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# Investigation of *Ocimumcanum* from Chhattisgarh for Antibacterial Attributes and Phytochemical Analysis

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**ABSTRACT**
*Ocimumcanum*, known as Kali tulsi, is a versatile medicinal plant abundant in phytochemicals, offering a spectrum of therapeutic applications for various ailments. It has been extensively studied for its phytochemical composition and antimicrobial traits. Particularly focusing on locally available strains in the Chhattisgarh region, an investigation has been undertaken. Results garnered significant scientific attention confirming important beneficial contents inherent in the plant extracts. High-Performance Thin-Layer Chromatography (HPTLC) analysis unveiled distinct compounds with Rf values at 0.099 and 0.183, exhibited the presence of anti-oxidants, while Fourier Transform Infrared (FTIR) analysis revealed the presence of intricate organic compounds like aldehydes, phenols, and carboxylic groups. Antioxidant evaluations using DPPH scavenging assays revealed strong antioxidant activity, which can be attributed to the significant phenolic content present in the plant extracts.Total phenol content determination provided valuable insights into the chemical makeup of the plant. Experimental assays demonstrated potent antimicrobial effects against pathogenic bacteria and fungi, highlighting its therapeutic significance.
**Keywords:** *Ocimumcanum;* phytochemical constituents; HPTLC; FTIR; phenolic contents; Antimicrobial activity.

## 1. INTRODUCTION

The botanical realm harbours an extensive reservoir of potential pharmaceuticals, where medicinal plants emerge as pivotal sources of bioactive compounds and therapeutic agents(Mushtaq *et al*., 2018). Vaou*et al*. (2021) demonstrated that, herbal plant extracts play a crucial role in fostering growth and combatting infections, offering practical and cost-effective remedies for numerous ailments. Rich in secondary metabolites like tannins, phenolics, alkaloids, and flavonoids, medicinal plant extracts bolster innate immune responses, enhance disease resistance, and deter pathogenic microorganisms' proliferation. Traditional medicines derived from medicinal plants, boasting a diverse array of properties including antifungal, anticancer, and antibacterial attributes, are embraced by roughly 80% of individuals in affluent societies (Al-Khayri*et al*., 2023). Exploration and research of the many secondary metabolites found in medicinal plants, some of which have unknown roles, are essential to contemporary medication development. They are a good alternative for many populations, particularly in underdeveloped nations, because they provide a wide range of bioactive chemicals with various medicinal characteristics. Anand *et al*., (2019) reported that, medicinal plant integration into contemporary healthcare systems can aid in the creation of long-term, reasonably priced remedies for a range of health issues.

Promoting further investigation into the chemical compositions, biological activities, and potential applications of medicinal plants is paramount, alongside advocating for their conservation and sustainable utilization. Vaou*et al*. (2021) suggested that leveraging the wealth of information and bioactive compounds found within these plants could unveil novel avenues for disease treatment, health enhancement, and medical progress.

*Ocimumcanum,* is a multipurpose medicinal herb with several therapeutic benefits. Itis a plant rich in phytochemicals, including tannins, saponins, terpenoids, alkaloids, glycosides, steroids, lactones, oils and fats, proteins, carbohydrates, phenols, and anthraquinones. Its strong anti-inflammatory and antioxidant properties are ascribed to the presence of flavonoids, phenolic compounds, and other phytochemicals. This plant is a possible source of natural antimicrobial agents since it has antimicrobial activity against a variety of harmful bacteria and fungi. Dharsono*et al*. (2022) and Hasan *et al*. (2023) reported the antipyretic (fever-reducing), anti-diabetic, analgesic (pain-relieving), and wound-healing qualities of *Ocimumcanum*for traditional utilizations.

Alamgir (2018) and Bhattacharjya*et al*. (2019) reported *Ocimumcanum*aspotential valuable medicinal plant and suggested its synergistic effects with diverse applications in both traditional and modern medicine, further research is warranted to comprehensively understand its therapeutic capabilities and determine optimal dosage regimens. Additionally, the flavonoids present in the plant extracts are known for their antioxidant and anti-inflammatory properties, helping prevent oxidative stress-related diseases and reducing inflammation and tannins exhibit astringent and antimicrobial properties, useful for treating infections and as natural preservatives(Ullah *et al.*, 2020a). Terpenoids and alkaloids contribute to its antimicrobial, analgesic, and antipyretic effects, while glycosides may play a role in its potential anti-diabetic properties(Singh *et al*., 2022). Steroids and lactones may also contribute to its anti-inflammatory and analgesic activities. Essential oils and fats impart a characteristic aroma and possess therapeutic properties. Based on these perspectives, a study has been initiated to explore the potential of native *Ocimumcanum*for antimicrobial properties.

