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# Pharmacognostic Standardization, Phytochemical Characterization and Anti-Inflammatory Potential of Ocimum kilimandscharicum Guerke

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**Abstract**

The present study was undertaken to evaluate the pharmacognostic characteristics, phytochemical profile, and anti-inflammatory potential of O. kilimandscharicum leaves. Macroscopic and microscopic evaluations were performed for authentication of the plant material. Essential oil was isolated using a Clevenger apparatus and subjected to phytochemical screening, thin-layer chromatography (TLC), gas chromatography–mass spectrometry (GC-MS), UV-visible spectroscopy, and Fourier-transform infrared spectroscopy (FTIR). Preliminary phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, terpenoids, saponins, and glycosides. TLC analysis showed an Rf value of 0.48, closely matching the standard 1,8-cineole (Rf 0.50). GC-MS analysis identified several bioactive compounds including 1,8-cineole, camphor, limonene, linalool, and borneol, with 1,8-cineole and camphor as major constituents. FTIR and UV analyses confirmed the characteristic functional groups and absorption maxima of the isolated compound. Molecular docking studies of 1,8-cineole against protein target 5U7N demonstrated a binding affinity of −4.3 kcal/mol with significant amino acid interactions. In vitro anti-inflammatory activity using the protein denaturation assay showed concentration-dependent inhibition with 55.84% inhibition at 100 µg/mL. The findings validate the traditional use of O. kilimandscharicum and highlight its potential as a natural source of anti-inflammatory agents for future pharmaceutical applications.

**Keywords:** Ocimum kilimandscharicum, Pharmacognostic evaluation, Phytochemical screening, 1,8-Cineole, Anti-inflammatory activity.

## 1. Introduction

Medicinal plants have served as valuable sources of therapeutic agents since ancient times and continue to play a crucial role in healthcare systems worldwide. According to the World Health Organization (WHO), nearly 80% of the global population relies on herbal medicines for primary healthcare needs, particularly in developing countries.[1-2] The increasing demand for safer and more effective natural remedies has led to extensive research on medicinal plants possessing pharmacological properties such as anti-inflammatory, antioxidant, antimicrobial, and anticancer activities. The genus Ocimum, belonging to the family Lamiaceae, comprises several aromatic and medicinally important species. Among them,

Ocimum kilimandscharicum Guerke, commonly known as Kapur Tulsi or Camphor Basil, is a perennial aromatic shrub native to East Africa and widely cultivated in India. The plant is characterized by a strong camphoraceous aroma due to the presence of volatile essential oils rich in terpenoid compounds. Traditionally, the leaves and essential oil of O. kilimandscharicum have been used for the treatment of cough, cold, bronchitis, fever, wounds, digestive disorders, and inflammatory conditions.[3-5]

Phytochemical investigations have revealed that O. kilimandscharicum contains a wide variety of bioactive constituents including 1,8-cineole, camphor, limonene, linalool, borneol, flavonoids, tannins, and saponins. These compounds are known to exhibit diverse pharmacological activities such as antimicrobial, antioxidant, anti-inflammatory, analgesic, and immunomodulatory effects. Among these constituents, 1,8-cineole is considered one of the major active compounds responsible for the therapeutic properties of the plant.[6-9] Inflammation is a complex biological response triggered by infection, injury, or harmful stimuli. Although inflammation is essential for tissue repair and host defense, chronic inflammation is associated with several diseases such as arthritis, cardiovascular disorders, diabetes, and neurodegenerative conditions. Conventional anti-inflammatory drugs are effective but often produce adverse effects including gastric irritation, ulceration, and renal toxicity. Therefore, there is growing interest in identifying plant-derived anti-inflammatory agents with improved safety profiles.[10-12]

Pharmacognostic standardization is an important step in ensuring the identity, purity, and quality of medicinal plants. Morphological, microscopic, and phytochemical evaluations provide essential information for authentication and quality control of herbal materials. In addition, advanced analytical techniques such as TLC, GC-MS, UV spectroscopy, and FTIR spectroscopy enable the characterization of active constituents and support the development of standardized herbal formulations.[13-17] Recent advances in computational biology have introduced molecular docking as an effective tool for predicting ligand–protein interactions and understanding the mechanism of action of bioactive compounds. Molecular docking studies help identify potential therapeutic targets and provide evidence for pharmacological activities at the molecular level.[18-20]

The present study was designed to perform pharmacognostic standardization, phytochemical characterization, and anti-inflammatory evaluation of Ocimum kilimandscharicum leaves. The study further aimed to isolate and characterize the major bioactive constituent, 1,8-cineole, using chromatographic and spectroscopic techniques and to investigate its interaction with an anti-inflammatory protein target through molecular docking analysis.

