

How to Cite:

JadheerAhsan, K., Jaleel, A., Chaudhary, H., Bhattacharjee, S., Dan, S., & Dilshani, J. (2022). Comparative studies of antibacterial activity of *Ocimum Sanctum* and *curcuma longa* leaf extracts against *E. coli* and *Staphylococcus Aureus*. *International Journal of Health Sciences*, 6(S5), 3694–3701. <https://doi.org/10.53730/ijhs.v6nS5.9322>

Comparative studies of antibacterial activity of *Ocimum Sanctum* and *curcuma longa* leaf extracts against *E. coli* and *Staphylococcus Aureus*

JadheerAhsan Kp

BSc Microbiology, Calicut University, Malappuram, Kerala, India, 676517

Orcid Id =0000-0001-6002-3923

Corresponding author email: sayyidjadheer@gmail.com

Abdul Jaleel

BSc Microbiology, Calicut University, Malappuram, Kerala, India, 676517

Orcid Id=0000-0002-1648-3778

Email: abduljaleelr1@gmail.com

Himanshi Chaudhary

Department of Bioscience and Biotechnology, Banasthali University, Tonk, Vanasthali, Rajasthan 304022

Swagato Bhattacharjee

Department of Biotechnology, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala, India, 695014

Siddhartha Dan

Department of Biotechnology, I.K Gujral Punjab Technical University

Email: siddharthadan7@gmail.com

Jasmine Dilshani

BSc Microbiology, Calicut University, Malappuram, Kerala, India, 676517

Email: jasmindilnashi6@gmail.com

Abstract---Due to their traditional therapeutic use, *Ocimum Sanctum* (Tulsi) and *Curcuma Longa* (Turmeric) have been subjected to numerous antibacterial tests. To compare the antibacterial effects of tulsi (*Ocimum sanctum*) as well as *Curcuma Longa* (Turmeric) against Gram-positive (*Staphylococcus aureus*) as well as Gram-negative (*Escherichia coli*) bacteria. The method of agar well diffusion was used. In addition, different phytochemical tests were carried out. Aside from the froth test, tannin tests; Sterol tests showed positive. All of the

results are presented in the form of a graph and various tables. When turmeric was used, it exhibited a 22 mm Zone of Inhibition (ZOI) in *E. coli* and a 17 mm ZOI in *Staphylococcus aureus*. When tulsi was applied, however, roughly 20mm of ZOI was found in both *E. coli* and *Staphylococcus aureus*. At six different concentrations, the diameter of ZOI was measured, methanol extract of both the Turmeric and tulsi, including 0.2 g/ml, 0.3 g/ml, 0.4 g/ml, 0.5 g/ml, 0.6 g/ml, and 0.7 g/ml. In conclusion, Tulsi leaf extract and Turmeric curcumin extract were collected to perform various antibacterial activities. The agar well diffusion method was used in this study. The bactericidal activity of different concentrations of extracts varied significantly. Tulsi is more effective against *E. coli* and *Staphylococcus aureus* than other herbs. On the other hand, turmeric has a variant that is more potent against *E. coli* than *Staphylococcus aureus*. As a result, these plants are extremely effective in treating enteric disorders such as diarrhea, vomiting, and urinary tract infection, among others.

Keywords---*Ocimum Sanctum*, *Curcuma longa*, Antibacterial, Phytochemical Zone of inhibition.

1. Introduction

Medicinal plants have been utilized in traditional medicine to treat human ailments and disorders since prehistoric times [1]. The existence of one or more of the plant's active ingredients could be attributed to the plants' therapeutic capabilities [2]. Phytochemicals such as alkaloids, flavonoids, and terpenoids have been found to be responsible for medicinal plants' antibacterial properties [3]. Plant screening has been intensified in recent years to uncover novel antibacterial medicines [4]. As a result, exploratory screening of medicinal plants for antimicrobial activity and the presence of phytochemicals might lay the groundwork for future study in this field.