## 2. MATERIALS AND METHODS

### 2.1 Sample

Authentic specimens of *Ocimumcanum* were procured from the Chhattisgarh State Biodiversity Board and subsequently housed at the Bioresource Complex of Government V.Y.T. PG Autonomous College, Durg (C.G.) for ongoing research endeavours.

### 2.2. Sample Processing

The leaves of *Ocimumcanum* underwent a cleaning process with water to eliminate impurities before being dried. Following drying, the biomass was finely ground into powder using an electric grinder and stored in a sealed container for subsequent use.

### 2.3 Soxhlet Extraction

A Soxhlet device was used to hold the 100g dried powder, and 500 ml of distilled water, methanol, and petroleum ether were used as separate solvents.Extraction proceeded until the solvent in the thimble appeared clear. The resultant extract was dried in a digital water bath until a dark green residue developed, and it was then concentrated using a Rotavapor. After that, these extracts were kept in a refrigerator for further usage. Using the formula, the extract's percentage yield was calculated:

$$\text{Percentage yield} = \frac{\text{Final weight of the dried extract}}{\text{Initial weight of the powder}} \times 100$$

### 2.4 Qualitative analysis for Phytochemicals

The phytochemical constituents of the *Ocimumcanum* extract were assessed through a series of phytochemical studies, with minor adaptations was reported by Adigüzel *et al.*, 2005.

#### 2.4.1 Test for proteins

- **Biuret Test:** A volume of 2 ml of extract was mixed with 2 ml of Biuret reagent. The resulting mixture was vigorously shaken and then gently heated for five minutes.A reddish or violet tint appeared, indicating the presence of proteins.
- **Million's test:** Combining 2 ml of Millon's reagent with extract, for any precipitation followed by a red color change upon mild heating indicated the presence of proteins.
- **Ninhydrin test:**A mixture of 2 ml of 0.2% Ninhydrin solution and *Ocimumcanum* extract was heated until boiling, observing the emergence of a purple color, indicative of the presence of proteins and amino acids.

### 2.4.2 Test for carbohydrates

- **Fehling's Test:** Equal volumes of Fehling A and Fehling B reagents were mixed, and 2 ml of this mixture was added to the extract. The sample was gently heated, and the formation of a brick-red precipitate indicated the presence of reducing sugars.
- **Benedict's Test:** Upon adding 2 ml of Benedict's reagent to the extract and heating the mixture to boiling, the appearance of a reddish-brown precipitate indicated the presence of carbohydrates.
- **Molisch's Test:** A combination of 0.5 ml of the extract and 2 ml of Molisch's reagent was thoroughly mixed, followed by the gentle addition of 2 ml of concentrated $H_2SO_4$ to the side of the test tube. The appearance of a violet ring at the interface suggested the presence of carbohydrates.
- **Iodine Test:**A mixture of 0.5–1 ml of Ocimumcanum extract and 2 ml of a solution of iodine was added; the appearance of dark blue or violet coloration showed the presence of carbs for energy.

### 2.4.3 Test for tannin

To 0.5 ml of the extract, 1 ml of distilled water and two to three drops of ferric chloride solution was added. The presence of tannin was indicated by a black coloration.

### 2.4.4 Test for saponins

5ml of distilled water and 1 ml of the extract were combined in a test tube and shaken vigorously. It was believed that the development of stable foam was a sign that saponins were present.

### 2.4.5 Test for terpenoids

2ml of chloroform were combined with the extract, which was then evaporated until it was completely dry. This was cooked for approximately two minutes after adding 2 ml of concentrated $H_2SO_4$. The interface was coloured grey, which suggested the presence of terpenoids.

### 2.4.6 Test for flavonoids:

- **Shinoda test:**A little quantity of magnesium was combined with *Ocimumcanum*extract, and drops of strong hydrochloric acid was added. After a few minutes, the hue became crimson scarlet, signifying the presence of flavonoids.
- **Alkaline reagent test:**Upon combining 0.5 ml of *Ocimumcanum* extract with 2 ml of 2% NaOH solution, a bright yellow coloration developed. The addition of a few drops of diluted acid resulted in the disappearance of this yellow tint, indicating the presence of flavonoids.

### 2.4.7 Test for alkaloids

*Ocimumcanum* extract was mixed with 2 ml of 1% HCl and gently heated. Subsequently, the mixture was subjected to the addition of Mayer's and Wagner's reagents. The formation of a precipitate in the reaction mixture indicated the presence of alkaloids.

