



Antibacterial activity of *Ocimum teniflorum* leaf extracts

Nivetha G¹ and Govindarajan B^{2*}

¹PG & Department of Microbiology, V.H.N.Senthikumara Nadar College (Autonomous), Virudhunagar, India

²Dr.BGR Re-Search Services, Tuticorin, India

*Corresponding author Email: drbgrtuticorin@gmail.com

Abstract

In the recent years we need to discover new antimicrobial compounds because of the new diseases. In the present study methanol extract of *Ocimum teniflorum* was evaluated for antibacterial activity against clinically important bacteria *Staphylococcus sp.* and *Klebsilla sp.* under in vitro study. The tulsi leaf extract, along with the controls, was then subjected to microbiological investigation to determine which concentrations of the leaf extract gave a wider inhibition zone.

Keywords: *Ocimum teniflorum*, Tulsi, Antibacterial

Introduction

Mosquitoes are one of the most vexing bloodsucking insects that humans face. Mosquito management and personal protection from mosquito bites are the most effective ways to combat the disease right now. The best way to avoid contracting this disease is to avoid mosquito bites (Chavare *et al.*, 2015). Mosquito control strategies include habitat modification, biological control, physical control and chemical control, as well as individual mosquito protection. Interrupting disease transmission by preventing mosquitos from biting humans is one of the ways for controlling mosquito-borne diseases. This might be accomplished by use repellents (Musau *et al.*, 2016). Because of the transmission of disease, increase in mosquitos' population is a perennial problem for many developing countries (Fradin *et al.*, 2002).

Several mosquito species belonging to the genera *Anopheles*, *Culex* and *Aedes* serve as vector for diseases (Adeogun *et al.*, 2012). The most common vector control methods rely on the use of chemical pesticides containing DEET (N,N-diethyl-3-methyl benzamide), which is known to infiltrate the skin, produce allergic and harmful reactions, and have an odour that some people

dislike. The use of synthetic insecticides to control mosquito have disturbed the natural biological control system, allowing mosquito population to rebound. It has also resulted in the rise of opposition (Hsu, *et al.*, 2013).

Plants are a rich source of bioactive compounds and could be used as an alternative mosquito control agent. Source of medicinal plant used as a resistance from illness can be traced back to over five million years in the early civilization of India, China, and North east, which is as old as mankind (Chawala *et al.*, 1992). In the present study we are to extract of *Ocimum tenuiflorum* aromatic plant give in Vietnam and to determine the antibacterial activity.

Objectives

1. To collect and to identify medicinal plant of *Ocimum tenuiflorum* leaves.
2. To extract from the *Ocimum tenuiflorum* leaves using methanol solvent.
3. *Ocimum tenuiflorum* methanol extracts antibacterial against *Staphylococcus sp.* and *Klebsiella sp.*

Materials and Methods

Source of plant: The experimental plant *Ocimum tenuifloram* was collected from Muruganeri, Madurai (District), Tamilnadu.



Figure 1: Plant sample collection site satellite View

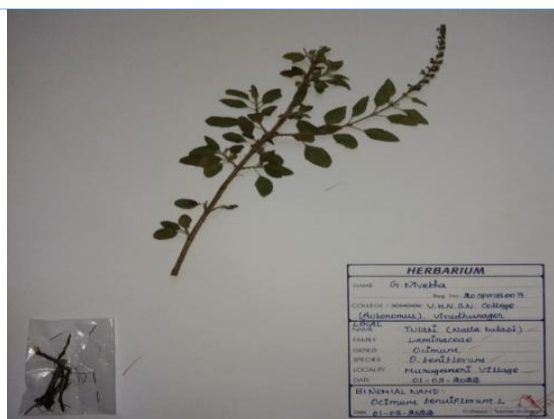


Figure 2: Herberium sheet - *Ocimum tenuiflorum*

Plant identification: The experimental plant Herbarium specimen was verified by the Dr. V.Siva, Assistant Professor of Botany, Department of Microbiology, V.H.N.S.N College (Autonomous), Virudhunagar.