Because of its medicinal properties, *Ocimum sanctum*, often known as tulsi or holy basil, has been utilized in Ayurveda for centuries. It is also regarded as the "king of herbs" and belongs to the kingdom Plantae, making it one of India's holiest herbs. It is known as the elixir in Ayurveda and increases longevity due to its potent flavor. Tulsi extract is often used to cure colds, headaches, gastrointestinal problems, and other ailments [5]. The anticancer effect of *O. sanctum* extract is demonstrated by a decrease in cell growth, an increase in reactive oxygen species, and a shift in the potential of the mitochondrial membrane [6]. Tulsi dried leaves have been used to repel insects in grains for centuries. Tulsi has been shown to improve lipid profiles and basal metabolic rate [7].

The gram-negative bacterium *Escherichia coli*, is the leading cause of diarrheal illnesses, peritonitis, colitis, and newborn mortality [8]. The pathogenicity of *E. coli* is acquired by virulence factors [9]. A severe case of diarrhoea can lead to bloody diarrhoea or hemorrhagic colitis, both of which are lethal. *E. coli*, which causes urinary tract infections, has developed resistance to the drug that was previously used to treat it, necessitating the development of new and plant

extract-based medicines, whereas *Staphylococcus aureus*, a Gram-positive bacterium (member of the Firmicutes), can cause the life-threatening disorders like sepsis and endocarditis. In wastewater treatment plant sites, *S. aureus* is common. *S. aureus* is well-known for its capacity to develop drug resistance and antibiotic resistance to antibiotics like penicillin and methicillin [10].

Antibiotic resistance among bacteria has risen dramatically over time due to evolution, adaptability, and abuse of synthetic medications against them. As a result, there will always be a need to produce efficient antimicrobial medications to combat them, and due to the huge plant diversity and phytochemicals that include secondary metabolites with antimicrobial activity, there is a constant need to do so, it can be an alternative to synthetic drugs because it is less expensive and has fewer side effects [11]. These plants can be used in a particular mixture to prevent and treat various illnesses and ailments. Formulations have a lot of potential in the food and pharma industries. *S. aureus* is the most common cause of dermal infections and septicemia among microbial health concerns, while pathogenic forms of *E. coli* can cause vomiting, diarrhea, and, nausea [10,11].

2. Materials and Methods

2.1. Plant materials.

The leaves of *Ocimum Sanctum* and *Circuma Longa* were taken from Botanical Garden in Manjeri, Kerala, and Manually sorted, washed thoroughly under running tap water and dried under sunlight, and air-dried leaves took 4–5 days. Turmeric was thoroughly cleansed, with all fibers removed and stored in a septic environment.

2.2. Preparation of extracts.

250 ml of methanol was added to a separate conical flask with 50 g of each leaf powder to create a stock solution with a concentration of 0.2 g/ml using a cold maceration extraction technique. Following that, working solutions of each extract were made using the formula " $C_1V_1=C_2V_2$ " at concentrations of 0.2 g/ml, 0.3 g/ml, 0.4 g/ml, 0.5 g/ml, 0.6 g/ml, and 0.7 g/ml. Where C_1 is the stock solution's concentration, C_2 is the new solution's final concentration, V_1 is the stock solution's volume, and V_2 is the new solution's final volume.

Curcuminoids from turmeric were selectively recrystallized using a variety of organic solvents and their combinations. The optimum recrystallization solvent for purifying curcuminoids was determined to be a 1:15 mixture of heated isopropyl alcohol and hexane.

2.3. Test pathogens.

To test antibacterial activity against *E. coli* and *Staphylococcus aureus*, pure cultures of the bacteria were chosen. Organisms were taken from the "Medical lab of Almas hospital.", Kerala, India. All the test bacteria were stored on a nutrient agar slope at 4 °C. These bacteria were sub-cultured for 24 hours before use.

2.4. Phytochemical analysis.

Each test sample of all two plants was subjected to phytochemical analysis. Standard methods were used to determine the content of tannin, saponin, flavonoids, anthraquinonoid, glycosides, sterol and, antimicrobial activity in *Ocimum Sanctum* and *Circuma Longa* leaf extracts [12]. There has been a surge in interest in using phytochemicals to treat dementia in recent years [13], and most phytochemicals are extracted from plants [14].

2.5. Test for tannin.

FeCl₃ (a few drops) was added to 2 ml of aqueous extract, and a dark green color developed, indicating the presence of condensed tannin.

2.6. Test for saponin.

The presence of stable foam after vigorous mixing and warming of 5 mL aqueous extracts with 5 mL distilled water can be used to estimate the saponin concentration.