### 2.4.8 Test for glycosides

- **Liebermann's test:**Ice was used to cool a mixture that contained the extract, 2ml of acetic acid, and 2ml of chloroform. $H_2SO_4$ was introduced cautiously. When the glycone portion of the glycoside or the steroidal nucleus were present, the hue changed from violet to blue to green.

- **Salkowski's test:**After adding 2 ml of concentrated $H_2SO_4$ and giving it a gentle shake, the extract was combined with 2 ml of chloroform. The tint turned reddish-brown, indicating the presence of glycosides.
- **Keller-kilani test:**2 to 3 drops of a 2% $FeCl_3$ solution were added to a mixture that contained 0.5 ml of Ocimumcanum extract and 2 ml of glacial acetic acid. The mixture was then mixed with 2 mi of concentrated $H_2SO_4$. The emergence of a brown ring at the interface suggested the presence of cardiac glycosides.

**2.4.9 Test for steroid**

- The *Ocimumcanum* extract was combined with 2 ml of chloroform. Subsequently, the mixture was treated with 2 ml of concentrated $H_2SO_4$ and acetic acid, respectively. The appearance of a green coloration in the reaction mixture indicated the presence of steroids.
- A mixture of chloroform and *Ocimumcanum* extract was prepared, followed by the gradual addition of concentrated $H_2SO_4$. The lowest layer exhibited a red tint, suggesting the presence of steroids.

**2.4.10 Test for lactones**:

- **Baljet's Test:** The extract was treated with sodium picrate solution. The resulting mixture transitioned from yellow to orange, signifying the presence of lactones.

**2.4.11 Test for Oils & Fats**

A minute quantity of the extract was passed through two filter sheets successively. If the filter paper exhibited an oily appearance, it indicated the presence of fats and fixed oil.

**2.4.12 Test for phenol:**

1ml of the extract was combined with 2 ml of alcohol and two to three drops of ferric chloride solution. The appearance of a blue-green or black color confirmed the presence of phenols.

**2.4.13 Test for anthraquinone:**

2.0 ml of the extract was mixed with 5 ml of chloroform and 5 ml of ammonia solution. The presence of anthraquinone was identified by observing a pink, crimson, or violet appearance.

**2.5 Confirmation of phytochemicals:**

**2.5.1 FTIR spectroscopy**

The *Ocimumcanum* extract was subjected to FTIR analysis to confirm the presence of typical functional groups. A Thermo Nicolet Avtar 370 FTIR spectrophotometer was used to record FTIR spectra. KBr pellets were utilized to create samples within the approximate range of 500–4000 cm-1.

**2.5.2 HPTLC**

The HPTLC system utilized for analysis consisted of the CAMAG III automated TLC sampler, the CAMAG dual-chamber chamber (20 cm × 10 cm), and the CAMAG Cats-3 software, along with a 25 µL HPTLC syringe. Pre-coated silica gel aluminum HPTLC plates 60-254 (SG60 F254, 20 cm x 10 cm, layer thickness 0.02 mm, Merck, Germany) were employed.

A constant application rate of 0.1 µL/sec and a distance of 5 mm between two strips were maintained during sample application. The sample size was kept at 5 mm × 0.45 mm, and the scan speed was set to 10 mm/s with a monochrome bandwidth of 20 nm. Chromatographic enhancement was achieved using a linear mixture of n-hexane and ethyl acetate (9.8:0) (v/v) in a 20 cm x 10 cm two-slot glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The chamber was optimized for saturation at room temperature (25℃±2) and relative humidity of 60% ±5%, with a saturation time of 30 minutes. The length of the chromatogram was 8 cm. After scanning, the TLC plate was air-dried with the aid of a hair dryer. Densitometric analysis was conducted using the CAMAG TLC Scanner-III with a scan size of 4 mm x 0.1 mm and a scan speed of 1 mm/s. The

wavelength measured was 254 nm in reflection-absorbance mode, utilizing deuterium light radiation emitting a transient UV spectrum between 190 and 400 nm.

**Calibration curves for standards:**Calibration curves for standards were established as follows:

1. A stock solution of standards was prepared by dissolving the standard compounds (50 mg/ml) in acetonitrile.
2. 1 mL of the stock solution was quantitatively transferred to a 10 ml measuring bottle and then diluted with acetonitrile to obtain a series of standard solutions with concentrations ranging from 0.1 to 0.6 mg/ml.

This process ensured the generation of solutions of different concentrations suitable for constructing calibration curves.

