Phytochemical Analysis and Antimicrobial Activity of *Ocimum Tenuiflorumrom*

11

Ruqquiya Shaikh
Neha Agarwal
Mubashiroddin Farooqui
Syeda Ummul Khair Asema*

Abstract

Recently, the antimicrobial properties and phytochemical analysis of extracts from Ocimum tenuiflorum have been investigated. Ocimum tenuiflorum is a member of the Lamiaceae family, which comprises approximately 200 general and 150 species. Various parts of this plant are utilized for medicinal purposes, primarily the leaves and flowering tops, which are used for oil extraction. Both the seeds and oil exhibit antibiotic properties. The sample of Ocimum tenuiflorum analysed contained tannins, saponins, proteins, steroids, carbohydrates, alkaloids, flavonoids and glycosides. Furthermore, the presence of certain phytochemicals such as tannins, saponins and steroids underscores the medicinal potential of the plant in therapeutic applications. This study demonstrated that extracts of Ocimum tenuiflorum are a valuable source of bioactive compounds with various medicinal uses. The extracts were tested against four different bacterial strains using the disc diffusion method. The antimicrobial activity was assessed against human pathogens E. coli, P. vulgaris, S. typhi and S. aureus through an agar diffusion assay. The plant extract exhibited a minimum

Ruqquaiya Shaikh
Department of Chemistry, Maulana Azad College of Arts, Science and Commerce, Chhatrapati Sambhaji Nagar, 431001, Maharashtra, India.
Neha Agarwal
Department of Chemistry, Navyug Kanya Mahavidyalaya, Lucknow
Mubashiroddin Farooqui
Department of Chemistry, Maulana Azad College of Arts, Science and Commerce, Chhatrapati Sambhaji Nagar, 431001, Maharashtra, India.
Syeda Ummul Khair Asema
Department of Chemistry, Maulana Azad College of Arts, Science and Commerce, Chhatrapati Sambhaji Nagar, 431001, Maharashtra, India.
Publisher: Anu Books
Book Name : Chemical Sciences at the Nexus of Sustainability: Bridging Disciplines

inhibitory concentration of 285 mg/ml for S. typhi. The findings from the phytochemical screening and antimicrobial activity support the observed effects and validate the use of this plant in herbal medicine.

Keywords: *Phytochemical Analysis, Proximate Analysis, Antimicrobial Activity, Ocimum tenuiflorum*

1. Introduction

Medical plants are abundant in India. All natural products can be thought of as bioactive molecules because each unique molecule has a biological function. One type of biologically indirect pharmacological activity or several types of them. As more and more nations in the U.S. and Europe appreciate nature's green gift, they are introducing herbal medications to their patients. In traditional medical practices, *Ocimum tenuiflorumrom* has a distinct identity since different sections of it are utilized to cure different ailments. Because of its anti-hyperglycemic, anti-pyretic, analgesic, antibacterial, expectorant, anti-cancer activity the adrenal cortex, soothes itching, and insecticidal qualities.

Nature has served as a source of medicinal agents for countless generations. Plants are crucial in delivering primary health care. They function as therapeutic agents and also provide essential raw materials for the production of both traditional and modern medicines, largely due to the prevailing belief that green medicine is safer and more reliable compared to expensive synthetic drugs, many of which have negative side effects.

Leaves of *Ocimum tenuiflorumrom* its own identity in medical, pharmaceutical and chemical sciences [1-5]. Additionally, the leaves of *Ocimum tenuiflorumrom* are used as a cough cold and to treat a variety of rheumatic conditions. Massive research on natural products was carried out by various researchers [6-10], with a focus on the close-up, phytochemical, physiochemical and spectroscopic study of different plants as a part of the current research on natural products being done in this lab. It was intriguing enough to conduct a close investigation of *Ocimum tenuiflorumrom* leaves. The solubility of the medication directly impacts drug absorption, transmission and drug effects. The moisture and ash content also affects these properties; as a result, the solubility of a sample of *Ocimum tenuiflorumrom* leaves in cold water, hot water, 1% NaOH and 1% HCl has been tested.

