

Research Article

**Phytochemical Analysis and Antimicrobial Activity of *Ocimum tenuiflorum* (Tulsi), A Known Indian Folk Medicinal Plant**

P. Saravanakumar, S. Thangapandiyan*, R. Dharanipriya, S. Gowri Shankar

PG & Research Department of Zoology, PSG College of Arts & Science, Coimbatore, Tamil Nadu, India.

*Corresponding author's E-mail: stp.nano@gmail.com

Received: 20-08-2018; Revised: 15-10-2018; Accepted: 18-11-2018.

ABSTRACT

In recent years researchers have been paid attention to find out new alternative sources of antimicrobial agents especially from plant sources. *Ocimum tenuiflorum* (tulsi) has served human kind as sources of medicinal agents since its earliest beginnings. Phytoconstituents present in leaves, barks & flower spikes and to characterize the compounds responsible for microbial activity. Phytochemical screening of the plant leaves reveals that the presence of saponins, alkaloids, flavonoids, steroids, phenols, tannins and glycosides. Different leaf extracts of *O. tenuiflorum* L were prepared and its antimicrobial activity were evaluated by agar well diffusion method against both gram positive & gram negative bacterial strains as well as three fungal pathogens. Ethanol extract had maximum zone of inhibition (18 ± 1.25) against *salmonella gallinarum*, whereas ethanol & methanol extracts were exhibited minimum zone of inhibition (7 ± 0.47) against *staphylococcus aureus*. Acetone extract had maximum zone (17 ± 0.81) of antifungal activity against *Aspergillus Niger*. Antimicrobial activity of various extract of leaves of *O. tenuiflorum* was carried in attempt to develop a new pharmaceutical drug from natural origin for the treatment of pathogenic microbes.

Keywords: *O. tenuiflorum* leaves, phytoconstituents, antimicrobial activity.

INTRODUCTION

Synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often associated with adulterations and side effects. Therefore, there is need to search new infection fighting strategies to control microbial infections. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy while there are some advantages of using medicinal plants, such as often fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature. For these reasons, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against multiple drug resistant microbial strains.^{1,2} The primary benefits of using plants derived medicines are that they are relatively safer than synthetic alternatives offering profound therapeutic benefits and more affordable treatment.³ Phytoconstituents are the natural bioactive compounds found in plants. Phytochemicals have antioxidant or hormone-like effect which helps in fighting against many diseases including cancer, heart disease, diabetes, high blood pressure and preventing the formation of carcinogens on their target tissues.⁴ These phytoconstituents work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions. Phytochemicals are basically divided into two groups, i.e. primary and secondary constituents according to their functions in plant metabolism. Primary constituents comprise

common sugars, amino acids, proteins and chlorophyll while secondary constituents consist of alkaloids, terpenoids, steroids and flavonoids.⁵

O. tenuiflorum or *Ocimum sanctum* is the most sacred herb and it is otherwise called Krishna tulsi.⁶ Tulsi is known as queen of medicinal plants. It is known to be an important medicinal plant from ancient period in India. Studies show that many *Ocimum* species are useful for the treatment of disorders in central nervous system (CNS) and also as antidepressant.⁷ In traditional medicine, aqueous extract of *Ocimum tenuiflorum* leaves is used for common cold and fever. Whole plant is also used for treating jaundice and for alleviating blood pressure.⁸

The novel medicinal plant *Ocimum sanctum* has been reported for its antimicrobial activity and it exhibited profound activity against various pathogenic microorganisms. The antimicrobial activities of the plant extract can be attributed to the presence of secondary metabolites including glycosides, alkaloids, steroids, terpenoids, flavonoids and saponins. These active components usually interfere with the growth or metabolism of microorganisms in a negative manner resulting in cell death.⁹

Antimicrobial drug resistance is a global problem today as the resistant microorganisms have emerged and spread throughout the world because of their genetic plasticity. Natural products of plants have been considered as the active ingredients of most of the modern medicines. Therefore, the aim of the current study has been focussed to screen the effect of *O. tenuiflorum* on diversified pathogenic microorganisms such as *Staphylococcus*



aureus, *Bacillus subtilis*, *Pseudomonas fluorescense*, *Salmonella gallinarum* and fungal pathogens *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus*, and *Trichoderma viride*. The microorganisms were chosen in this study are ubiquitous in our environment and are the primary causative agents of most common infections.