### 2.6 Quantitative Analysis:

#### 2.6.1 Test for total phenolic contents (TPC)

The determination of phenol in the *Ocimumcanum* extracts was conducted using Folin-Ciocalteu (FC) reagent, following the method outlined by Siddhuraju et al., (2002) and Barapatre et al. (2015).Each Ocimumcanum extract solution sample included 0.5 ml. To this, 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of $Na_2CO_3$ (2% w/v) were added (1 mg/ml). The mixture that was produced was shaken and incubated for 15 minutes at 45°C. By utilizing a UV spectrophotometer, the samples' absorbance was determined at 765 nm. Amounts of gallic acid equivalent (GAE) in milligrams (µg/mg) of extract were used to express the results. At doses of 2.5 mg to 15 mg/mL, the dry extract was dissolved in methanol. Following the mixing of these solutions with 5 mL of potassium ferricyanide (1%), and 5 mL of phosphate buffer (0.2 M, pH 6.6), they were incubated for 20 minutes at 50°C. Following the addition of 5 mL of 10% TCA, the reaction mixture was centrifuged for 10 minutes at 3,000 x g. At 700 nm, the absorbance of the 5 mL solution was measured after the top layer was combined with 5 mL of distilled water and 1 mL of 1% ferric chloride. Reduced extract potency was shown by an increase in absorbance.

#### 2.6.2 Anti-oxidant analysis by 2, 2-diphenyl-1-picrylhydrazyl Radical Scavenging Assay

A modified version of Ebrahimabadi's method (Ebrahimabadi et al., 2010) was employed to assess the antibacterial and DPPH radical scavenging capabilities of *Ocimumcanum* extracts. The extracts were mixed with 3 mL of DPPH solution (40 mg/L in methanol) and incubated at room temperature in the dark for 30 minutes. Following incubation, the solutions were analyzed using a UV-VIS spectrophotometer to measure absorbance at 517 nm. Negative controls consisted of a methanol solution of DPPH, while positive controls included ascorbic acid and BHT at the same concentration. *Ocimumcanum* extracts were utilized, with an equivalent of lacking DPPH serving as negative controls. The following formula was employed to determine the percentage of DPPH radical inhibition:

$$I\ \% = \frac{(A_{control} - A_{sample})}{(A_{control})} \times 100$$

Where $A_{control}$ and $A_{sample}$ are the absorbances of the control and test samples, respectively.

### 2.7 Antimicrobial analysis:

#### 2.7.1 Antibacterial

*Escherichia coli* (NCIM 2065) obtained was sub-cultured on nutritive agar media (NAM) and maintained at 4°C. The bacteria used in the antibacterial activity test were cultured for 24 hours. Each plant extract was then evaluated for its antibacterial properties using the well diffusion method on NAM. The diameter of the zone of inhibition (ZOI) around each well was measured in millimetres.

#### 2.7.2 Antifungal

The obtained *Alternaria* was sub-cultured on Potato Dextrose Agar Media (PDA) and preserved at 4°C. The fungi intended for the antifungal activity test were cultivated for 48 hours. Subsequently, each plant extract was assessed for its antifungal properties using the well diffusion method on PDA. The diameter of the zone of inhibition (ZOI) surrounding each well was measured in millimetres.

## 3 RESULT AND DISCUSSION

### 3.1 Sample description

*Ocimumcanum*, a perennial herbaceous plant, belongs to the mint family and is indigenous to the Indian subcontinent, particularly abundant in the Chhattisgarh region. Renowned for its aromatic foliage, it has a long history of utilization in traditional remedies. Morphologically, it presents as an erect, branched herb with quadrangular stems and oblong-lanceolate leaves. The upper surface of the leaves exhibits a vibrant green hue, while the lower surface appears purple or reddish-purple. Glandular hairs adorn the leaves, contributing to their rough texture and distinctive aroma. The plant produces petite flowers, typically in purplish-white or pinkish-white hues, and its fruits consist of small nutlets ensconced within the calyx.

**3.2 Phytochemical Profiling**

Phytochemical analysis serves as the foundational step in identifying the valuable secondary metabolites present in a sample. The qualitative analysis of *Ocimumcanum* is summarized in Table 1. It was noted that the *Ocimumcanum* specimens utilized in the study exhibited proteins, carbohydrates,tannins, saponins, terpenoids, flavonoids, alkaloids,glycosides, steroid, lactones, oil & fats, phenol and Anthraquinone across their water, methanolic, and petroleum ether extracts.The methanolic extract showed the highest concentration of phytochemicals, indicating the affinity of phytochemicals for polar solvents.Methanol is a polar solvent due to the presence of the hydroxyl group (-OH), that allows methanol to dissolve a wide range of substances and its compatibility with water make it particularly useful in diverse applications where both polar and non-polar solvents are needed.