2. Preparation of Sample

Initially, the collection of leaves was carried out. The leaves of *Ocimum tenuiflorum* were separated using scissors and subsequently air-dried. They were dried at ambient temperature. The leaves were then ground in a mill equipped with

a screen featuring a 5mm diameter hole to obtain a particle size of 40-60 mesh. This finely ground powder was utilized as a sample for various analyses. All chemicals employed are of analytical reagent grade.

2.1. Method of extraction for Ocimum tenuiflorum

The extraction of the *Ocimum tenuiflorum* was performed utilizing the ethanol-benzene Soxhlet extraction method. Approximately 5 grams of leaves powder were sequentially extracted in a Soxhlet extractor with 400 ml of ethanol-benzene. The resulting extracts were then evaporated using a rotary evaporator. The filtrates were subsequently combined and concentrated to dryness under controlled temperature and pressure [6, 7].

2.2. Phytochemical analysis

The phytochemicals present in the were assessed through elemental analysis of magnesium, calcium, sulfur, iron, sodium and chlorine, which were examined using color tests with suitable chemicals and reagents. Additionally, the filtrate was utilized to test for phenols, tannins, saponins, glycosides, flavonoids, steroids and alkaloids[11-13]**.** Phytochemical analysis was conducted on the stem extract following standard methods [14, 15]

2.3. Moisture content and ash content

Silica crucible was taken and it was weighed and kept in oven till it showed constant weight. The leaves sample was analyzed for moisture and ash content by known method and the percentage of the moisture and ash of sample is calculated by applying the following formula, loss of weight of sample

$$\% \text{ of Moisture and ash} = \frac{\text{loss of weight of sample}}{\text{Weight of sample taken}} \times 100$$

2.4. Cold water solubility, hot water solubility

1% NaOH solubility and 1% HCl solubility. The cold water, hot water, 1% NaOH and 1% HCl solubility of leaves sample was analyzed by known method and percentage of solubility of each sample is calculated by applying the following formula,

$$\% \text{ of solubility} = \frac{\text{Weight loss of samples}}{\text{Weight of sample taken}} \times 100$$

The literature survey on Herbal drugs showed that the results obtained during this study are good.

2.5. Phytochemical analysis

The phytochemicals present in the leaves were analyzed through elemental analysis of magnesium, calcium, sulfur, iron, sodium and chlorine. Which were

examined using color tests with suitable chemicals and reagents. Additionally, the filtrate was utilized to test for phenols, tannins, saponins, glycosides, flavonoids, steroids and alkaloids. A phytochemical analysis of the stem extract was conducted following standard methods.

a) **Detection of alkaloids was performed using Mayer's test**: The filtrate was treated with Mayer's reagent, and the formation of a yellow cream precipitate indicates the presence of alkaloids.
b) **Detection of tannins was carried out using the Gelatin test:** To the extract, a 1% gelatin solution containing sodium chloride was added and the formation of a white precipitate indicates the presence of tannins.
c) **Detection of saponins was conducted using the Froth test**: The extract was diluted with distilled water to a volume of 30 ml and shaken in a graduated cylinder for 20 minutes; the formation of a layer of foam indicates the presence of saponins.
d) **Detection of flavonoids was performed using the Lead acetate test:** The extract was treated with a few drops of lead acetate solution and the formation of a yellow color precipitate indicates the presence of flavonoids.
e) **Detection of carbohydrates was conducted using Molisch's test**: The filtrate was treated with 2 drops of alcoholic á-naphthol solution in a test tube and 2 ml of concentrated sulfuric acid was carefully added along the sides of the test tube; the formation of a violet ring at the junction indicates the presence of carbohydrates.
f) **Detection of proteins was performed using the Xanthoproteic test**: The extract was treated with a few drops of concentrated nitric acid solution; the formation of a yellow color indicates the presence of proteins.
g) **Detection of steroids was carried out using Libermann Burchard's test**: The extract was treated with chloroform and filtered, then the filtrate was treated with a few drops of acetic anhydride, boiled, and then cooled. Concentrated sulfuric acid was carefully added to the test tube, and the formation of a brown ring at the junction indicates the presence of steroids.
h) **Detection of glycosides was performed using Legal's test**: The extract was treated with sodium nitroprusside in pyridine; the formation of a pink to blood red color indicates the presence of glycosides.

