

COMPARATIVE STUDY ON ANTIMICROBIAL ACTIVITY OF TULSI (*OCIMUM SANCTUM*) AND NEEM (*AZADIRACHTA INDICA*) METHANOL EXTRACTVIPUL KUMAR^{1,2}, ANURAG CHAKRABORTY¹, MANPREET KAUR¹, SONY PANDEY¹, MANOJ KUMAR JENA^{1*}¹Department of Biotechnology, School of Bioengineering and Biosciences, Lovely Professional University, Punjab, India. ²Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology, Delhi, India. Email: manoj.20283@lpu.co.in

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ABSTRACT**Objective:** This study was focused on to compare the antimicrobial activity of methanolic leaf extracts of tulsi and neem.**Methods:** We have chosen tulsi (*Ocimum sanctum*) and neem (*Azadirachta indica*) to compare their antimicrobial activity toward Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. Phytochemical extracts have been made by the use of methanol as solvent and dried leaf powder by the cold maceration extraction process. Phytochemical analysis for some secondary metabolites has been done using standard protocols. Nutrient agar plates were inoculated with the above-mentioned microorganisms by spreading bacterial inoculum on the surface of the media. Wells (6 mm in diameter) were punched in the agar. The phytochemical extracts of neem and tulsi were allowed to diffuse into the medium, and after incubation of 24 h at 37°C, the zones of inhibition were observed.**Results:** Statistical analysis showed that tulsi was more effective toward *S. aureus* while neem was more effective toward *E. coli*. Minimum inhibitory concentration (MIC) of tulsi for both the microorganisms was 0.4 g/ml, and the MIC of neem for both the microorganisms was 0.2 g/ml. When both the tulsi and neem extract were mixed with each other for every concentration at equal volume, they have shown better effects in comparison to individual neem or tulsi extract and also the MIC got reduced to 0.2 g/ml for both the bacteria.**Conclusions:** Leaf extracts of both the *A. indica* and *O. sanctum* have shown antimicrobial activity against *E. coli* and *S. aureus*. *A. indica* has higher antimicrobial activity against *S. aureus*, whereas the *O. sanctum* was found to be more effective against *E. coli* (indicated by the zone of inhibition). When both the extracts were mixed with each other, they have shown a better effect toward both bacteria.**Keywords:** Minimum inhibitory concentration, Tulsi, Neem, Phytochemical, Zone of inhibition.© 2018 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2018.v11i12.28756>**INTRODUCTION**

Azadirachta indica, an eminent and sacred gift of nature, is commonly known as neem, and it is mainly cultivated in the tropical regions of the Indian subcontinent. The Latinized name of neem, *A. indica*, is derived from the Persian word "Azad" means free, "dirakht" means tree, and "i-Hind" means of Indian origin. Hence, it literally means "the free tree of India" [1]. Neem is a member of the Meliaceae family. Neem possesses many constituents that are of great importance for the treatment of various diseases, and the constituents also modulate the genetic pathways or metabolic activities of the host body [2]. Neem is widely used in the field of pharmaceuticals, agriculture, as fertilizer, etc., due to the presence of biologically important phytochemicals [3]. Acetone extract and chloroform extract of neem leaf found to be a strong candidate for cancer therapy and as strong antibiotics against pathogenic bacteria [4]. Flavonoids are used in food and beverages of plant origin, such as tea, cocoa, and wine, and hence, they are also called dietary flavonoids [5].

Ocimum sanctum, commonly known as tulsi or holy basil, has been used since ages in Ayurveda due to its healing property. It is also known as "queen of herbs" and is one of the holiest herbs in India with Kingdom - Plantae, Subkingdom - Tracheobionta, Superdivision - Spermatophyta, Division - Magnoliophyta, Class - Magnoliopsida, Subclass - Asteridae, Order - Lamiales, Family - Lamiaceae, Genus - *Ocimum*, and Species - *O. sanctum*. Due to its powerful savor, it is regarded as the elixir in Ayurveda and believed to promote lifespan. The extract of tulsi is commonly known for the treatment of cold, headache, stomach disorders, etc. [6]. *O. sanctum* extract shows anticancer activity by decreasing cell proliferation,

increasing reactive oxygen species, and by altering mitochondrial membrane potential [7]. Since ages dried leaves of tulsi have been used for repelling insects in grains. Tulsi has been effective in improving the lipid profile and basal metabolic rate [5]. The study of immune diffusion and inflammation shows that intake of a leaf extract before food can increase immune response by increasing the level of helper T cells and natural killer cells.

