

ANTIBACTERIAL ACTIVITY OF KRISHNA TULASI (*Ocimum tenuiflorum* Linn.)

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Abstract

Antibiotics was the efficiently combating microbial infections but now a day's antibiotics resistance to human pathogenic bacteria has been commonly reported from all over the world. Hence the people of developing countries face the troubles by the morbidity and mortality associated with infectious diseases. Therefore whole world look towards natural products to develop alternative antimicrobial drugs for the treatment of infectious diseases. The medicinal plants used for treating infectious diseases since ages. In ancient literature of Ayurveda there are so many references for the utilization of plants for infections on the name of Krumighna, Jantughna, Rakshoghna. In this study *Ocimum tenuiflorum* Linn. leaves extract tested against *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC-25923), *Klebsiella pneumoniae* (ATCC-27736) bacteria at different concentration. The result of the present study showed the antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*.

Keywords: Antibacterial; *O. tenuiflorum*; Well-diffusion method.

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INTRODUCTION

Ayurveda an ancient medical science have been utilized medicinal plants to treat and cure infectious diseases since ancient period. The antimicrobial drugs have been mentioned in Ayurveda text since ages on the name of krumighna or rakshoghna.^{[1][2]} Today about 80% of people relays on traditional medicine in developing countries for their primary health care. Herbal medicines are currently in demand and their popularity is increasing day by day. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine. India is a vast repository of medicinal plants that are used in traditional medical treatments.^[3]

The infectious diseases cause the 26 per cent of annual deaths worldwide. Nearly 30 per cent of 1.49 billion disability-adjusted life years (DALYs) are lost every year to diseases of infectious origin.^[4] Mostly the people of developing countries face the troubles by the morbidity and mortality associated with infectious diseases. Infants and children particularly affected by these. About three million children die each year from malaria and diarrheal diseases alone.^[5] India too has its share of infectious epidemics and though mortality owing to these is decreasing, it is a significant part of the disease burden our society carries. The disease burden is high in India because of obvious reasons like poor sanitation, lack of access to fresh water, poor hygiene etc. though exact dependable statistics are not available, a good percent of cases go unreported and infection not recognized till it become symptomatic.^[6]

The efficiency of antibiotics in combating microbial infections was very promising shortly after their introduction. However, resistance to these agents developed rapidly afterward and the problem of antibiotic resistance has remained a menace threatening the benefits of antibacterial agents. As a result,

a solution to the issue of antimicrobial resistance is a matter of urgent importance.^[7] Therefore whole world look towards herbal medicine for best option against microorganisms. Natural products are viewed as a privileged group of structures which have evolved to interact with a wide variety of protein targets for specific purposes. Also the same protein structure with little or no variation serves different purposes in different organisms. As a result, it is anticipated that the search for antimicrobial leads from natural sources will yield better results than from combinatorial chemistry and other synthetic procedures.^[8]

Ocimum tenuiflorum Linn. is the holy herb and also known as Krishna tulsi, it belonging to family Lamiaceae. It is very important for its therapeutic potentials. Traditionally it is used in skin diseases, cold, cough, fever, vomiting, swelling etc. and the various parts like leaves, flowers, seeds, root and stems are being used in the treatment. The main chemical constituents present in *O. tenuiflorum* are eugenol, oleanolic acid, ursolic acid, rosmarinic acid, carvacrol, Linalool, and β -caryophyllene.^[9]

Tulsi (*Ocimum tenuiflorum* Linn.) belonging to family Lamiaceae are very important for their therapeutic potentials. Traditionally leaves are used for common colds and fever. Whole plant powder is also used for treating jaundice and for alleviating blood pressure.^[10]

Traditional important use of Tulsi is in respiratory problems such as Kasa (cough), Shwasa (Asthma), Pratishyaya (cold). It is an erect, much branched, softly hairy, annual herbs, 30-75 cm high. Leaves are elliptic - oblong, entire or serrate, pubescent, minutely gland-dotted. Flowers are purplish or crimson, in close whorled racemes. Nutlets are subglobose or broadly ellipsoid, slightly compressed, pale brown or reddish.^[11]

The plant is bitter, acrid, aromatic, stomachic, demulcent, diaphoretic, digestive, diuretic, expectorant, febrifuge, vermifuge and alexeteric. It is useful in cardiopathy, blood disorders, leucoderma, asthma, bronchitis, catarrhal fever, otalgia, hepatopathy, vomiting, lumbago, hiccough, ophthalmia, gastropathy in children, genitourinary disorders, ringworm, verminosis and skin diseases.^[12]

For the evaluation of antimicrobial activity of *Ocimum tenuiflorum* Linn. leaves, the present study was under taken.

MATERIAL AND METHODS

Plant material

The fresh leaves of *Ocimum tenuiflorum* Linn. were collected in the month of 21 July to 30 September 2014 from village Dabha, Wardha District (M.S.). This village situated at 20.57 N and 78.81 E. For authentication of plant, herbarium prepared and sent to Botanical Survey of India, Pune Maharashtra. The voucher specimen number is BSI/WRC/Tech./2014/447- MSD-3.

Leaves were subjected for washing under the tap water to remove adherent soil, dirt etc. for 2-3 times and finally followed by ethanol wash and then allowed to shade dry at room temperature for seven days. Finally leaves of three plants powdered individually to a coarse powder with mixer grinder. Stored in the air tight container and labelled it for study.