**Table 1: Qualitative analysis of phytochemicalsin different parts of *Ocimumcanum.***

| SI. No | Phytochemical | Solvent | | |
|---|---|---|---|---|
| | | Water | Methanol | Petroleum ether |
| 1. | Proteins | - | - | - |
| 2. | Carbohydrates | + | + | - |
| 3. | Tannins | - | + | - |
| 4. | Saponin | - | + | - |
| 5. | Terpenoids | - | + | + |
| 6. | Flavonoids | + | + | + |
| 7. | Alkaloids | + | + | - |
| 8. | Glycoside | + | + | - |
| 9. | Steroid | - | - | - |
| 10. | Lactones | - | - | - |
| 11. | Oils & Fats | - | + | + |
| 12. | Phenol | + | + | + |
| 13. | Anthraquinone | - | - | - |

Through various studies, researchers have identified the presence of significant compounds such as tannins, saponins, terpenoids, flavonoids, alkaloids, glycosides, steroids, lactones, oils and fats, proteins, carbohydrates, phenols, and anthraquinones in its water, methanol, and petroleum ether extracts. These phytochemicals are believed to contribute to the plant's potential medicinal properties and therapeutic applications. For instance, flavonoids are known for their antioxidant and anti-inflammatory activities, while tannins possess astringent and antimicrobial properties(Ullah *et al.*, 2020). The presence of such a diverse range of phytochemicals in *Ocinumcanum* has sparked considerable interest among researchers exploring its potential as a source of natural remedies and pharmaceuticals(Dharsono*et al.*, 2022a).

**3.3 FTIR analysis**

FTIR spectra using KBr pellet technique of leaves of *Ocimumcanum*at wavenumbers between 500-4000 $cm^{-1}$ is shown in Figure. 1. The peaks at different wavenumber range can be observed in the figure. The characteristic peaks were seen to be noted at 3286.13which demonstrated the O-H stretch, and H-bond, the peak at 1606.86 shows the C-C stretch in the ring, the peak at 1731.28 shows the C=O stretch, the peaks at 1242.78, 1017.22 shows the C-O stretch and the peaks at 773.89 shows the bending of =C-H. This shows the presence of functional groups such as phenol, aldehydes and carboxylic group in *Ocimumcanum*.

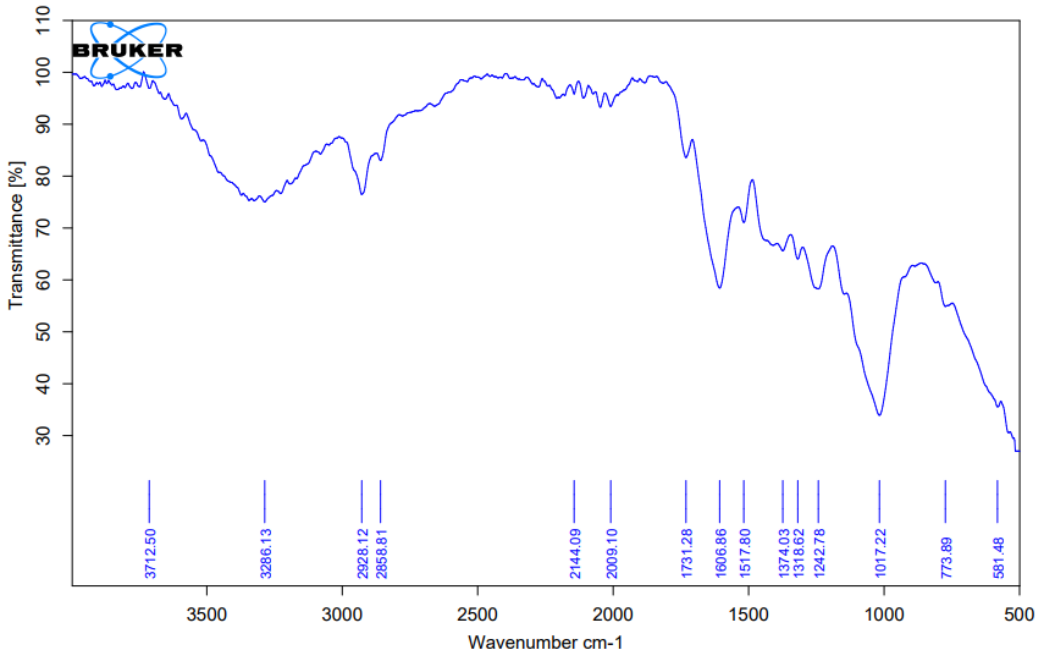


***Figure 1: FTIR spectra of Ocimumcanum***

The FTIR analysis of *Ocimumcanum*indicating the presence of complex organic compounds in the plant. This consistency suggests that the chemical constituents present may share similarities, possibly reflecting the plant's biological functions or metabolic pathways (Wongsa*et al*., 2022).The occurrence of these specific functional groups, renowned for their medicinal and pharmacological

properties, hints at the potential existence of bioactive compounds in *Ocimumcanum*.Phenolic compounds, recognized for their antioxidant properties, and aldehydes and carboxylic groups, commonly found in bioactive molecules, indicating the plant's therapeutic potential(Dharsono*et al*., 2022).

