. black (Krishna Tulsi) and green (Rama Tulsi), their chemical constituents are similar[5]. *Ocimum tenuiflorum* is widely distributed covering the entire Indian sub-continent, ascending up to1800 m in the Himalayas and as far as the Andaman and Nicobar Islands[6]. Tulsi is a Sanskrit word which means "the incomparable one" and has a very

special place in the Hindu culture. Several medicinal properties have been attributed to the Tulsi plant not only in Ayurveda and Siddha but also in Greek, Roman and Unani systems of medicine[7].

The Tulsi plant is even known to purify the atmosphere and also works as a repellent to mosquitoes, flies and other harmful insects. The major effects of tulsi leaves are antifertility effect[8], antidiabetic effect, antiallergic and immunomodulator effects[9], stress resilience[10,11], anti-ageing effects[12], antioxidant activity[13], immunity tune-up[14], lung and bronchial support, nutrition, allopathic medicine complement, antimicrobial properties[15].

Sustainability of good health of living organisms depends upon the purity of water. Water is used for several purposes by human beings and the level of purity of water consumed is very crucial as it has a direct effect on health. Provision of clean and safe water in rural areas is a great challenge for the developing countries of the world since most communities rely on poor traditional sources that often provide unsafe domestic water. Nearly 1.6 million people are constrained to use contaminated water and more than a million people die from diarrhoea every year due to water borne diseases especially in developing countries.

In the process of identifying a plant based substitute for killing water borne pathogens, *in vitro* antimicrobial studies were carried out on the leaf extracts of *Ocimum tenuiflorum* against water borne bacterial pathogens.

MATERIAL AND METHODS

Collection of Plant

Fresh leaves of *Ocimum tenuiflorum* were collected in and around Coimbatore, Tamilnadu were authenticated by Botanical Survey of India, Coimbatore. The voucher specimens were kept in the Department of Microbiology, PSG College of Arts and Science, Coimbatore, Tamilnadu, India. The collected plant leaves were washed thoroughly 2-3 times with running water and with distilled water. The leaves were air-dried under shade. The leaves were crushed to make possible fine powder with the help of mortar and pestle and stored in airtight bottles for further analysis.

Preparation of Herbal Extract

A total of 10g of air dried plant leaf powder was weighed and was placed in 100ml solvents (water, chloroform, ethanol and methanol) in a conical flask and then kept in a rotary shaker at 200-220 rpm for 24h. Then it was filtered with the help of muslin cloth and centrifuged at 10000 rpm for 5 min. The supernatant was collected and the solvent was then removed under reduced pressure in a rotary evaporator. The residue was air dried thoroughly to remove the traces of solvent used. The dried crude extracts were dissolved in dimethyl sulfoxide (DMSO) and were stored at 4°C until required for testing.

Microbial Strains

The bacterial strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Shigella dysenteriae* were obtained as water isolates from Aharam mineral waters private limited, Sengapalli, Erode. The reference strains of bacteria were maintained on nutrient agar (HiMedia, Mumbai, India) slants at 4°C with a subculture period of 30 days.

Antibacterial Assay-Disc Diffusion Method

The plant extracts prepared with different solvents were used to test their antibacterial activity. Antibacterial activity was demonstrated using a disc diffusion method[16] which is widely used for the antimicrobial susceptibility testing. Muller Hinton agar medium was prepared and the inoculum suspensions of respective bacteria were spread over the agar plates using sterile swabs, for uniform distribution of bacteria. Whatmann No.1 filter paper was cut into small discs of diameter 3-6 mm in size and autoclaved. Each sterile disc was loaded with 10µl of test extract and placed on the agar plates inoculated with respective microorganisms. Later the plates were placed at room temperature for an hour to allow diffusion of extract into the agar. Then the plates were incubated for 24h at 37°C temperature. Negative controls were prepared using DMSO. Ampicillin (10µg per disc) was

used as positive reference standard to determine the sensitivity of each bacterial species tested. The results were recorded by measuring the diameter of inhibition zone at the end of 24 h. Zone of inhibition surrounding the discs was measured using a transparent ruler and the diameter was recorded in mm.