MATERIALS AND METHODS

Collection and identification of plant

Healthy, disease free, mature leaves of *O. tenuiflorum* were collected from the region of Annur, Coimbatore District, and Tamil Nadu. The collected plant material was identified and authenticated by Botanical Survey of India, Tamil Nadu Agricultural University, Tamil Nadu, where the voucher sample was preserved.

Preparation of leaf material

Leaves of selected plant were plucked and washed thoroughly with running tap water. It was again washed with sterile distilled water to remove dirt prior to drying process. The leaves were dried in shade at room temperature for a week to remove the moisture content and powdered using mixer grinder. Finally, powdered sample was stored at room temperature for further studies.

Preparation of plant extract

2.5 g of powdered sample was taken in air tight bottles. To this, 50 ml of different solvents such as ethanol, methanol, acetone and distilled water was added. After 2 days, the contents were stirred well thoroughly and filtered using Whatmann No.1 filter paper. The filtrate was collected and stored in sterile bottle at 4°C until further use. For antibacterial studies, each extract was prepared by dissolving 250 mg in 5 ml of 10% (v/v) aqueous dimethyl sulphoxide (DMSO).

Phytochemical analysis

Freshly prepared leaf extracts were subjected to standard phytochemical analyses using standard procedure.¹⁰ In order to find out the presence of various phytoconstituents such as alkaloids, terpenoids, flavonoids, tannins, steroids, anthroquinones, saponins, resins, glycosides and phenols.

Antimicrobial Studies of Plant Extracts

Bacterial culture

The following bacterial pathogens namely *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Salmonella gallinarum*, and *Proteus vulgaris*, were procured from the Microbial Type Culture Collection (MTCC), Chandigarh, India. All the cultures were grown on nutrient agar plates and maintained in the nutrient agar slants at 4°C. Overnight culture in the nutrient broth was used for the present experimental study.

Assay to evaluate antibacterial activity of plant extracts

The antibacterial activity of plant extract was evaluated using agar well diffusion method.¹¹ Pure cultures of each bacterial strain were sub cultured in nutrient broth on a rotary shaker at 200 rpm for 24 hours at 37°C. For preparing Mueller Hinton agar (MHA) plates, the MHA medium was boiled to dissolve completely and sterilized by autoclaving at 15 lbs pressure (121°C) for 30 minutes. After sterilization, 20 ml of MHA media was poured into the sterile petri plates and kept at room temperature for solidification. Then, each strain was swabbed uniformly onto the individual Mueller Hinton agar plates using sterile cotton swabs. Wells of 6 mm diameter were made on Mueller Hinton agar plates using sterile cork borer and 50 µl of plant extracts were poured into each well on all plates. The plates were incubated overnight at 37°C and the results were observed by the presence of bacterial growth inhibition zone around the sample loaded well and their diameters (mm) were measured using measuring scale. The assay was performed in triplicates.

RESULTS AND DISCUSSION

Collection and identification of plant

Healthy, disease free, mature leaves of *O. tenuiflorum* were collected from the region of Annur, Coimbatore District, and Tamil Nadu. Fig.1 shows the *O. tenuiflorum* plant with fresh leaves. *O. tenuiflorum* is commonly known as Tulsi is cultivated for religious and medicinal purposes. It is widely known across the Indian Subcontinent as a medicinal plant and an herbal tea.



Figure 1: Morphology of *O. tenuiflorum*

Preparation of leaf material

Fresh Leaves of *O. tenuiflorum* were collected and washed thoroughly with running tap water. It was again washed with sterile distilled water to remove dirt prior to drying process. Afterwards the leaves were dried in shade at room temperature for a week to remove the moisture content and powdered using mixer grinder. Finally, powdered sample was stored at room temperature for further studies. Fresh drying powdered from *O. tenuiflorum* leaves were presented in Figs 2a, 2b



Figure 2: Fresh leaves (a) Powder form of *O. tenuiflorum* (b)

Preparation of plant extracts

The leaf extracts of *Ocimum tenuiflorum* was prepared with four different solvents such as ethanol, methanol, acetone and distilled water. 2.5g of leaf powder was dissolved in 50ml of each solvent. These four leaves extract with different solvents.

