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An Evaluation of the Antimicrobial Activity of Various Concentrations of *Ocimum sanctum* Against Various Species of Bacteria: an *Invitro* Study

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ABSTRACT

Plants are one of the most important sources of medicines. Among the plants known for medicinal value, the plants of genus Ocimum belonging to family Labiatae are very important for their therapeutic potentials. *Ocimum sanctum L*., known as 'Tulsi' in Hindi and 'Holy Basil' in English is an erect softy hairy aromatic herb or under shrub found throughout India. The antimicrobial activity is done by using Soxhlet apparatus method. For antimicrobial activity, Nutrient broth was used for inoculating the culture. Then wells were cut on the solidified agar medium which were swabbed with microorganism. Thus agar well diffusion method is carried out. The extracts were poured into the wells at different concentrations like 25%, 50% and 100%. After incubation, zone of inhibition was observed. As the concentration of extract increases, the activity also increases and thus the zone of inhibition too increases. Significant results were shown by *Escherichia coli* and *Staphylococcus aureus*. Hence, *Ocimum sanctum* can be used in developing drugs and medicines against various activities of Bacteria.

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1. INTRODUCTION

The large number of drugs in use is derived from plants, like morphine from *Papaver somniferum*, Atropine from *Atropa belladonna* etc. The medicinal plants are rich in secondary metabolites (which are potential sources of drugs) and essential oils of therapeutic importance.

Plants are one of the most important sources of medicines.Among the plants known for medicinal value, the plants of genus Ocimum belonging to family Labiatae are very important for their therapeutic potentials. *Ocimum sanctum L*., known as 'Tulsi' in Hindi and 'Holy Basil' in English is an erect softy hairy aromatic herb or undershrub found throughout India. Tulsi is commonly cultivated in gardens. Two types of *Ocimum sanctum L*., are met within cultivation. (i) Tulsi plant with green leaves known as Sri Tulsi and (ii) Tulsi plants with purple leaves known as Krishna Tulsi [1]. Extracted essential oils have also been shown to contain biologically active constituents that are insecticidal, nematicidal and fungistatic [2]-[5]. These properties can be frequently attributed to predominate essential oil constitutes such as methyl chavicol, eugenol linalool, camphor and methyl cinnamate. Two minor components of the essential oil of sweet basils *(Ocimum basilicum)*: Juvocimene I and II have been reported as potent juvenile hormone analogs [2].

Several medicinal properties have been attributed to *Ocimum sanctum*. Different parts of tulsi plant e.g. leaves, flowers, stem, root, seeds etc are known to possess therapeutic potentials and have been used by traditional medical practitioners as expectorant, analgesic, anti cancer, anti asthmatic, antiemetic, diaphoretic, anti diabetic, anti fertility, hepatoprotective, hypotensive, hypolipidimic and anti stress agents. Tulsi has also been used in treatment of fever, bronchitis, arthritis, convulsions etc. Eugenol is a phenolic compound and is the major constituent of essential oils extracted from different parts of tulsi plant.

The use of plants to heal diseases, including infectious one, has been extensively applied by people. Data from the literature as well as our results reveal the great potential of plants for therapeutic treatment, inspite of the fact that they have not been completely investigated. Therefore, more studies need to be conducted to search for new compounds. Once extracted, and before being used in new therapeutic treatments, they should have their toxicity tested *in vivo*. Bioassays have demonstrated the toxicity of extracts from different plants. The antimicrobial activity was tested by the method based on the principle of Kirby Bauer test. An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, all protozoans (*Merriam-Webster Online Dictionary)*. The disc diffusion method is the commonly used technique to check the antimicrobial activity. The pattern of growth of an organism on the inoculated plate begins at the point distant from the crude extract reservoir and proceeds inwards until a point is reached where the concentration of crude extract is inhibitory for the organisms.

2. MATERIALS AND METHODS

Sample collection: Samples of Tulsi (*Ocimum sanctum*) were collected from 5 different locations in Kerala viz; Alappuzha, Kollam, Kottayam, Pathanamthitta and Trivandrum. The leaves of the collected samples were used for the study. The methodology has been optimized in the department research laboratory.

Table 1. Geographical locations of the accessions of *Ocimum sanctum*.

Accessions (Acc.)	Places from where samples are collected
Acc.1	Alappuzha
Acc.2	Kollam
Acc.3	Kottayam
Acc.4	Pathanamthitta
Acc.5	Trivandrum

The following steps are involved to assess the anti microbial activity of *Ocimum sanctum*

2.1 Crude extract preparation

1. The samples were dried in shade place.
2. After drying, the samples were ground to powder and were used for extraction.
3. 20 g of each ground samples was placed in Soxhlet extractor.
4. Methanol was used as the solvent to extract the bioactive compounds in Ocimum.
5. After extraction, the excess solvent from the extract was distilled out using a distillating apparatus.
6. The collected extracts were concentrated by exposing them in a laminar air flow and stored at 4°C.
7. Three different concentrations of the extract from the sample were prepared; 25, 50 and 100% by using DMSO (Dimethyl Sulphoxide) as the solvent.
8. Three different concentrations such as 100%, 50% and 25% were prepared by the following method.