Tables: 1 Medicinal plant tested for their antibacterial activity in the study

Species / Family / Voucher specimens	Common name	Part used	Popular uses
<i>Ocimum tenuiflorum</i> / <i>Lamiaceae</i>	Thulasi	Leaves	Thulasi essential oil is used as a medicine for fever, Headache, Lung disorders, Heart disorders and many other diseases.
Plant Systematic position		Mosquito systematic position	
Kingdom: Plantae		Kingdom: Animalia	
Order: Lamiales		Phylum: Arthropoda	
Family: Lamiaceae		Class: Insecta	
Genus: <i>Ocimum</i>		Order: Diptera	
Species: <i>tenuiflorum</i>		Family: <i>Culicidae</i>	
		Genus: <i>Culex</i>	

Plant extracts Preparation: The collected *Ocimum tenuiflorum* plant leaves was shade dried for 7 days at room temperature and grind. Plant sample mixed with the methanol solvent in a 1:10 ratio (10gm of plant leaves 100ml of methanol solvent was used). It was 24 hours at 37⁰ C in a shaker set to 150 rpm. After a mixed solvent was filtered using a rotary evaporated at 35⁰ C.



Figure 3: *Ocimum tenuiflorum* leaves



Figure 4: *Ocimum tenuiflorum* power



Figure 5: control



Figure 6: *Ocimum tenuiflorum* powder mixed with methanol solvent



Figure 7: Leaf extract of *Ocimum tenuiflorum*

Table: 2 Yield percentage of methanolic extract of plant used in the study

Plant	Solvent	Extract yield
<i>Ocimum tenuiflorum</i>	Methanol	10

Preparation of Subculture media for Inoculum: All the microorganisms were obtained from Department of Microbiology, V.H.N. Senthikumara Nadar College (Autonomous) Virudhunagar, Tamilnadu, India. Gram-positive, Gram-negative bacteria were selected for this work. A loopful of Microorganism was transferred from laboratory-maintained culture into the test tubes containing sterilized nutrient broth medium. The tubes were incubated for 18 - 24 hours at 37°C. *Staphylococcus*, *Klebsilla* Stock culture were maintained at refrigeration.

Gram positive	Gram negative
<i>Staphylococcus sp.</i>	<i>Klebsilla sp.</i>

Antibacterial activity: Kirby bauer disk diffusion assay 1 ml of each bacterial suspension was uniformly spread on a solid growth medium in a Petri dish. Four sterile paper disks (6 mm in diameter; Becton, Dickinson & Co.) were placed on the surface of each agar plate and were impregnated with 10 μ L of the diluted plant extract. Plates were incubated for 24 h. Antibacterial activity as MIC was determined as the lowest concentration of plant extract which produced an inhibition zone around a disk following the 24-h incubation. Disks impregnated with sterile distilled water and ethanol served as negative controls and a disk with an antibiotic ampicillin served as a positive control.

Results

The *Ocimum tenuiflorum* leaf extract was tested against *Staphylococcus sp.* and *Klebsiella sp.* at different concentrations such as 10 μ l, 15 μ l, 20 μ l. The result indicated that the methanol extract of *Ocimum tenuiflorum* showed antibacterial activity against *Staphylococcus sp.* 9mm, 7mm, 5mm and antibacterial activity against *Klebsiella sp.* 8mm, 5mm 2mm.

Table: 3 Diameter of zone of inhibition (mm) of plant extract against microorganisms at 50mg concentration.

Test organisms	Leaf extract <i>Ocimum tenuiflorum</i>		
<i>Staphylococcus sp.</i>	9mm	7mm	5mm
<i>Klebsilla sp.</i>	8mm	5mm	2mm

Table: 4 Biochemical test characterization of *Staphylococcus sp.* and *Klebsiella sp.*

Test	<i>Staphylococcus sp.</i>	<i>Klebsilla sp.</i>
Gram straning	+	-
Indole test	-	-
Methyle red	+	-
Citrate utilization	+	+
Voges prokauer	+	+
Coagulase	+	-
Gas	-	+
Gelatin Hydrolysis	+	-
H2S	-	-
Catalase	+	+
Motility	-	-
Nitrate reduction	+	+
Pigment Mostly	+	-
Oxidase	-	-
Urease	+	+