2.6 Antimicrobial activity

The antimicrobial activity was assessed utilizing the agar disc diffusion method. Negative controls were established by employing the same solvents used

to dissolve the samples. The inhibition zones were measured and compared to the standard reference antibiotic amoxicillin. Each extract underwent serial dilution using dimethyl sulphoxide (DMSO) as a solvent to achieve a 2 mg/ml solution. The concentration of the amoxicillin standard utilized for this study was 2 mg/ml. The prepared concentration of the extract was evaluated for its antimicrobial activity against 1 gm of positive bacteria and 3 gm of negative bacteria on nutrient agar plates through the disc diffusion method. All plates were incubated at 37 °C for 24 hours. The evaluation of antimicrobial activity was conducted by measuring the diameter of the zones of inhibition against the tested bacteria.

3. Results and Discussion

Results are discussed in **Table 1 -5.**

Table 1: Proximate analysis of *Ocimum tenuiflorum*

Sr. No.	Parameters	Percentage (%)
1	Moisture content	20.8
2	Ash content	14.4
3	Cold water solubility	71.2
4	Hot water solubility	91
5	1% NaOH solubility	25.5
6	1% HCl solubility	51

Table 2: Phytochemical analysis of *Ocimum tenuiflorum*

Sr. No.	Content	Test	Result
1	Alkaloids	Mayers test	+
2	Tannin	Gelatin test	+
3	Saponin	Froth test	+
4	Flavonoids	Lead acetate test	+
5	carbohydrate	Molish test	+
6	Protein	Xanthoproteic test	+
7	Steroids	Libermann -Burchard test	+
8	Glycosides	Legal's reagent	+

"+" = present, "-" = absent

Table 3: Nutrient Analysis of *Ocimum tenuiflorum*

Sr. No.	Content	Result
1	Magnesium	+
2	Calcium	+
3	zinc	+
4	Potassium	+
5	iron	+
6	Phosphorus	+
7	Manganese	+

"+" = present, "-" = absent

Total four isolated compounds were studied for their antimicrobial activities. All the pathogens tested during analysis are human pathogen. Activities of compounds were tested against all the pathogens by disc diffusion method. It was found that all the compounds are active against bacteria.

Table 4: Antimicrobial Activity of Compounds

Comp.	*E. coli*	*P. vulgaris*	*S. typhi*	*S. aureus*
E1	Active	Inactive	Active	Active
E2	Active	Inactive	Active	Active
E3	Active	Inactive	Active	Active
E4	Active	Inactive	Active	Active

Table 5: Minimum Inhibitory Concentration (MIC) values of active compounds in mg/ ml

Comp.	*E. coli*	*P. vulgaris*	*S. typhi*	*S. aureus*
E1	815	3892	812	1094
E2	748	3726	798	1827
E3	445	3599	385	772
E4	1538	3542	2232	2340

MIC value = 3500-1900 Inactive;
1800-1500 Weakly active;
1400-1000 Moderately active;
< 1000 Highly active.

It contains a significant number of proteins, which are crucial as enzymes, hormones and antibodies. A high carbohydrate intake is vital for sustaining life in both plants and animals and it also serves as raw material for various industries [16].

The presence of flavonoids indicates that the stem possesses biological functions such as acting as an antioxidant, providing protection against allergies, free radicals, inflammation, ulcers, hepatotoxins, tumours and viruses [17]. Flavonoids are water-soluble antioxidants that prevent oxidative damage to cells and exhibit strong anti-ulcer and anticancer properties [18]. The saponin content suggests as a productivity agent, although the saponin levels are low compared to findings from other studies. Alkaloids are recognized as highly effective bioactive compounds in plants. Both alkaloids and their synthetic derivatives are utilized as medicinal agents due to their bactericidal and analgesic effects. These compounds are water-soluble phenolics that can precipitate proteins and are found in all plants. Tannins contribute to the bio-unavailability of proteins [19].