2.7. Test for flavonoids.

A yellow precipitate occurred when acid extract (2 mL) was combined with sodium hydroxide solution (2 mL), showing the presence of flavonoids.

2.8. Test for anthraquinonoid.

2 ml methanolic extract was boiled in chloroform and combined with 1 ml ammonium hydroxide. The existence of anthraquinonoid was confirmed by the presence of rose-red color.

2.9. Test for glycosides.

In 2 ml of aqueous extract, a few drops of Molisch reagent (10 percent alcoholic solution of alpha-naphthol) were added, and then sulfuric acid was applied drop by drop along the test tube's wall. The presence of a violet ring verified the good result of the glycosides test.

2.10. Test for sterol.

2 mL chloroform was added to 2 mL methanol extract and heated, and then acetic anhydride & sulfuric acid were added. The existence of sterols was confirmed by the reddish-brown color.

2.11. Antimicrobial activity.

The "Agar well diffusion method" was used in this experiment. The organisms mentioned above were inoculated into a nutrient agar plate. After Spreading the bacterial inoculum over the plate (Spread plate method), Agar media was punched with 6 mm diameter wells. 50 mL of plant extracts were applied to the wells of various Petri plates and incubated for 24 hours at 37 degrees Celsius. After that, the zone of inhibition (ZOI) was observed, estimated, and compared using a Vernier scale. The "Agar Cut Well Method" is another name for this method.

3. Results and Discussion

3.1. Statistical analysis

At six different concentrations, the diameter of ZOI was measured, for the quantitative assessment of antibacterial activity of the methanol extract of both the turmeric and tulsi, including, such as 0.2 g/ml, 0.3 g/ml, 0.4 g/ml, 0.5 g/ml, 0.6 g/ml, and 0.7 g/ml. The ZOI measurements were taken as absolute values with a standard error of ± 0.2 mm. Aside from the froth test tannin tests, the Sterol tests showed positive. All of the results are presented in a graph and various tables. When turmeric was used, it exhibited a 22 mm Zone of Inhibition (ZOI) in *E. coli* and a 17 mm ZOI in *Staphylococcus aureus*. When Tulasi was applied, however, roughly 20mm of ZOI was found in both *E. coli* and *Staphylococcus aureus*. As a result, turmeric has been shown to have more antibacterial activity than *Ocimum Sanctum*, and both medicinal plants are effective against the majority of gastrointestinal illnesses that cause nausea, vomiting, and other enteric diseases (Figure 1). For *E.coli* and *S.aureus*, a comparative examination of ZOI for varying concentrations of *Ocimum Sanctum* and *Circuma Longa* was performed and shown in (Figure 2).

Comparing ZOI of Tulsi extract on *E. coli* and *Staphylococcus* at various concentrations (Table 1). Comparing ZOI of Turmeric extract on *E. coli* and *Staphylococcus aureus* at various concentrations (Table 2). We discovered that *Ocimum Sanctum* and *Circuma Longa* differ in terms of the presence of certain phytochemicals after performing phytochemical analysis for several secondary metabolites. The final result can be seen in (Table 3).

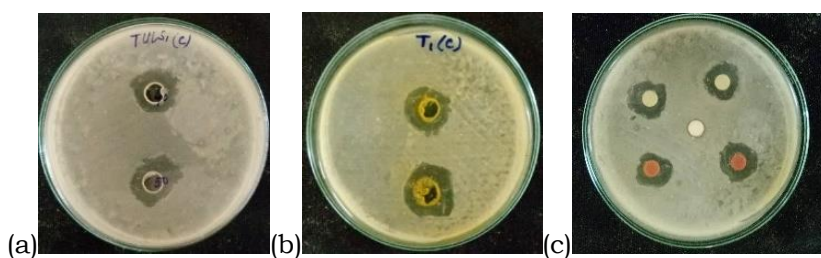


Figure 1. (a) Antibacterial activity of *Ocimum Sanctum* against *Staphylococcus aureus*; (b) Antibacterial activity of *Circuma Longa* against *Staphylococcus aureus*; (c) Antibacterial activity *Ocimum Sanctum* and *Circuma Longa* against *E. coli*