+ Positive; - Negative



Figure 8: *Klebsilla sp* pure culture

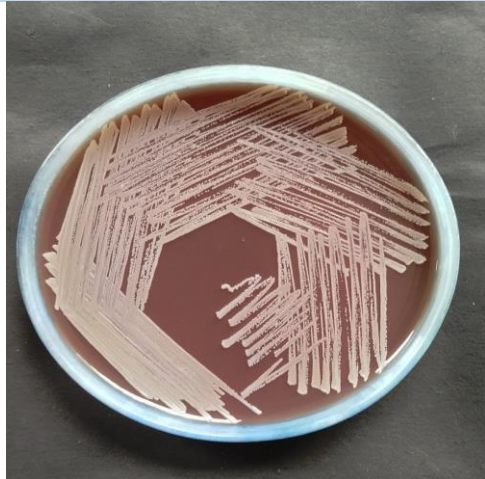


Figure 9: *Staphylococcus sp.* pure culture

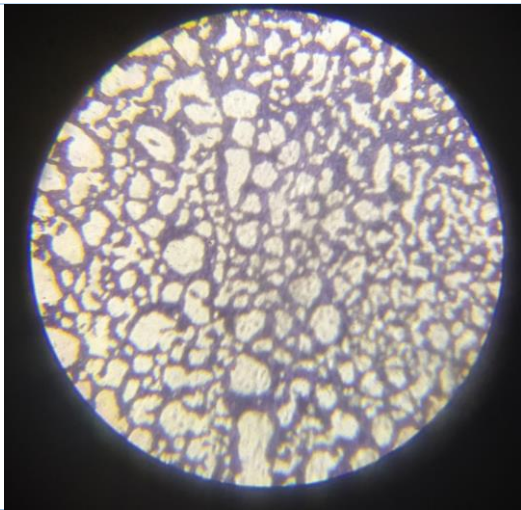


Figure 10: Gram staining of *Staphylococcus sp.*

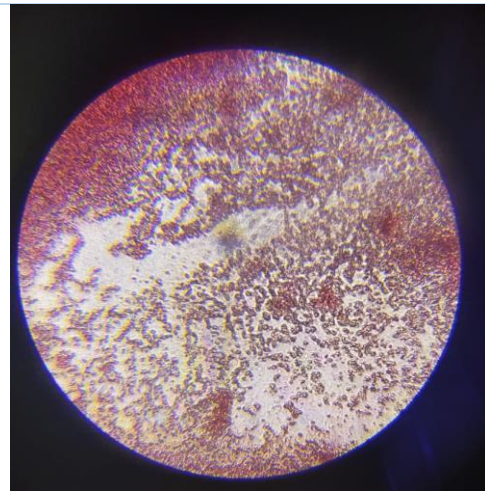


Figure 11: Gram staining of *Klebsiella sp.*



Figure 12: Zone inhibition of *Staphylococcus sp.*



Figure 13: Zone inhibition of *Klebsiella sp.*

Summary

The objectives of the present investigation were to study the, to collected and to identify the medicinal plant of *Ocimum tenuiflorum* leaves; To extract the selected from *Ocimum tenuiflorum* leaves using methanol solvent; Antibacterial against *Staphylococcus sp.* and *Klebsiella sp.*; The *Ocimum tenuiflorum* leaf extract was tested against *Staphylococcus sp.* and *Klebsiella sp.* at different concentrations such as 10 µl, 15µl, 20µl. The result indicated that the methanol extract of *Ocimum tenuiflorum* showed antibacterial activity against *Staphylococcus sp.* 9mm, 7mm, 5mm.

Antibacterial activity against *Klebsiella sp.* at different concentrations such as 10 µl, 15µl, 20µl. The result indicated that the methanol extract of *Ocimum tenuiflorum* showed antibacterial activity against *Klebsiella sp.* 8mm, 5mm 2mm. The tulsi extract demonstrated an antimicrobial property.

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