Phytochemical analysis

The results of the phytochemical composition of the extracts of *O. tenuiflorum* is given in Table 1. The results of the phytochemical studies showed that all tested

extracts (ethanol, methanol, acetone and aqueous) contains the presence of alkaloids, terpenoids, flavonoids, tannins, steroids, anthraquinones, saponins, resins, glycosides and phenols. Phytochemical analysis revealed that *O. tenuiflorum* contained rich source of bioactive compounds such as alkaloids, terpenoids, flavonoids, tannins, steroids, anthraquinones, saponins, resins, glycosides and phenols. Phytochemicals are divided into two groups, which are primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consist of alkaloids, terpenoids and phenolic compounds.¹² *O. tenuiflorum* leaf extracts contained various phytochemical compounds such as saponins, alkaloids, flavonoids, cardiac glycosides, steroids, phenols and tannins.¹³ The presence of tannins suggests the ability of this plant to play a major role as antidiarrhoeic and antihemorrhagic agent.¹⁴ The presence of glycosides has been used for over two centuries as stimulants in cases of cardiac failure.¹⁵

Table 1: Qualitative phytochemical analysis of *O. tenuiflorum* leaf extracts

Compounds	Test Adopted	Ethanol Extract	Methanol Extract	Acetone Extract	Aqueous Extract
Alkaloids	Mayer's Test	+	+	+	+
Terpenoids	Salkowski Test	+	+	+	+
Flavonoids	Sodium hydroxide Test	+	+	-	-
Tannins	Lead Acetate Test	+	+	—	+
Steroids	Chloroform Test	+	+	—	+
Anthraquinones	Free anthraquinones Test	—	—	—	—
Saponins	Foam Test	+	+	—	+
Resins	Sodium hydroxide Test	+	+	+	+
Glycosides	Keller Killiani's Test	+	+	+	—
Phenols	Ferric chloride Test	+	+	+	+

+ = Present; - = Absent

Antimicrobial study Of *O. tenuiflorum* Leaf Extracts

The antimicrobial effect various leaf extracts of (ethanol, methanol, acetone and aqueous extracts) *O. tenuiflorum* were studied against selected bacterial and fungal pathogenic strains such as *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas fluorescens*, *Salmonella gallinarum*, *Staphylococcus aureus*, *Aspergillus flavus*, *Aspergillus niger* and *Trichoderma viridea* by agar well diffusion method and the results are presented in Figs 3&4. Organic extracts of *Ocimum sanctum* L. showed wide zones of inhibition against *Escherichia coli*, *Staphylococci* sp., *Shigella* sp., *Staphylococcus aureus* and *Enterobacteria* sp.¹⁶ The antibacterial effect of ethanolic extract of *O. tenuiflorum* exhibited maximum inhibition zone (18 mm) against *Salmonella gallinarum*. Whereas the minimum inhibitory zone (7 mm) was noticed against

Staphylococcus aureus. Regarding antifungal effect of ethanolic extract of *O. tenuiflorum*, maximum inhibition zone (14 mm) and minimum inhibitory zone (8 mm) was observed against *Trichoderma viridae* and *Aspergillus flavus* respectively.