Determination of Minimal Inhibitory Concentration

The minimal inhibitory concentration was followed with the bacterial strains sensitive to the extracts in the disc diffusion assay. The MIC helps to measure more exactly the concentration of an antimicrobial necessary to inhibit growth of standardized inoculums under defined conditions. Minimum inhibitory concentrations of *Ocimum tenuiflorum* was determined by the broth dilution method according to the recommended standards of the National Committee for Clinical Laboratory Standards (NCCLS; now renamed the Clinical and Laboratory Standards Institute, CLSI). The inoculum of the bacterial strains were prepared from 12 h broth cultures and the suspensions were adjusted to 0.5 McFarland standard turbidity. The ethanolic extracts of *Ocimum tenuiflorum* dissolved in DMSO, were first diluted to the highest concentration (512mg/ml) to be tested, and then serial twofold dilutions were made in order to obtain a concentration ranging from 512 to 0.5 mg/ml in 9 ml sterile test tubes containing the nutrient broth. An aliquot of 1.0ml of standardized suspension of bacteria as previously described were added to each tube and incubated at 37°C for24 h. Negative controls were equally set up by using solvents and test organisms without extracts. Tubes with medium only were set as controls to check the sterility of the medium.

Determination of Minimal Bactericidal Concentration

From the tubes showing no visible sign of growth/turbidity in MIC, 0.1 ml of the sample was inoculated onto sterile nutrient agar using streak plate method and incubated at 37°C for 24 h. The least concentration that did not show growth of the test organism was considered as the MBC[17]. The experiments were replicated three times on different occasions with duplicate samples analysed per replicate.

RESULTS AND DISCUSSION

Solvent Extraction and Yield of the Plant Extracts

The active components are normally extracted from all plants, but the concentrations of these components vary from plant to plant. However, parts known to contain the highest concentration of the principles are preferred to therapeutic purposes and it can either be the leaves, stems, barks, roots, bulks, corms, rhizomes, woods, flowers, fruits or the seeds[18]. In the present study, leaves of *Ocimum tenuiflorum* were selected for active component isolation and testing of *in vitro* antibacterial activity.

Screening the crude plant extracts for the desired bioactivity is among the most important operations in medicinal plant research, and extraction is the first crucial step of the process. Solvent extraction is the most popular method of extraction. For selection of solvents 'like dissolves like' principle is applicable. Thus polar solvents will extract out polar substances and non-polar material will be extracted out by non-polar solvents. Extraction Efficiency refers to the yield of extraction, whereas efficacy refers to the potency (magnitude of bioactivity / the capacity to produce an effect) of the extract.

In the present study, four solvents namely ethanol, methanol, water and chloroform were selected for the isolation of active principles from the plant. In terms of efficiency, Maximum yield was obtained from the aqueous leaf extract (4.52%), while minimum yield was obtained from chloroform leaf extract (2.55%). Percentage yield of various extracts of *Ocimum tenuiflorum* were represented in Table 1.

Antibacterial Activity (Disc Diffusion Test)

The *in vitro* antibacterial activities of ethanol, methanol, water and chloroform extracts of *Ocimum tenuiflorum* was studied and their potentials were qualitatively assessed by the presence or absence of inhibition zones and the zones diameters being showed in Tables 2,

Table 1: Percent yield of the different solvent extracts of the dried and powdered leaf- *Ocimum tenuiflorum*

Plant name	**Plant part**	**Solvent**	**Amount of solvent (ml)**	**Amount of plant powder (gm)**	**Percent yield (w/w)[a] (Average ± SD)**
Ocimum tenuiflorum	Leaf	Ethanol	100	10	3.02±0.14
		Methanol	100	10	2.85±0.12
		Water	100	10	4.52±0.20
		Chloroform	100	10	2.55±0.05

[a]*based on the mass of plant material extracted*

Table 2: Antibacterial activity of the different extracts of *Ocimum tenuiflorum* against the bacterial strains tested based on disc diffusion method