Preparation of extracts

20 g *Ocimum tenuiflorum* Linn., leaves powdered material was extracted in 200 ml ethanol by soxhlet apparatus at 40-50°C. Filtered extract kept at room temperature for elimination of ethanol, 2.29 g extract was collected. And it is diluted in 10 ml ethanol. This extract stored in a refrigerator for further use and called as the mother solution. Made the different concentration of extract from

mother solution as 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml, 100 mg/ml.

Microorganisms

The pure culture of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC-25923), *Klebsiella pneumoniae* (ATCC-27736) bacteria procured from the American Type Culture Collection (ATCC).

Preparation of subculture media for Inoculum

A loopful of organism was transferred from laboratory maintained culture (agar slant) into the test tubes containing sterilized nutrient broth medium. The tubes were incubated for 18-24 hours at 37°C.

Preparation of Assay Medium and Pour plates

The sterile Mueller Hinton Agar (MHA) is dispensed to previously sterilized Petri dishes and allowed them to cool at 40°C, the medium will solidify. The Petri dishes provide a large surface area for growth and cultivation. The mixture of microorganisms (prepared subculture media) was lawn over the surface of solid MHA medium with sterile swab stick. The six well (hole) of 8 mm diameter were bored. The *Ocimum tenuiflorum* Linn. leaves extract of different concentration (20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL, 100 mg/mL) poured in each well and standard solutions (99% ethanol) poured in one well with sterile pipette. The volume of all test solution and standard solutions was 50µl. The Petri dishes were kept in incubator for 18 to 24 hr at 37°C. All the procedures were carried out in an aseptic area.^[13]

RESULTS AND DISCUSSION

The present study was to carry out for the evaluation of antibacterial activity of *Ocimum tenuiflorum* Linn.

Figure 1: Inhibition zone of *Ocimum tenuiflorum* Linn. leaves extract



Table 1: Zone of Inhibition in *Escherichia coli*

Bacteria		<i>Escherichia coli</i>				
Plant extract	Control (ethanol)	20mg/ml	40mg/ml	60mg/ml	80mg/ml	100mg/ml
Zone of Inhibition (mm)	8	8	8	8	9	10

Table 2: Zone of Inhibition in *Staphylococcus aureus*

Bacteria		<i>Staphylococcus aureus</i>				
Plant extract	Control (ethanol)	20mg/ml	40mg/ml	60mg/ml	80mg/ml	100mg/ml
Zone of Inhibition (mm)	10	16	17	18	18	19

Table 3: Zone of Inhibition in *Klebsiella pneumoniae*

Bacteria		<i>Klebsiella pneumoniae</i>				
Plant extract	Control (ethanol)	20mg/ml	40mg/ml	60mg/ml	80mg/ml	100mg/ml
Zone of Inhibition (mm)	10	11	11	11	11	12

The plant extract tested against gram positive (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*). Zone of inhibition of *Ocimum tenuiflorum* Linn. ethanol extract in this study indicated the antibacterial activity in *Staphylococcus aureus* and *Klebsiella pneumoniae* from the concentration 20mg/ml to 100 mg/ml. (Figure 1) There was less antibacterial activity against *Escherichia coli*. 8mm, 8mm, 8mm, 9mm and 10mm zone was present at 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml, and 100 mg/ml respectively. (Table 1 and Figure 1)

The zone of inhibition against *Klebsiella pneumoniae* was 11 mm, 11 mm, 11 mm, and 11 mm at 20 mg/ml, 40 mg/ml, 60 mg/ml, and 80 mg/ml respectively. And 12 mm at 100 mg/ml, control drug shows 10 mm zone. (Table 2) The zone of inhibition present against *Staphylococcus aureus* was 16 mm, 17 mm, 18 mm, 18 mm, 19 mm at 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml, 100 mg/ml respectively and control drug was shoed 10 mm zone. (Table 3)

Staphylococcus aureus and *Klebsiella pneumoniae* both are respiratory pathogens and

causes of nosocomial pneumonia. High mortality rates have been recorded during influenza epidemics as a result of cardiorespiratory failure or secondary bacterial pneumonia caused by *Staphylococcus aureus*.^[14] *Ocimum tenuiflorum* Linn. may be the best solution for this troubles. Sushruta also recommended Krishna tulsi in respiratory diseases.^[15] The results of this study proved its efficacy against respiratory tract bacteria.

E. coli that is capable of causing disease in healthy individuals three general clinical syndromes can result from infection with one of these pathotypes: enteric/diarrhoeal disease, urinary tract infections (UTIs) and sepsis/meningitis.^{[16][17]} But the *Ocimum tenuiflorum* Linn. had showed the less activity against this pathogen. That means it may be not indicated in infection of digestive and urinary system.

CONCLUSION

Ocimum tenuiflorum Linn. belongs to Krumighna gana in Charaka and Sushrut samhita, so that it's important therapeutic indications may be infectious diseases caused by bacteria. Result of this study reveals that *Ocimum tenuiflorum* Linn. has potential activity against *Staphylococcus aureus* and *Klebsiella pneumonia* respiratory tract bacteria, so that it is useful in respiratory tract infection. And it may be not indicated in digestive and urinary tract infection because of less activity found against *Escherichia coli*.

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