Potassium is the most abundant element present. It plays a crucial role in regulating body weight and enhancing water and electrolyte balance in blood and tissues. The calcium content has been assessed, as it is essential for muscle

contraction, which is necessary for the development of bones and teeth in infants and fat uses [20]. The sodium concentration is low, making this vegetable beneficial for treating heart-related conditions. Excessive sodium intake can lead to hypertension. Iron is a vital component of the diet for pregnant women, nursing mothers, infants, and the elderly to prevent anaemia. Magnesium also plays significant roles in most reactions involving phosphate transfer[20]. It is essential in. It is important in structural stability of nucleic acids. It plays a powerful role in internal absorption of electrolyte in the body. Its defect in man includes severe diarrhoea and migraines [21, 22].

3.1 Activity against *E. coli*

E. coli is a gram-negative parasite that resides exclusively in the intestines of humans or animals. Clinical infections attributed to E. coli include urinary tract infections, diarrhoea, pathogenic infections and septicaemia. Typically, patients suffering from diarrhoea are observed between February and July. It is evident that the compounds E1, E2 and E3 exhibit high activity at minimal concentrations, whereas the E4 compound demonstrates moderate activity. Therefore, these synthesized drugs may serve as optimal alternative treatments for diseases caused by *E. coli,* provided that their pharmaceutical, biochemical, and medicinal significance is established, and that they do not present adverse or toxic effects [23-25].

3.2 Activity against *S. aureus*

Staphylococcus aureus is a gram-positive bacterium commonly found in wounds and is known to cause wound infections. It appears in clusters resembling grapes. Its capacity to develop resistance to penicillin and other antibiotics underscores its significance as a human pathogen. This bacterium is responsible for two types of diseases: infection and intoxication. Due to its ability to resist common antibiotics, there is a continual need to synthesize and test new types of drugs against it. Four compounds have been evaluated against the *S. aureus* pathogen, with E3 demonstrating high activity, E1 showing moderate activity and E2 and E4 exhibiting weak activity. These active compounds may be utilized for the treatment of wound infections, provided they undergo thorough biological, pharmaceutical and medical studies to ensure they do not possess any toxic effects [23-25].

3.3 Activity against *S. typhi*

S. typhi is a gram-negative bacterium that is the causative agent of typhoid, with an incubation period ranging from 7 to 14 days. Patients typically exhibit mild pyrexia, which can escalate into a fatal fulminating disease. Since bile serves as an excellent culture medium for the bacteria, it proliferates abundantly in the gall bladder and is continuously discharged into the intestine, where it affects the Peyer's

patches and lymphoid follicles of the ileum. Four compounds have been tested against the *S. typhi* pathogen, revealing that E1, E2 and E3 exhibit high levels of activity, while E4 shows weak activity. Therefore, these synthesized drugs may serve as alternative treatments for diseases caused by *S. typhi*, but only after thorough investigation in the fields of pharmaceutical, biochemical, and medicinal sciences. These drugs could potentially replace traditional medications, provided they do not present toxic effects or other side effects [23-25].

3.4 Activity against *P. vulgaris*

Proteus vulgaris is a gram-negative bacterium that resides in the intestinal tracts of both humans and animals. It can be located in soil, water and fecal matter. This bacterium acts as an opportunistic pathogen in humans and is recognized for causing urinary tract infections as well as wound infections. Four compounds have been tested against the *P. vulgaris* pathogen, all of which are inactive[23-25].

4. Conclusion

This type of research will be relevant to the fields of pharmaceuticals, medicine, agriculture, industry and biochemistry. The study also demonstrated that the proximate, phytochemical and mineral analyses of *Ocimum tenuiflorum* leaves indicate it is a balanced and abundant source of both macro- and micronutrients. Additionally, rural communities utilized the plant as a medicine, which suggests its role in providing medicinal property. Therefore, further research will be conducted on this plant.

