

# Phytochemical and Antimicrobial Potential of *Ocimum gratissimum* (Clove Basil)

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**Abstract-** The aim of this study was to evaluate the antimicrobial activities of the aqueous and alcoholic (ethanol and acetone) extracts of *Ocimum gratissimum* leaves against different bacterial and fungal strains. Agar well diffusion method has been used to determine the antimicrobial activities of different plant extracts against Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and fungi (*Candida albicans*, *Penicillium crysogenum*, *Aspergillus niger* and *Aspergillus flavus*). The extracts exhibited both antibacterial and antifungal activities against tested microorganisms. The plant leaves were tested positive for alkaloids, saponins, flavonoids, tannins and other tested phytochemicals in a preliminary phytochemical screening analysis. This study showed that the phytochemicals of leaf extract of *Ocimum gratissimum* have antimicrobial properties.

**Key words:** *Ocimum gratissimum*, antimicrobial, phytochemical screening

## Introduction

Medicinal plants play a vital role in the treatment and prevention of various diseases and their promotion and there is growing interest in the search for new drugs from natural

resources (Ullah et al., 2018). Medicinal plants offer a substantial opportunity as they contain various bioactive chemical constituents (phytochemicals) that can act as antimicrobial agents.

*Ocimum gratissimum* (clove basil) as the medicinal herb. Basil is the main ingredient of pesto sauce but is also used to flavour other sauces and soups. Different parts including the leaves, stems, flowers, roots, seeds, and even the whole plant are useful. The seeds are edible, and when soaked in water become mucilaginous. The leaves can be eaten as a salad. Basil is widely used in traditional medicine. It is used in Ayurveda and in traditional Chinese medicine for treating digestive system disorders, such as stomach ache and diarrhoea, kidney complaints, and infections. *Ocimum gratissimum* belongs to the group of plants known as spices. *Ocimum gratissimum* commonly known as clove basil belonging to family Lamiaceae is an important aromatic and medicinal plant existing wild or cultivated in various tropical and subtropical parts of the globe. The plant possesses two unique features firstly it contains essential oil with diversity in chemical composition and water stress tolerance capacity. Plant oil is considered the Mother Nature's chemical factory (Tanko et al., 2008; Akara et al., 2021)

shows a unique composition of alcohols, aldehydes, ketones, ethers, esters, lactones, oxides, peroxides (Venuprasad et al., 2014) tannins (Irondi et al., 2016) and flavonoids (Benitez et al., 2009; Melo et al., 2019). It is also known as African basil, exhibits significant antimicrobial activity, particularly against bacteria and fungi.

## Material and Methods

### Collection of plant material

The leaves and flowers of *Ocimum gratissimum* were collected from the Botanical Garden, Patanjali yoga peeth, Haridwar, Uttarakhand. The leaves were washed with distilled water and subjected to crude extract preparation at room temperature for further studies.

### Preparation of crude extract

The leaves of the plant *Ocimum gratissimum* were used for crude extract preparations separately for phytochemical and antimicrobial analysis. 25 mg and 50 mg (fresh weight) of leaves of *Ocimum gratissimum* were homogenized in 3-5 folds of aqueous and organic solvent (50% ethanol and acetone) separately in pestle and mortar at room temperature. The extracts were filtered through sterilised whattman filter paper and filtrate was centrifuged at 10000 rpm at 4°C for 5 minutes. The clear supernatant was used as crude extract for phytochemical analysis and antimicrobial testing. Extracts were kept at 4°C for the further analysis.

### Isolation, purification and identification of microbial strains from soil and spoiled fruits

The microbial strains were isolated from soil by serial dilution method. (Cappuccino and Sherman, 2005; Aneja, 2009) and by some spoiled fruits. The isolated colonies of *Penicillium crysogenum*, *Aspergillus niger* and *Aspergillus flavus*, *Candida albicans* were

streaked onto the fresh SDA plates while *E. coli*, *Bacillus* sp., *Staphylococcus aureus* and *Pseudomonas* sp. were streaked onto the fresh NAM plates.