Escherichia coli, Gram-negative bacteria, is the major cause of diarrheal diseases, peritonitis, colitis, and infant mortality [8]. *E. coli* acquires its pathogenicity through virulence factors [9]. In the serious case of diarrhea, the patient may end up with bloody diarrhea or hemorrhagic colitis and can become life threatening. *E. coli* responsible for urinary tract infection has become resistant to the drug that has been used to cure it, so need of new and plant extract as a medicine is increasing several folds, whereas *Staphylococcus aureus*, which is a Gram-positive bacteria (member of Firmicutes), can cause life-threatening diseases such as sepsis, endocarditis, and pneumonia. *S. aureus* is prevalent in the sites of wastewater treatment plants. *S. aureus* is considered notorious due to its ability to become drug resistant and resistant to antibiotics such as penicillin and methicillin.

There is an enormous increase in antibiotic resistance among bacteria over a period of time due to evolution, adaptation, and overuse of synthetic drugs against them. Hence, there is always a need of developing potent antimicrobial drugs against them, and due to huge plant diversity and phytochemicals possessing secondary metabolites having antimicrobial activity, it can be a great alternative for synthetic drugs due to its cheaper cost and fewer side effects [10]. We can use these plants as a special formulation for preventing and curing various infections and

diseases. Formulations can be used in food and pharmaceutical industry effectively. Among various microbial health threats, *S. aureus* is the most common for skin infections and septicemia, and pathogenic strains of *E. coli* can lead to various diseases such as diarrhea, vomiting, and nausea.

METHODS

Plant leaf collection

The leaves of *A. indica* (neem) and *O. sanctum* (tulsi) were collected from Botanical Herbal Garden, Lovely Professional University, Phagwara, Punjab. Leaves were manually separated, cleaned, and air-dried for 4–5 days. Subsequently, the leaves were pulverized to a coarse powder through mortar and pestle. Approximately 50 g powder of both the leaves was weighed and stored in an airtight bottle [10].

Preparation of extracts

About 250 ml of methanol was added in a separate conical flask with 50 g of each leaves powder to make a stock solution of 0.2 g/ml concentration using a cold maceration extraction process. Afterward, working solution of each extract at concentrations 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 was prepared on the basis of the formula "C1V1=C2V2."

Where,

C1=Concentration of stocks solution,

C2=Final concentration of a new solution,

V1=Volume of stock solution,

V2=Final volume of new solution

Then, mix solutions of neem and tulsi were also prepared 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 by mixing of each extract concentration at 1:1 ratio.

Test organisms

Two bacterial cultures were used to evaluate the antimicrobial activity: *E. coli* (MTCC40) and *S. aureus* (MTCC740) from the stock of Microbiology Laboratory of Lovely Professional University.

Antimicrobial activity test

Nutrient agar plates were inoculated with the above-mentioned microorganisms by spreading the bacterial inoculum on the surface of the media (spread plate technique). Wells (6 mm in diameter) were punched in the agar [11]. 50 μ l extract of neem, tulsi, and their mixture of each concentration is allowed to diffuse out into the agar medium in separate Petri dishes, after incubation of 24 h at 37°C, the zones of inhibition were observed uniformly circular as there was a confluent lawn of growth. Moreover, finally, the diameter of the zone of inhibition (ZOI) was measured in millimeters by Vernier scale [12].

Phytochemical analysis

Test for tannin

In 2 ml of aqueous extract FeCl_3 (few drops) was added, and dark green color appeared, which confirmed the presence of condensed tannin [13].

Test for saponin

Observation of stable foam after vigorous mixing and warming of 5 ml aqueous extracts with 5 ml distilled water indicated the presence of saponin [13].

Test for flavonoids

Acid extract (2 ml) was added in sodium hydroxide solution (2 ml), yellow precipitate was observed, which confirmed the presence of flavonoids [13].

Test for anthraquinone

Methanolic extract (2 ml) was heated with chloroform and mixed with 1 ml of ammonium hydroxide. Observation of rose red color confirmed the presence of anthraquinone [14].

Test for glycosides

Few drops of Molisch reagent (10% alcoholic solution of alpha-naphthol) were added in 2 ml of aqueous extract, and then, sulfuric acid was added drop by drop along the wall of the test tube. Observation of violet ring confirmed the positive test for glycosides [15].

Test for sterol

About 2 ml of chloroform was added in 2 ml methanol extract and heated up, and then, acetic anhydride and sulfuric acid were added. The observation of reddish-brown color confirmed the presence of sterols [15].

RESULTS AND DISCUSSION

The quantitative assessment for antimicrobial activity of the extracts was performed by measuring the diameter of ZOI at six different concentrations 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 methanol extract of both the neem and tulsi.

ZOI has been taken as absolute values neglecting ± 0.2 mm of standard error.