Methanolic extract of *O. tenuiflorum* against selected bacterial pathogens exhibited maximum inhibition zone (16 mm) against *Proteus vulgaris* followed by inhibitory zone of about 15 mm against *Pseudomonas fluorescens*, *Salmonella gallinarum*. Least inhibitory zone of 7 mm was observed against *Staphylococcus aureus*. Meanwhile, the antifungal effect of *O. tenuiflorum* methanolic leaf extracts exhibited maximum activity against *Aspergillus niger* (14 mm), *Aspergillus flavus* (12 mm) and *Trichoderma viride* (9 mm) respectively. Alcoholic extract showed wider zone for *Vibrio cholera*.¹⁷

The antimicrobial effect of acetone extracts of *O. tenuiflorum* demonstrated greatest susceptibility towards bacterial pathogens (17 mm for *Escherichia coli*, 16 mm for *Salmonella gallinarum*, 13 mm for *Pseudomonas fluorescens*, 9 mm for *Staphylococcus aureus* and 7 mm for *Proteus vulgaris*) whereas the least susceptibility was noticed on fungal pathogens (17 mm for *Aspergillus niger*, 12 mm for *Aspergillus flavus* and 11 mm for *Trichoderma viridae*). The aqueous extract of *O. tenuiflorum* showed greatest inhibitory zone against *Pseudomonas fluorescens* (17 mm) followed by *Escherichia coli* (16 mm), *Trichoderma viridae* (14 mm), *Aspergillus niger* (13 mm), *Salmonella gallinarum* and *Staphylococcus aureus* (12 mm), *Aspergillus flavus* (10 mm) and *Proteus vulgaris* (8 mm). Aqueous and acetone extracts of *Ocimum sanctum* L. were also found to be sensitive to many plant fungi such as *Alternaria tenuis*, *Helminthosporium* sp. and *Curvularia penniseli*. Essential oil of Tulsi was tested on plant pathogenic fungi such as *Alternaria solani*, *Candida guilliermondii*, *Colletotricum capsici*, *Curvularia* sp., *Fusarium solani*, *Helminthosporium oryzae* and other bacterial strains like *Anthrobacter globiformis*, *Bacillus megaterium*.¹⁸

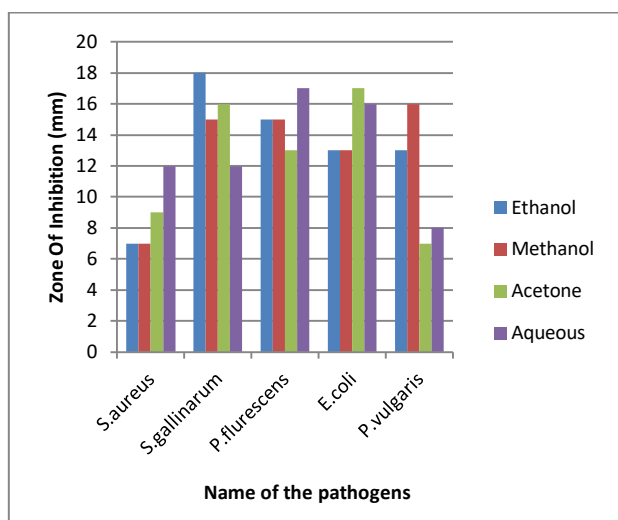


Figure 3: Zone inhibition of various extracts of *O. tenuiflorum* leaves against bacterial pathogens

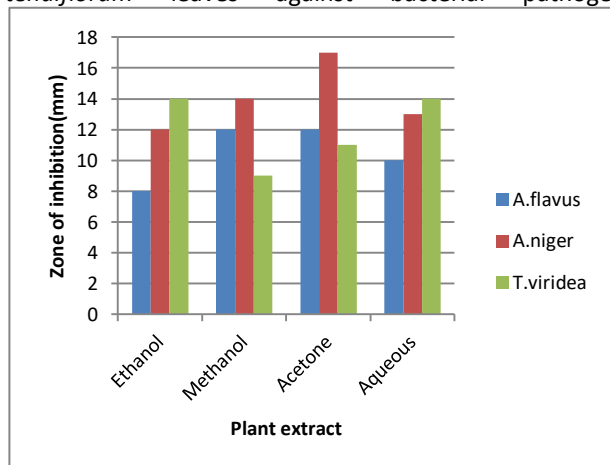


Figure 4: Inhibition zone of various extracts of *O. tenuiflorum* against Fungal pathogens

CONCLUSION

The frequency of life threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients in developing countries like our India. Results on bactericidal effect revealed that leaf extracts of *O. tenuiflorum* showed varying degrees of antibacterial property against the selected pathogens used. In conclusion, the present findings clearly indicated that *O. tenuiflorum* leaves possess the capabilities of being a good candidate in the search for a natural antibacterial agent against diseases caused by both gram-positive and gram-negative pathogenic strains.