**Screening of antimicrobial activity:** Antimicrobial activity of plant extract was screened by agar disc diffusion method on SDA plate for fungal strains and NAM plates for bacterial strains and measure the zone of inhibition in mm. (Khokra et al, 2008). Sterile agar plates were prepared, and the fresh standardized broth culture of each test organism was inoculated. Subsequently, a sterile cork borer of 5 mm diameter was used to punch 4 wells on each of the plates. sixty micro-litres (60 µl ) of each of the varying concentrations (25mg/L, 50 mg/mL) of the test extracts was dropped into three of the wells, while the remaining one well was filled with sterile distilled water to serve as the control. The plates were then left for one hour to allow the contents in the well to diffuse into the agar, followed by incubation at 37°C for 24 hours for NAM plate while 28°C for SDA plate. The diameter zones of inhibition were then measured in millimetres (mm).

### Phytochemical screening of different parts of *Ocimum gratissimum*

Phytochemical tests were carried out in the aqueous and organic extract of *Ocimum gratissimum* using standard methods to identify the phytochemical constituents as described by Sofowara (1993), Trease and Evans (1989), Omoya and Akharaiyi (2012), Jyothi Prabha and Venkatachalam (2016), Harborne and Williams (2000).

### Screening for Tannins

5 ml each of the extracts were stirred separately with 100 ml distilled water and filtered. One ml ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate was an indication of the presence of tannins.

### **Screening for Terpenoids**

5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3 ml of concentrated sulphuric acid. Formation of reddish-brown layer at the junction of two solutions confirms the presence of terpenoids.

### **Screening for Flavonoids**

A pinch of zinc dust was added to 2 ml of extract followed by the addition of 1 ml concentrated HCl. Appearance of pink colour indicate the presence of flavonoids

### **Screening for Saponins**

5 ml each of the extracts were mixed with distilled water and shaken separately in a test tube. Frothing, which persists on warm heating was taken as preliminary evidence for the presence of the saponins.

### **Screening for Anthraquinone**

One drop of concentrated ammonium hydroxide was added to 10 mg of each extract, previously dissolved in isopropyl alcohol. After two minutes, formation of red colour indicated the presence of anthraquinone.

### **Screening for Glycosides**

5 ml extract was mixed thoroughly with 1 ml of glacial acetic acid and 1 ml of 5% FeCl<sub>3</sub> solution in a test tube. 1ml of concentrated sulphuric acid was added to the above reaction mixture carefully along the side of test tube. Development of green-blue colouration shows the presence of glycosides. (Kellar- Kiliani test).

### **Screening for Phytosterols**

(Salkowski Test) 5 mL of extract mixed with 2 ml of chloroform then 2 mL of concentrated H<sub>2</sub>SO<sub>4</sub> were added into it. Red colour was

observed in lower layer of chloroform indicates the presence of phytosterols.

### **Screening for Alkaloids**

**Mayer's test-** 1 ml of every extract mix with a drop of Mayer's chemical agent is additional by the aspect of the test tube. A creamy or white precipitate indicates the presence of alkaloids.

**Screening of Phenols-** Few drops of 10% lead acetate solution were added to 5ml of test solution. Formation of white precipitates indicates the presence of phenol in the test solution.

### **Results and Discussion**

Various extracts, including ethanolic extracts, have shown effectiveness against a range of pathogens. This study was to evaluate the antibacterial activities of the aqueous and the alcoholic extracts of *O. gratissimum* leaves against different bacterial strains. (Table:1) The antibacterial activity against gram positive and gram-negative strains was performed using the agar diffusion technique. The inhibition zone was determined through the microtiter broth dilution method. Studies have shown *O. gratissimum* effectiveness against *Bacillus*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Aqueous and ethanolic extracts of the leaves of *O. gratissimum* were active against *E. coli*, *P. aeruginosa*, and *S. aureus*, and the aqueous extract of the leaf was active against *P. aeruginosa* at the investigated concentrations. The extract of *O. gratissimum* was more effective in inhibiting *E.coli* than *P. aeruginosa* bacteria. All fractionated extracts showed the highest inhibition zone diameter against gram positive bacterial strains.