ZOI for *S. aureus*

With neem for the above-discussed concentrations, 4 mm, 7 mm, 8 mm, 8 mm, 10 mm, and 12 mm diameter, ZOI has been observed, respectively (Table 1). In tulsi for 0.2 g/ml and 0.3 g/ml concentrations, no ZOI was observed, whereas for other concentrations, 4 mm, 12 mm, 17 mm, and 20 mm diameter ZOI was observed, respectively (Table 2). The ZOI of 8 mm and 4 mm of *S. aureus* from neem and tulsi at concentration 0.4 g/ml has been shown in Figs. 1 and 2, respectively.



Fig. 1: ZOI of 8mm of *S.aureus* from 0.4g/ml of Neem methanolic extract

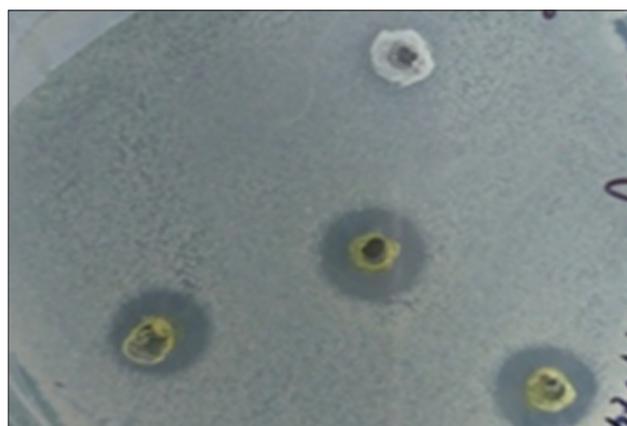
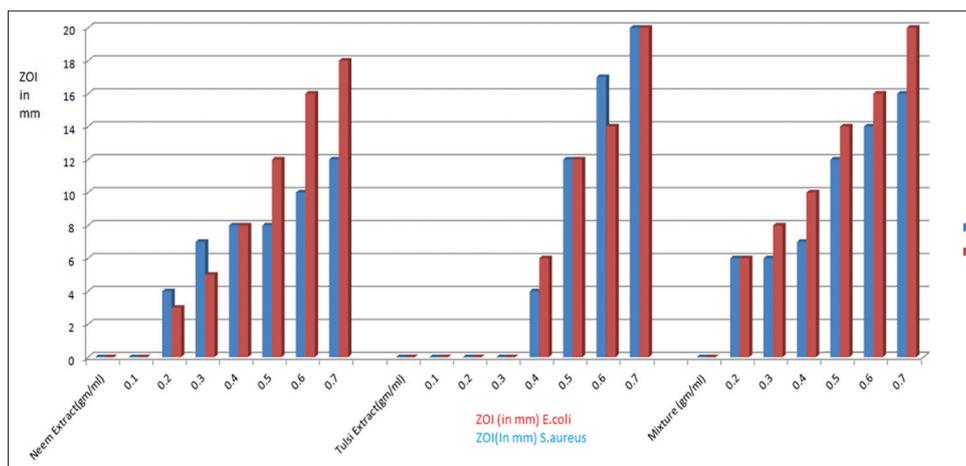


Fig. 2: ZOI of 4 mm of *S.aureus* from 0.4g/ml of Tulsi methanolic extract



Graph 1: Comparing zone of inhibition to methanol extracts at various concentrations (0.1–0.7 g/ml) of each extract for both the *Escherichia coli* and *Staphylococcus aureus*

Table 1: Neem methanolic leaf extracts at various concentrations (0.2 g/ml–0.7 g/ml) and their particular ZOI for *S. aureus*

Concentration (Neem) (g/ml)	ZOI (<i>S. aureus</i>) (mm)
0.2	4
0.3	7
0.4	8
0.5	8
0.6	10
0.7	12

S. aureus: *Staphylococcus aureus*, ZOI: Zone of inhibition

Table 4: Tulsi methanolic leaf extracts at various concentrations (0.2 g/ml–0.7 g/ml) and their particular ZOI for *E. coli*

Concentration (Tulsi) (g/ml)	ZOI (<i>E. coli</i>) (mm)
0.2	0
0.3	0
0.4	6
0.5	12
0.6	14
0.7	20

ZOI: Zone of inhibition, *E. coli*: *Escherichia coli*

Table 2: Tulsi methanolic leaf extracts at various concentrations (0.2 g/ml–0.7 g/ml) and their particular ZOI for *S. aureus*

Concentration (Tulsi) (g/ml)	ZOI (<i>S. aureus</i>) (mm)
0.2	0
0.3	0
0.4	4
0.5	12
0.6	14
0.7	20

