

Essential Oils Extracted From *Ocimum Tenuiflorum* (Tulasi) Leaves Show Antimicrobial Activity in Vitro



Medical Science

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ABSTRACT

The aim of this study was to evaluate the antibacterial activity of the essential oils extracted from the leaves of *Ocimum tenuiflorum* (Tulasi) against some microbial pathogens namely *Salmonella enterica serovar typhi*, *Escherichia coli* and *Staphylococcus aureus*. Higher antibacterial activity was shown against gram-positive bacteria compared to Gram-negative bacteria. According to the results of this study it was found that highest antimicrobial activity was noticed against *Staphylococcus aureus*. In conclusion, *Ocimum* extracts found to be containing chemical compounds may be useful in controlling many microbial pathogen, especially the enteric pathogen like *Salmonella enterica serovar typhi* and drugs can be developed using these essential oils.

1. Introduction

Ocimum tenuiflorum, also known as holy basil, or **tulasi** (also spelled thulasi), is an aromatic **plant** in the family Lamiaceae which is native to the Indian subcontinent and widespread as a cultivated **plant** throughout the Southeast Asian tropics.

Plant extracts has been used to treat for microbial disease from ancient time in traditional medical systems. Ability of using most of the medicinal plants for the treatments for various diseases may lie the antimicrobial effect of phytochemicals [1].

Antimicrobial activities of some phytochemicals have been investigated and the possibility of using them in the development of new antimicrobial drugs also been documented [2]. Due to the development of resistance in pathogenic microorganisms to antibiotics used in modern medical science, there is a growing attention towards plant extracts as a source of new antimicrobial drug discoveries. As such investigations on the composition, activity, as well as validation of the use of extracts obtained from medicinal plant is important [3].

Ocimum tenuiflorum is a grassy annual plant originated from India [4, 5, 6]. Some of the phytochemicals of medicinal importance present in *Ocimum tenuiflorum* have already been identified [7]. Some of these phytochemicals have been shown to possess useful biological activities belonging mainly to phenolic, flavonoid, and carotenoid compounds [8].

The ability of this plant to be used in traditional medicine in the treatment of headaches, cough, diarrhea, constipation, warts, kidney malfunctions, nasal polyps and ulcers has also been reported [4,5,9]. Further, its action as insecticide, nematicide, fungicide and antimicrobial compound also has been reported [7, 10, 11, 12, 13, 14].

Salmonella enterica serovar typhi, *Escherichia coli* and *Staphylococcus aureus* are pathogenic bacteria causing typhoid and gastroenteritis of human. *Salmonella* is one of the major food and water borne pathogenic bacteria which cause the disease called salmonellosis [15, 16].

Escherichia coli is a bacterium present in the intestinal tract of warm-blooded animals as its normal micro flora. Most of the *E. coli* strains are nonpathogenic but some serogroups, such as enterohemorrhagic O157:H7, are pathogenic and cause severe diarrhea and fever [18].

Staphylococcus aureus causes staphylococcal food poisoning.

The aim of present study was to investigate the antibacterial activity of essential oil extracts of *Ocimum tenuiflorum* leaves against above microbial pathogens.

2. Materials and Methods

2.1 Isolation of essential oil from *Ocimum tenuiflorum* [20]

Fresh leaves of *Ocimum tenuiflorum* were harvested. **The leaves were chopped or bruised.**

A bundle of fresh tulasi leaves were chopped into two or three pieces, or mashed with a clean cup base, so more oils will be exposed to the liquid. Dried leaves can be crumbled by hand instead, or left mostly whole. Fresh tulasi leaves should be washed before chopping. The stems were removed.

The tulasi leaves and 90–95% alcohol was packed into a sealable jar. The jar was stuffed with tulasi leaves, leaving as little as ½ inch (1.25 centimeters) of space if stronger extracts wanted. Once the leaves is in, the alcohol was poured into the jar, completely covering the leaves. The lid of the jar was closed tightly. The leaves may float at first. They were being pushed down with a spoon, but they should sink on their own after a few days.

The jar was left for several weeks with occasional shaking. The exact length of time simply depends on how strong one's extract to be, but it took about four to eight weeks. Most people prefer to store the jar in a dark place, since sunlight could lower the extract's shelf life. Once or twice a week, the jar was shaken for a couple minutes to speed up the dissolving process.

The liquid was strained into a brown glass container. The liquid was poured through a coffee filter to remove the leaves and the sediment. The extract was stored in a brown glass container to protect it from sunlight and increase its shelf life. It can last for six months or more, although it may lose its potency gradually.

If the extract has a alcoholic smell to it, or isn't as strong as it was required, The jar was left out for another week with a fresh coffee filter or cloth over the lid. Some of the alcohol will evaporate. To remove the oil from the alcohol, the container was placed in the freezer. The oil will congeal on top of the alcohol – which won't freeze – and can be scraped off.

For the highest content of oils in plants, they were picked around 10 am, after the dew is gone, but before the sun gets too hot. Essential oils are usually made using a lengthy process of distillation by steam, not viable for the average laboratory.

2.2 Antibacterial activity assay [3]

Prepared cultures of *Salmonella enterica serovar typhi*, *Escherichia coli* and *Staphylococcus aureus* were used for this assay. From each cultures, 1 ml were cultured on Mueller Hinton Agar plates. Then, with the help of a sterile cork borer wells of 4 cm diameter were prepared on each of those plates. 50 µl of each *Ocimum tenuiflorum* extracts were separately added to the wells in

plates which were inoculated with each bacteria. Then those were incubated at 37°C for 24 hours. After incubation a clear zone of inhibition

were observed around the wells. Diameters of those inhibition zones were measured. Antimicrobial activity of each *Ocimum tenuiflorum* extracts against the tested pathogenic microorganisms was evaluated using those measurements.

3. Results and Discussions

Antibacterial activity of different *Ocimum tenuiflorum* extracts against microbial pathogens, *Salmonella enterica*, *Escherichia coli* and *Staphylococcus aureus* were studied. Results of the studies are shown in the table 1.

Table 1: Antibacterial activity of different *Ocimum tenuiflorum* extracts against *Salmonella enterica*, *Escherichia coli* and *Staphylococcus aureus*

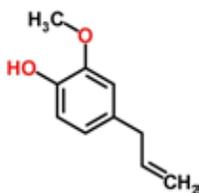
Extract	Microorganisms		
Basil Oil	<i>Salmonella enterica</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
	Zone diameter (18 mm)	Zone diameter (16 mm)	Zone diameter (20 mm)

According to the results, extracts obtained from *Ocimum tenuiflorum* leaves shown antibacterial activity against the above three microorganisms. In this study^[14] *Ocimum* extract was found to be with antimicrobial properties against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enterica*. Significantly higher antibacterial activity of *Ocimum tenuiflorum* extract was shown against *Salmonella enterica*. In this present study, highest and lowest antimicrobial activity was shown against *Staphylococcus aureus* and *Escherichia coli*

respectively.

Essential oils usually obtained from *Ocimum tenuiflorum* are linalool, (-)-bornyl acetate, eugenol, eucalyptol and methyl chavicol.

This can be mainly due to the presence of eugenol, a phenolic compound, which has been reported to have antimicrobial properties^[21, 22, 23].



4. Conclusion

Essential Oils extracted from *Ocimum tenuiflorum* obtained by alcoholic method showed antimicrobial activity against three microbial pathogens. Those were found to be more effective in controlling gram positive bacteria than gram negative bacteria. So, *Ocimum tenuiflorum* extract can be useful in the development of drugs against tested microbial pathogens.

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