REFERENCES

- Benkeblia N, Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*), Lebensm Wiss.Technol, 37, 1968-2004, 263-268.
- Akerele O, Summary of WHO Guidelines for the Assessment of Herbal Medicines, Herbal Gram, 22, 1993, 13-28.
- Chopra RN, Nayer SL, Chopra IC, Glossary of Indian Medicinal Plants, Council of Scientific and Industrial Research, 37, 1992, 7-246.
- Daniel VN, Daniang IE, Nimyel ND, Phytochemical analysis and mineral elements composition of *Ocimum basilicum* obtained in jos metropolis, plateau state, Nigeria, International journal of engineering & technology, 11, 2011, 161-165.
- Koche D, Shirsat R, Imran S, Bhadange DG, Phytochemical screening of eight traditionally used ethnomedicinal plants from Akola district (MS) India, International journal of Pharma and bioscience, 1, 2010, 253-256.
- Londhe AM, Kulkarni AS, Lawand RV, In-Vitro Comparative Study of Antibacterial and Antifungal Activities: A Case Study of *Ocimum kilimandscharicum*, *O. tenuiflorum* and *Ocimum gratissimum*, International journal of Pharmacognosy and Phytochemical Research, 7, 2015, 104-110.
- Adebolu TT, Oladimeji S. Antimicrobial activity of leaf extracts of *Ocimum gratissimum* selected diarrhoea causing bacteria in southwestern Nigeria. African Journal of Biotechnology, 47, 2005, 682-684.
- Ravi A, Elumalai A, Eswarhi MC, Kasarla R, A Review on Krishna tulsi, *O. tenuiflorum* Linn. International Journal of Research In, 2012, 3, 291-293.
- Biswas P, Evaluation of antibacterial activities of leaf extracts of two medicinal plants *Ocimum canum* Sims and *O. tenuiflorum* Linn, Journal of Microbiology and Biotechnology Research, 3, 2013, 20-23
- Trease GE, Evans WC, Pharmacognosy, W.B Scandars Company Ltd. London, 14, 1989, 269-300.
- Panthi MP, Chaudhary RP, Antibacterial activity of some selected folklore medicinal plants from west Bengal, Scientific World, 4, 2006, 16 – 21.
- Krishnanaih D, Sarbatly R, Bono A, Phytochemical antioxidants for health and medicine, Nature Biotechnology of molecular Biology Reviews, 1, 2007, 97-104.

13. Naik SL, Shyam PK, Marx P, Baskari, Devi VR, Antimicrobial Activity and Phytochemical Analysis of *O. tenuiflorum* Leaf Extract, International Journal of Pharm Technology and Research, 8, 2015, 88-95.
14. Price KR, Johnson TI, Fenwick GR, The chemistry and biological significance of Saponins in food and feeding stuffs, Critical Reviews in Food Science and Nutrition, 26, 1987, 22-48.
15. Sood S, Narang D, Thomos MK, Gupta YK, Maulik SK, Effect of *Ocimum sanctum* Linn. On cardiac changes in rats subjected to chronic restraint stress, Journal of Ethnopharmacology, 108, 2006, 423-7.
16. Rahman A, Parvez MAK, Parvin T, Paul DK, Satter MA, Antimicrobial activity of three bioactive compounds from the stem bark of *Piper chaba* hunter, Bioscience in Research, 1, 16-20.
17. Geetha RK, Vasudevan DM. Inhibition of lipid peroxidation by potentialexttracts of *Ocimum sanctum*: *In vivo and in vitro* studies, Life science, 76, 2004, 21-28.
18. Mondal S, Mirdha RB, Mahapatra, The behind sacredness of Tulsi (*Ocimum sanctum* Linn), Indian Journal of Physiology and pharmacology, 53, 2009, 291-306.

Source of Support: Nil, **Conflict of Interest:** None.