Plant species & part	**Solvent**	**Diameter of zone of inhibition in mm**			
		Bacterial strains			
		Ec	***Pa***	***St***	***Sd***
Ocimum tenuiflorum- Leaf	Ethanol	18±1.27	16±2.40	28±0.79	12±0.97
	Methanol	14±0.69	12±1.25	25±2.35	11±1.26
	Chloroform	5±1.52	9±1.25	8±1.68	6±1.02
	Water	5±1.26	12±2.45	8±2.12	5±1.86
Ampicillin	-	22±1.22	23±4.12	30±1.20	24±1.89
DMSO	-	0±0.00	0±0.00	0±0.00	0±0.00

Ec: Escherichia coli; Pa: Pseudomonas aeruginosa; St: Salmonella typhi; Sd: Shigella dysenteriae

which clearly shows that all the extracts have shown antibacterial activity equivalent to that of standard against the entire tested organisms. The bacteria used in this study are associated with various forms of water borne diseases. According to the results, both the ethanol and methanol extracts of *Ocimum tenuiflorum* had a great potential of antibacterial activities against *Escherichia coli* (18mm& 14mm)and *Salmonella typhi* (28mm& 25mm) respectively.

The inhibition zones produced by these extracts indicated that, both extracts showed effective antimicrobial activities, although the ethanolic extract showed slightly higher activity, based on inhibition zone sizes. The chloroform and aqueous extracts had very low inhibition effects against all the tested organisms. Organic solvent extracts provided more potent antibacterial activity as compared to aqueous extracts. The polarity of Secondary metabolites and antibacterial compounds make them more readily extracted by organic solvents because Secondary metabolites are more soluble in organic solvents than water, and using organic solvents does not negatively affect their bioactivity against bacterial species suggesting that organic solvents are clearly better solvents of antimicrobial agents[19].

Being the most commonly used medicinal plant in Indian household its antibacterial activities have been studied against common pathogens. The essential oil from the leaves of Tulsi exhibited some inhibitory effect against *E. coli, B. anthracis, B. subtilis, Sal. newport, Sal. pullorum, Staph. aureus, P. vulgaris and P. aeruginosa*[20], *Mycobacterium tuberculosis, Arthobacter globiformis, B. megatherium*[21,22, 23,24], *Klebsiella aerogens, Proteus mirabilis, Shigella dysentriae, Vibrio cholerae* and *Staphylococcus aureus*[25], *Salmonella typhi, Salmonella paratyphi A* and *Salmonella typhimurium*[26, 27, 28].

MIC and MBC

The extent of antimicrobial activity was assayed by MIC and MBC assay.

Table 3: MIC and MBC of Ethanolic extract of *Ocimum tenuiflorum*

Solvent	Extract concentration (mg)	Bacterial strains							
		Escherichia coli		*Pseudomonas aeruginosa*		*Salmonella typhi*		*Shigella dysenteriae*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Ethanol	**0.5**	+	+	+	+	+	+	+	+
	1.0	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+
	4	-	+	+	+	+	+	-	+
	8	-	+	-	+	-	+	-	+
	16	-	-	-	+	-	+	-	+
	32	-	-	-	-	-	-	-	-
	64	-	-	-	-	-	-	-	-
	128	-	-	-	-	-	-	-	-
	256	-	-	-	-	-	-	-	-
	512	-	-	-	-	-	-	-	-
DMSO		+	+	+	+	+	+	+	+

(+) growth of microorganism; (-) No growth of microorganisms.

The minimum inhibitory concentration ranged between 4mg/mL to 8mg/mL and minimal bactericidal concentration ranging from 16mg/ml to 32 mg/ml depending on microorganism and results were shown in Table 3. Results obtained showed that MIC values were lower than MBC values, suggesting that the extracts were bacteriostatic at lower concentration and at higher concentration the extracts were bactericidal. The results of the assay suggest that ethanol extract of leaves of *Ocimum tenuiflorum* could possibly act as a bactericidal agent to these microorganisms.

CONCLUSION

The present study exhibited the antibacterial effect of various extracts of *Ocimum tenuiflorum*. The inhibitory effect of the extract justified the medicinal use of *Ocimum tenuiflorum*. These extracts could be promising natural antimicrobial agents with potential applications in controlling bacteria that cause water borne diseases. The extracts can provide a cheap and sustainable method toward disease reduction and can eventually improve the quality of life of the rural and peri-urban poor in developing countries.

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