**3.4 HPTLC analysis**

*Ocimumcanum*, is a highly observed medicinal plant in Ayurveda. In this study, four bioactive compounds, namely eugenol, luteolin, ursolic acid, and oleanolic acid, were measured from the leaves of black varieties of *Ocimumcanum*(Beltrán-Noboa *et al.*, 2023). High-performance thin-layer chromatography (HPTLC) with densitometry was used for analysis. The methods demonstrated precision, with low relative standard deviation (RSD) values for both intraday and inter day analyses. Instrumental RSD values were also low, indicating accurate measurements. The other $R_f$ values seen at 0.099,0.183 exhibited the presence of anti-oxidants. Recovery studies confirmed the methods' accuracy, with average recoveries ranging from 99.3% to 100.58% for the four compounds. Eugenol content in the samples ranged from 0.175% to 0.362%, while luteolin content ranged from 0.019% to 0.046%.

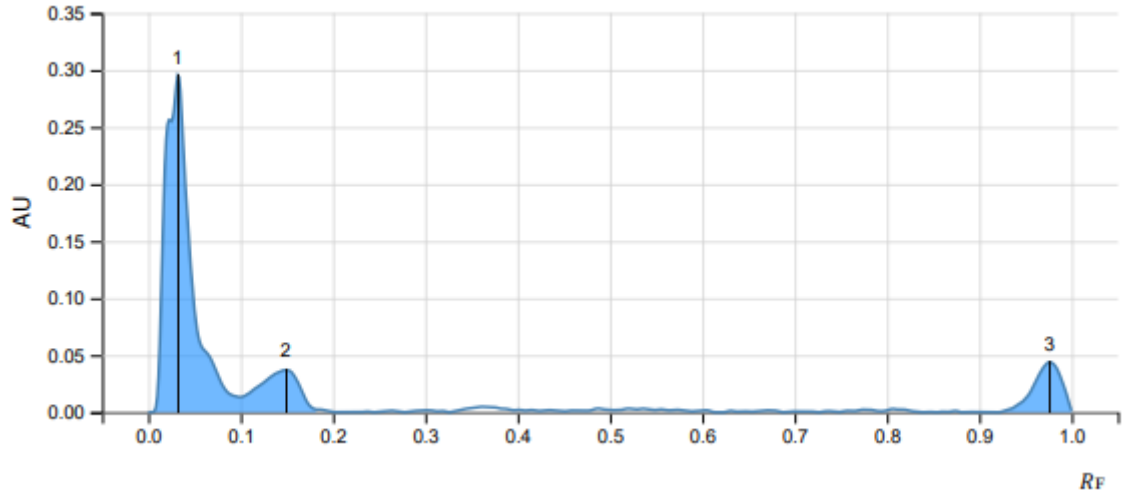


***Figure 2: HPTLC of extract of Ocimumcanum***

**3.5 Determination of total phenols**

In the investigation of the extract of *Ocimumcanum* using Folin Ciocalteu's reagent, the researchers found that the total phenolic content was measured at 2.18 mg/ml. Phenolic compounds play a crucial role in antioxidant activity, highlighting the significance of measuring their presence in assessing the antioxidant properties of the extract, as indicated byKatalinic*et al.*, 2006. This experiment sheds light on the importance of phenols as they contribute to the overall antioxidant capacity of the extract, reflecting its potential health benefits. Moreover, the observed sensible reducing power of the leaves in this study, as illustrated in Figure 3, further underscores the antioxidant prowess of *Ocimumcanum* and its potential applications in promoting health and combating oxidative stress.