**Table-1 Study of antibacterial activity of different leaves extracts of *O. gratissimum* samples on selected bacterial isolates**

S.No.	Test Organism (Bacterial Strains)	Inhibition zone in diameter (mm± SD)					
		25 mg/L			50mg/L		
		Aqueous	Ethanol	Acetone	Aqueous	Ethanol	Acetone
1	<i>E. coli</i>	15±0.22	20±0.28	19±0.25	17±0.29	25±0.34	20±0.28
2	<i>P. aeruginosa</i>	12±0.21	21±0.23	18±0.27	19±0.26	28±0.28	19±0.26
3	<i>S. aureus</i>	18±0.36	31±0.26	27±0.32	22±0.24	27±0.38	28±0.23
4	<i>Bacillus subtilis</i>	19±0.32	28±0.29	26±0.30	21±0.25	29±0.29	25±0.33

The antimicrobial activity of the leaves extracts of *O. gratissimum* were studied in different concentrations (25 and 50 mg/ml) on fungal strains (*Penicillium crysogenum*, *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*). The extracts of *O. gratissimum* as shown antifungal activity against *Candida albicans*

and other fungal pathogens (Table-2). The antifungal activities of the extracts increased linearly with increase in concentration of extracts (mg/ml). The growth inhibition zone measured ranged from 23 to 38 mm for all the sensitive fungal strains.

**Table-2 Study of antifungal activity of different leaves extracts of *O. gratissimum* samples on selected fungal isolates.**

S.No.	Test Organism (Fungal Strains)	Inhibition zone in diameter (mm)					
		25 mg/L			50mg/L		
		Aqueous	Ethanol	Acetone	Aqueous	Ethanol	Acetone
1	<i>Candida albicans</i>	25±0.32	23±0.28	25±0.25	27±0.29	28±0.34	23±0.28
2	<i>Penicillium crysogenum</i>	22±0.25	27±0.23	28±0.27	29±0.26	29±0.28	29±0.26
3	<i>Aspergillus niger</i>	28±0.36	34±0.26	29±0.32	32±0.24	32±0.38	19±0.23
4	<i>Aspergillus flavus</i>	29±0.32	38±0.49	31±0.30	30±0.25	29±0.29	22±0.33

The existence of phytochemicals with therapeutic potential was checked in the leaves of *O. gratissimum*. Preliminary phytochemical screening was done using standard techniques. The phytochemical components discovered in the leaves of *O. gratissimum* included alkaloids, flavonoids, saponins, and tannins. Among these, the tannin and flavonoids content were remarkably high. The current results could provide a rational support for the traditional use of *O. gratissimum* to treat infections. Cytotoxic qualities, anti-bacterial, anti-viral properties,

are credited to the presence of saponin (Bailly & Vergoten, 2020). The Flavonoids and phenols are major compounds that act as antioxidants or free radical scavengers (Bhandary *et al.*, 2012). Tannin shows an anticancer property that is perceptible from its inhibitory activity towards growth while the phenolic compound, tannin, terpenoid, flavonoids possess an ant-helminthic property so the plant *Zanthoxylum*, *Acorus* could be used to treat stomach problems (Nath & Yadav, 2016).

**Table-3 Screening of phytochemicals in different leaves extracts of *O. gratissimum*.**

S.No.	Phytochemicals	Preliminary qualitative Identification					
		25 mg/L			50mg/L		
		Aqueous	Ethanol	Acetone	Aqueous	Ethanol	Acetone
1	Tannin	++	+++	++	++	+++	+++
2	Alkaloids	+	+++	++	+	+++	+++
3	Glycosides	+	+++	++	+	+++	+++
4	Anthraquinone	+	+++	++	+	+++	++
5	Phytosterols	+	++	+	+	++	++
6	Terpenoids	+	++	++	+	+++	++
7	Flavonoids	++	+++	++	++	+++	+++
8	Saponin	-	+	+	+	+++	++
9	Phenols	+	++	++	+	+++	+++

### Conclusions

This study suggests the exploration of *O. gratissimum* as sources of natural products for future use in the management of bacterial infections. The findings could also be of commercial interest to both pharmaceutical companies and research institutes. Furthermore, further studies are required to be conducted concerning the botanical preparation of the traditional sources of medicinal plants in various fields, including pharmacology, phytochemistry, ethnobotany and other biological activities associated with drug recovery.

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### Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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