References

1. Jain, S. (1965). Medicinal plant lore of the tribals of Bastar. *Economic Botany, 19*(3), 236–250.
2. Lata, S., Maurya, A. K., & Jain, S. (2008). An overview of ethnobotanical studies on Uttarakhand state in North India. *Ethnobotany, 20*, 25–40.
3. Ijinu, T. P., George, V., & Pushpangadan, P. (2022). History of research on medicinal plants in India. In *Medicinal and aromatic plants of India* (Vol. 1, pp. 35–61). Springer.
4. Yadav, R., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology, 3*(12).
5. Patil, S. H., Kurlapkar, D. D., & Gaikwad, D. K. (2019). Dye-yielding plant resources of Maharashtra, India: A checklist. *Biodiversitas, 20*(1), 250–266.
6. Fagbohun, E., Egbebi, A., & Lawal, O. (2012). Phytochemical screening, proximate analysis, and in vitro antimicrobial activities of methanolic extract

of *Cnidoscolus aconitifolius* leaves. *International Journal of Pharmaceutical Sciences Review and Research, 13*(1), 28–33.

7. Thirumurugan, K. (2010). Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants.
8. Okunlola, G. O., et al. (2019). Proximate analysis, mineral composition, and antioxidant properties of bitter leaf and scent leaf. *International Journal of Vegetable Science, 25*(4), 346–354.
9. Nandagoapalan, V., Doss, A., & Marimuthu, C. (2016). Phytochemical analysis of some traditional medicinal plants. *Bioscience Discovery, 7*(1), 17–20.
10. Waghulde, S., Kale, M., & Patil, V. (2021). Cumulative phytochemical analysis and identification of drug lead compounds from medicinal plant extracts. In *Chemical Proceedings*.
11. Shukla, R. K., et al. (2012). Proximate analysis, nutritive value, total phenolic content, and antioxidant activity of *Litchi chinensis* Sonn. *Natural Products: An Indian Journal, 8*(9), 361–369.
12. Uahomo, P. O., Isirima, J. C., & Akoko, S. (2022). Evaluation of phytochemicals and bioactive properties in leaf and root parts of *Cyathula prostrata*: A qualitative and quantitative analysis. *Asian Plant Research Journal, 9*(3), 8–16.
13. Evans, W. C. (2009). *Trease and Evans' pharmacognosy*. Elsevier Health Sciences.
14. Ghosh, P., et al. (2019). Evaluation of phytochemical constituents and antioxidant property of leaf acetone extracts of five herbaceous medicinal weeds. *Journal of Pharmaceutical Sciences and Research, 11*(8), 2806–2813.
15. Kushagra, D., et al. (2020). In vitro evaluation of anti-urolithic activity of leaves extract of *Costus igneus*. *Research Journal of Pharmacy and Technology, 13*(3), 1289–1292.
16. Sengupta, P. S., et al. (2019). Pharmacognostical and analytical study of Kushmanda ghrita, a polyherbal formulation, for pharmaceutical standardization.
17. Oke, D. G. (2014). Proximate and phytochemical analysis of *Cajanus cajan* (pigeon pea) leaves.
18. Akindahunsi, A., & Salawu, S. (2005). Phytochemical screening and nutrient-antinutrient composition of selected tropical green leafy vegetables. *African Journal of Biotechnology, 4*(6).

19. Antia, B., et al. (2006). Nutritive and anti-nutritive evaluation of sweet potatoes (*Ipomoea batatas*) leaves. *Pakistan Journal of Nutrition, 5*(2), 166–168.
20. Okwu, D. (2001). Evaluation of chemical composition of indigenous species and flavouring agents. *Global Journal of Pure and Applied Sciences, 7*(3), 455–460.
21. Nagalingam, S., Sasikumar, C. S., & Cherian, K. M. (2012). Extraction and preliminary phytochemical screening of active compounds in *Morinda citrifolia* fruit. *Asian Journal of Pharmaceutical and Clinical Research, 5*(2), 179–181.
22. Shukla, A., Vats, S., & Shukla, R. K. (2014). Proximate composition, nutritive value, and evaluation of antioxidant potential of stem of *Dracaena reflexa* Lam. *International Journal of Pharmacy and Pharmaceutical Sciences, 6*(11), 360–364.
23. Nagar, P. P. (n.d.). B.Sc. Microbiology.
24. Ananthanarayan, R. (2006). *Ananthanarayan and Paniker's textbook of microbiology*. Orient Blackswan.
25. Brooks, G. F., Butel, J. S., & Morse, S. A. (2007). *Medical microbiology*. McGraw-Hill Medical.