   100µg plant extract = 100%

   50µg plant extract + 50µl DMSO = 50%

   25µg plant extract + 75µl DMSO = 25%

   On the basis of the calculation, three different concentrations were used for testing the anti microbial activity.

2.2 Preparation of wells

Take three sterile Petri dishes. Pour the nutrient agar medium into the plates and allow to solidify. Swab the microbes (*Escherichia coli* & *Staphylococcus aureus*) into the plates. Using a gel puncher, make appropriate wells on the plate. Different concentrations of plant extract (25%, 50%, and 100%) was weighed

out and then dissolved in DMSO. To each plate, three different concentrations of extracts were added in different wells and kept for incubation for 24 hours. Testing for anti microbial activity

1. A sterile wire loop was used to pick a colony of the test organism (*Escherichia coli & Staphylococcus aureus*) and was suspended in nutrient broth.
2. The broth was incubated for 5 hours at 37°C.
3. After incubation, a sterile cotton wool was immersed in to the cell suspension.
4. The excess suspension was drained by pressing against the wall of the tube.
5. The cell suspension was swabbed all over the surface of the nutrient agar plates in order to obtain a lawn growth of the organism.
6. Using a sterile gel puncher, three wells were made which are equidistant from each other.
7. The three different concentrations of plant extract were added to these wells.
8. All the steps were carried out under strict aseptic conditions.
9. The plates were incubated at 37°C for 24 hours.
10. The plates were observed for zones of inhibition around the well

3. RESULTS AND DISCUSSION

The activity of Tulsi extract against *Escherichia coli & Staphylococcus aureus* was found to be higher at a concentration of 100% followed by 50% & 25% respectively. The maximum zone of inhibition was found to be 16mm against *Escherichia coli* and 17mm against *Staphylococcus aureus*.

Table 2. Methanol Extract Sensitivity Pattern of E.Coli & S.Aureus.

Name of organism	Concentration (%) of extract	Zone of inhibition (mm)
E.coli	25	11
	50	13
	100	16
S.aureus	25	9
	50	13
	100	17

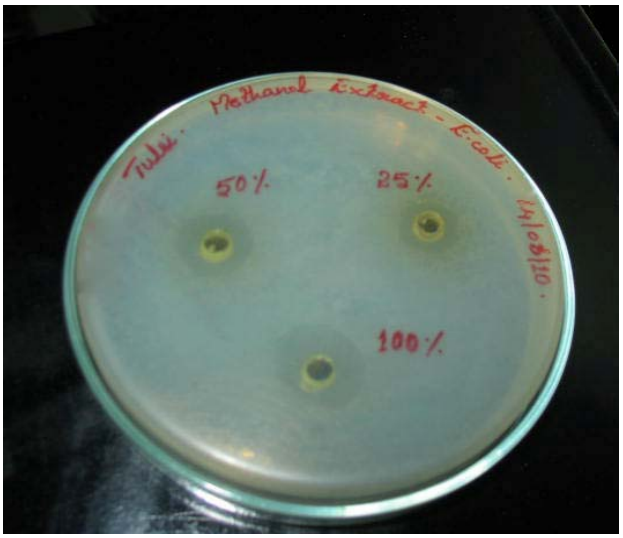

Figure 1. *Ocimum sanctum* Methanol Extract against *E.Coli*

Figure 2. *Ocimum sanctum* Methanol Extract against S. Aureus

Soxhlet apparatus method has been used to determine the antimicrobial activity. Substances that dissolve only in methanol diffuse out during methanolic extraction and those substances which dissolve only in water diffuse during water extraction while using a Soxhlet apparatus. Antimicrobial activity is mainly shown by methanolic extract. The methanolic extract of *Ocimum sanctum* gives carbohydrates, tannin, saponin, terpanoids, glycosides, anthraquinone, and alkaloids which are responsible for antimicrobial activity. The result shows that methanolic extracts of *Ocimum sanctum* was effective in antimicrobial activity. Agar well diffusion method is used here which ultimately results in clear zones. As the concentration of extract increases, the activity also increases and thus the zone of inhibition too increases. Significant results were shown by *Escherichia coli* and *Staphylococcus aureus*.

REFERENCES

[1] Llori. "Antidiarrhoeal activities of Ocimum gratissium", *J. Diarrhoeal Dis Res*, Vol/Issue: 14(4). Pp. 283-285, 1996.
[2] Nishida, R., W.S. Bowers and P.H. Evans. "Synthesis of highly active juvenile harmones analogsm Juvocimene I and II, from the oil of sweet basil Ocimum basilicum L", *J. Chem. Ecol*, Vol. 10. Pp. 1435-1450, 1984.
[3] Chatterjee, A., N.C. Sukul, S. Laskal, and S. Ghosmajumdar. "Nematicidal Principles from two species of Lamiaceae", *J. Nematol.*, Vol. 14. Pp. 118-120, 1982.
[4] Chavan, S.R. and S.T. Nikan. "Mosquito Larvicidal activity of Ocimum basilicum Linn", *Indian J. Med Res.*, Vol. 75. Pp. 220-222, 1982.
[5] Chogo, J.B. and G.Grank. "Chemical composition and biological activity of Tanzanian plant Ocimum suave", *J. Nat. Prod*, Vol/Issue: 44(3). Pp. 308-311, 1981.