S. aureus: *Staphylococcus aureus*, ZOI: Zone of inhibition

Table 5: Mixture (Tulsi and Neem) leaf extracts at various concentrations (0.2 g/ml–0.7 g/ml) and their particular ZOI for *E. coli* and *S. aureus*

Concentration (Neem and Tulsi) (g/ml)	<i>E. coli</i> (mm)	<i>S. aureus</i> (mm)
0.2	6	6
0.3	8	6
0.4	10	7
0.5	14	12
0.6	16	14
0.7	20	16

ZOI: Zone of inhibition, *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*

Table 3: Neem methanolic leaf extracts at various concentrations (0.2 g/ml–0.7 g/ml) and their particular ZOI for *E. coli*

Concentration (Neem) (g/ml)	ZOI (<i>E. coli</i>) (mm)
0.2	2
0.3	5
0.4	8
0.5	12
0.6	16
0.7	18

ZOI: Zone of inhibition, *E. coli*: *Escherichia coli*

ZOI for *E. coli*

For the discussed concentration of neem, 2 mm, 5 mm, 8 mm, 12 mm, 16 mm, and 18 mm ZOI has been observed, respectively (Table 3). In tulsi for 0.2 g/ml and 0.3 g/ml concentrations, no ZOI has been observed, whereas for other concentrations, i.e., 6 mm, 12 mm,

Table 6: Results of various phytochemical tests for both the neem and tulsi

Test	Neem	Tulsi
Flavonoid test	Present	Present
Tannin test	Present (condensed)	Present (condensed)
Froth test	Absent	Absent
Sterol test	Present	Present
Molisch test	Absent	Present
Anthraquinone test	Absent	Present

14 mm, and 20 mm diameter, ZOI has been observed, respectively (Table 4).

Mixture of tulsi and neem extract

In the case of mixture of the methanolic extracts of neem and tulsi in *E. coli* culture for discussed concentration 6 mm, 8 mm, 10 mm, 14 mm,

16 mm, and 20 mm, ZOI has been observed. In *S. aureus*, 6 mm, 6 mm, 7 mm, 12 mm, 14 mm, and 16 mm, ZOI has been observed, respectively (Tables 5 and 6).

Results of phytochemical analysis

After performing phytochemical analysis for various secondary metabolites we have found both tulsi and neem differs from each other in terms presence of certain phytochemicals. Result has been shown in (Table 6).

Comparative analysis

Comparative analysis of ZOI for various concentration of tulsi and neem for *E. coli* and *S. aureus* has been done and shown in (Graph 1).

Graph analysis

Neem extract

Minimum inhibitory concentration (MIC) for both the microorganisms was found to be 0.2 g/ml. It has been observed that neem extract was more powerful against *E. coli* in comparison to *S. aureus* at higher concentrations. Neem was more powerful against *E. coli* than tulsi.

Tulsi extract

MIC for both the microorganisms was found to be 0.4 g/ml. About similar effect has been shown by tulsi extract on both the microorganisms. Tulsi extract has shown a more powerful effect on *S. aureus* than that of neem.

Mixture (neem and tulsi)

MIC for both the microorganisms was found to be 0.2 g/ml. In a mixture, we have observed that it was more powerful than that of individual extracts if we see for both the bacteria simultaneously, and ZOI was linear for both the cultures pertaining to the concentrations.

CONCLUSION

In summary, the leaf extract of both the *A. indica* and *O. sanctum* has shown antimicrobial activity against *E. coli* and *S. aureus*. The methanolic leaf extract of *A. indica* has greater antimicrobial activity against *S. aureus*, whereas the *O. sanctum* was found to be more effective against *E. coli* (indicated by the ZOI). The MIC of neem and tulsi against *E. coli* was found to be 0.2 g/ml and 0.4g/ml, respectively. The MIC of neem and tulsi against *S. aureus* was 0.2 g/ml and 0.4g/ml, respectively. When leaf extracts of neem and tulsi were mixed and their effect was observed against *E. coli* and *S. aureus*, it showed that the combination of both works more effectively against *S. aureus* as well as *E. coli*, and the MIC of mix extract against them was 0.2 g/ml.

AUTHORS' CONTRIBUTION

All the authors have contributed equally in this project, but specifically concept of the project was formulated by Vipul Kumar and Anurag

Chakraborty, acquisition, analysis, and implementation of data have been done by all the authors equally, whereas drafting of manuscript and critical revision was done by Sony Pandey, Manpreet Kaur, and Manoj Kumar Jena. Each and every part of this project has been done under the supervision of Manoj Kumar Jena.

CONFLICTS OF INTEREST

There are no conflicts of interest among the authors declared.

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