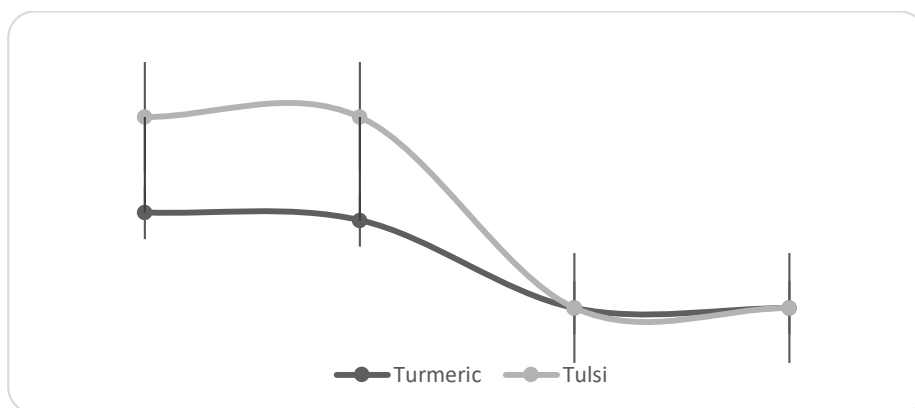


Figure 2. Comparing the overall zone of inhibition in *Ocimum Sanctum* (Tulsi) and *Circuma Longa* (Turmeric) to various doses of Methanol extracts (0.1-0.7 g/ml) of both *Escherichia coli* and *Staphylococcus aureus*.

Table 1. Comparing ZOI of Tulsi extract on *E. coli* and *Staphylococcus aureus* at various concentration.

Concentration of Tulsi (g/ml)	ZOI in <i>E.coli</i> mm	ZOI in <i>Staphylococcus</i>
0.2	0	0
0.3	4	4
0.4	6	8
0.5	12	12
0.6	16	15
0.7	20	20

Table 2. Comparing ZOI of Turmeric extract on *E. coli* and *Staphylococcus aureus* at various concentration.

Concentration of Turmeric(g/ml)	ZOI in <i>E.coli</i> mm	ZOI in <i>Staphylococcus</i>
0.2	0	0
0.3	6	5
0.4	8	10
0.5	16	12
0.6	18	14
0.7	22	17

Table 3. Comparing Phytochemical test results

Test	Turmeric	Tulsi
Flavonoid test	Present	Present
Tannin test	Present	Present
Froth test	Absent	Absent
Sterol test	Present	Present

Molisch test	Present	Present
Antraquinone test	present	Present

3.2. Discussion

In the present study, a leaf sample was collected from Kerala. Tulsi is a tropical plant that is native to the world's tropics and is widely farmed as well as an escaped weed. It is grown for its essential oil, as well as for religious and therapeutic purposes. Tulsi is a sacred plant in various Hindu religious traditions, and it is associated with Goddess figures. 'Tulsi' signifies 'the incomparable one' in Sanskrit.

There has been a resurgence of interest in investing in Tulsi's traditional health-promoting benefits in recent years. The nutritional and pharmacological characteristics of the whole plant in its natural form, as it has been traditionally used, could be the consequence of synergistic interactions between a variety of active phytochemicals. As a result, individual components or extracts cannot fully replicate Tulsi's overall effects. Tulsi standardisation has escaped modern research due to its inherent botanical and biochemical complexity. Tulsi has no caffeine or other stimulants, despite its reputation as a general vitalizer and enhancer of physical endurance.

4. Conclusions

In conclusion, Tulsi leaf extract and turmeric curcumin extract were collected to perform various antibacterial activities. The agar well diffusion method was used in this study. The bactericidal activity of different concentrations of extracts varied significantly. Tulsi is more effective against *E. coli* and *Staphylococcus aureus* than other herbs. Turmeric, on the other hand, has a variant that is more potent against *E. coli* than *Staphylococcus aureus*. As a result, these plants are extremely effective in treating enteric disorders such as diarrhea, vomiting, and urinary tract infection, among others.

Funding

This research received no external funding.

Acknowledgments

Mr. Jadheer Ahsan kp, Ms. Jasmine Dilshani, and Mr. Abdul Jaleel are thankful to Director University of Calicut, Malappuram, for their encouragement for this chapter. Ms. Himanshi Chaudhary is thankful to Principal Banasthali University, Tonk, for her support. The authors have not received any funding in this regard.

Conflicts of Interest

There are no conflicts of interest declared by the authors.

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