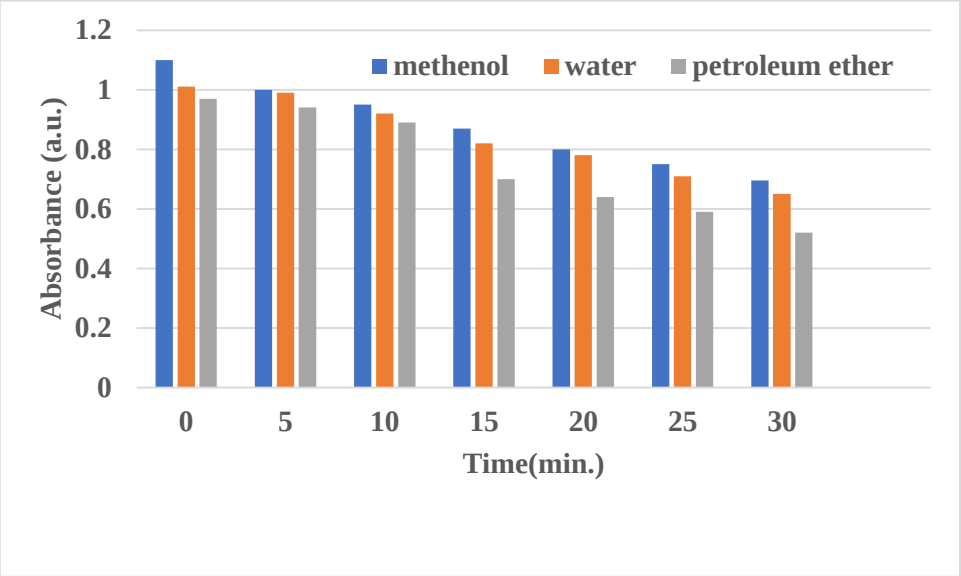


**Figure 3: Reducing power of total phenolics of*Ocimumcanum***

It was observed that, the of *Ocimumcanum*exhibit reducing power in terms of Ascorbic acid equivalent. However, the *Ocimumcanum*showed activity 2.31±0. µg/ml. The Methanolic extract was found superior among the others.Methanol's polarity and ability to dissolve both polar and non-polar substances make it a versatile solvent. It is commonly used in processes where a solvent with intermediate polarity is needed, bridging the gap between highly polar solvents like water and less polar solvents like diethyl ether or hexane.

**3.6 Evaluation of Antioxidant Activity (2, 2-diphenyl-1-picrylhydrazyl Scavenging Assay)**

The *Ocimumcanum* demonstrate an impressive ability to scavenge superoxide molecules. This scavenging prowess extends to the DPPH free radicals, as evidenced by the higher scavenging ability observed in *Ocimumcanum* extract(Chaudhary *et al.*, 2020). The elevated free radical scavenging capability is likely attributed to the presence of phenolics, key constituents found in *Ocimumcanum*. Further enhancing the antioxidant activity are the flavonoids present in the plant, highlighting their role in improving overall efficacy.Moreover, the extracts from the plant showcase a variety of substitutions of free hydroxyl groups, thereby contributing significantly to their anti-superoxide properties. Notably, the scavenging activity of the DPPH radical in all extracts exhibits a concentration-dependent pattern, showcasing a complex relationship between extract concentration levels and radical scavenging effectiveness. Among the various extracts, the ethanolic and methanolic extracts emerge as front runners, displaying the highest levels of activity(Saeed *et al.*, 2012).

Importantly, the antioxidant activity of the compounds is intricately tied to their concentration levels and unique structural features. With increasing concentration of the extract, there is a corresponding rise in the number of active groups present in a single molecule that actively participate in radical scavenging activities. This increase in active groups underscores the heightened antioxidant potential observed with escalating extract concentrations, ultimately contributing to the overall efficacy of the plant extracts in combating oxidative stress.

***Table. 2 DPPH free radical scavenging activity of Ocimumcanum***

| S.No. | Concentration (mg/ml) Concentration (mg/ml) Concentration (mg/ml) Concentration (mg/ml) | Samples | DPPH free radical scavenging activity (%) |
|---|---|---|---|

| | Concentration (mg/ml) Concentration (mg/ml) Concentration (mg/ml) Concentration (mg/ml) Concentration (mg/ml) | | |
|---|---|---|---|
| 1. | 1 | Ascorbic acid | 90.67 ± 1.02 |
| 2. | 1 | Water extract | 64.75 ± 0.52 |
| 3. | 1 | Methanolic extract | 69.32 ± 0.64 |
| 4. | 1 | Petroleum ether extract | 59.78 ± 0.98 |

### 3.7 Microbiological investigations on Antimicrobial Activity

#### 3.7.1 Antibacterial

In this research study, the focus was on investigating the antibacterial properties of *Ocimumcanum*, commonly known as holy basil. The experiments conducted aimed to assess the effectiveness of the plant extract in combating gram-negative bacteria. The obtained results, outlined in Figure- 4 of the study, revealed promising outcomes. The data illustrated that the extract exhibited significant antibacterial activity specifically against *E. coli*, a well-known gram-negative bacterium associated with severe infections.

The positive results obtained from the study highlighted the substantial antibacterial potential of plant-based extracts in comparison to synthetic antibacterial agents. These findings suggest that natural remedies, such as those derived from phytochemicals in plants like *Ocimumcanum*, could serve as effective alternatives with fewer associated side effects and supporting traditional medicinal uses of the plant for various ailments.Moreover, the study delved into the historical applications of phytochemicals in traditional medicines for treating a wide range of conditions, including fever, epilepsy, asthma, leprosy, and other ailments. Understanding the mechanisms by which these plant-based compounds work against harmful pathogens like *E. coli* provides valuable insights for future medicinal advancements.

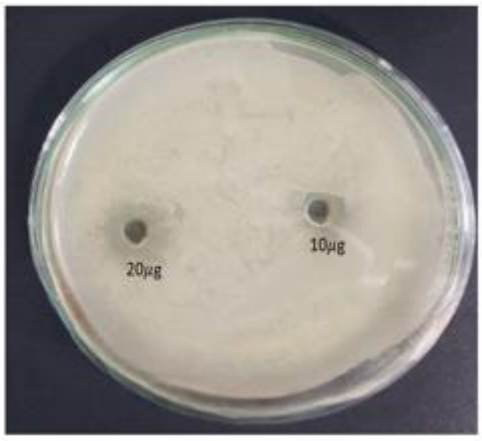


**Figure 4: Antibacterial of *Ocimumcanum*against *E. Coli***

***Table3: Measurements of Zone of inhibition***

| **Bacteria** | ***Ocimumcanum* Leaf extract** | |
|---|---|---|
| | **10 µg/ml** | **20 µg/ml** |

| | | |
|---|---|---|
| ***E. Coli*** | 7mm | 10mm |

### 3.7.2 Antifungal

In this research study, the investigation delved into exploring the antifungal properties exhibited by extracts derived from *Ocimumcanum*. Specifically, the study focused on analyzing the efficiency of these extracts in combating fungal infections. The findings outlined in Figures 5 clearly depict the favorable outcomes observed. The results revealed that the extracts displayed notable antifungal potential against the prominent fungi Alternaria, a species known for causing a wide range of infections in humans. This particular fungus is associated with various infections, ranging from superficial keratitis and onychomyeosis to more severe invasive and disseminated infections. The implications of these results highlight the significant therapeutic capabilities inherent in plant-based antifungals, which could offer a promising alternative to synthetic antifungal agents. Moreover, the study suggests that these natural remedies possess the ability to effectively target fungal pathogens while potentially minimizing the adverse side effects commonly associated with artificial antifungal treatments.

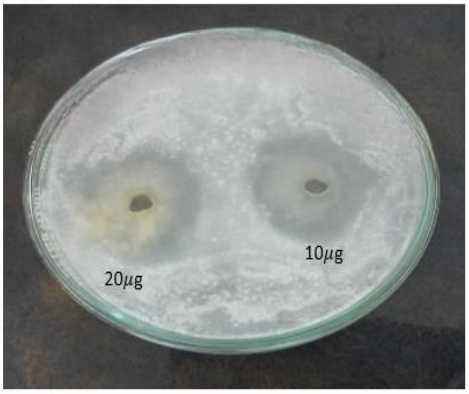


**Figure 5: Antifungal activity of *Ocimumcanum*against *Alternaria***

***Table3: Measurements of Zone of inhibition***

| **Fungi** | ***Ocimumcanum* Leaf extract** | |
|---|---|---|
| | **10 µg/ml** | **20 µg/ml** |
| ***Alternaria*** | 10mm | 15mm |

## 4 CONCLUSION

The current research project has been primarily dedicated to exploring the diverse phytochemical components found within *Ocimumcanum*. Through thorough analysis, it has been observed that this plant harbors a multitude of phytochemical compounds that offer valuable health benefits. The investigation has delved into identifying the specific functional groups that are prevalent within this botanical specimen. A comprehensive evaluation of its antimicrobial properties against *E. Coli* and the fungi *Alternaria* has underscored the remarkable antimicrobial efficacy possessed by this plant, making it a promising candidate for medicinal applications. Additionally, the phytochemical constituents were substantiated through a detailed HPTLC analysis, a technique known for its reliability, simplicity, and efficiency. By cross-referencing the collected data with established standards, the presence of diverse phytochemicals and antioxidants in *Ocimumcanum* was unequivocally confirmed. Noteworthy findings revealed elevated levels of total phenol content and significant antioxidant activity in this plant species. The abundance of phenolic compounds within the plant extract plays a pivotal role in driving its potent antioxidant capabilities, further highlighting its potential therapeutic value.

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**CONFLICT OF INTEREST**
All authors declare that there is no conflict of interest.

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