## 2. Materials and Methods

### Plant Material Collection and Authentication

Fresh leaves of O. kilimandscharicum were collected from Malda, Nandurbar District, Maharashtra, India, during November 2025. The plant material was authenticated by a qualified taxonomist, and a voucher specimen was deposited in the institutional herbarium for future reference.

**Pharmacognostic Evaluation**

Macroscopic characteristics such as color, odor, taste, size, shape, and texture were recorded. Microscopic studies were carried out using transverse sections stained with safranin and observed under a compound microscope.

**Extraction of Essential Oil**

Dried leaf powder (70 g) was subjected to hydrodistillation using a Clevenger apparatus for 3 hours. The obtained essential oil was collected and stored in amber-colored containers until analysis.

**Preliminary Phytochemical Screening**

The extract was screened qualitatively for alkaloids, flavonoids, tannins, saponins, terpenoids, and glycosides using standard phytochemical methods.

**Thin Layer Chromatography**

TLC analysis was performed using silica gel 60 F254 plates with toluene:ethyl acetate (8:2 v/v) as the mobile phase. The developed plates were visualized under UV light, and Rf values were calculated.

**GC-MS Analysis**

GC-MS analysis was carried out using a Shimadzu GC-MS instrument. Compounds were identified by comparing mass spectra with the NIST library database.

**Spectral Analysis**

FTIR spectroscopy was performed in the range of 4000–400 $cm^{-1}$. UV-visible spectroscopy was carried out between 200–400 nm to identify absorption maxima of the isolated compound.

## 3. Results and Discussion

Macroscopic evaluation revealed green ovate leaves with a characteristic camphoraceous odor and pungent taste. Microscopic examination showed dorsiventral leaf anatomy, diacytic stomata, and glandular trichomes.

Figure 1. Microscopic Evaluation of Ocimum kilimandscharicum Leaf Revealing Diacytic Stomata, Glandular Trichomes, and Vascular Bundle

Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, terpenoids, saponins, and glycosides.


Figure 2. Phytochemical screening of Ocimum kilimandscharicum

**Isolation of bioactive compounds**

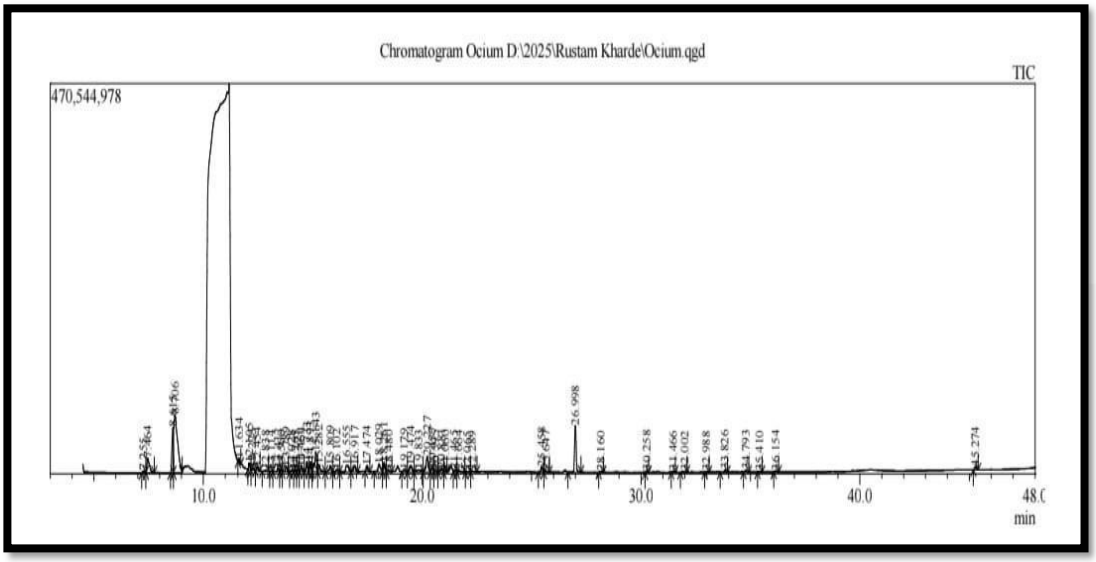
The essential oil of Ocimum kilimandscharicum was isolated using a Clevenger apparatus and further subjected to column chromatography for separation of bioactive constituents. Different fractions were collected and analyzed by TLC. The isolated fraction exhibited an Rf value comparable to the standard 1,8-cineole, indicating successful isolation of the major active compound. The obtained bioactive fraction was further characterized and used for subsequent analytical and pharmacological studies.

Figure 3. Column Chromatography and Separation of Phytoconstituent

**GC-MS Analysis**

GC-MS analysis of the isolated fraction of Ocimum kilimandscharicum revealed the presence of several volatile bioactive compounds. The chromatogram showed characteristic peaks corresponding to 1,8-cineole, camphor, limonene, linalool, borneol, and other terpenoid constituents. Among these, 1,8-cineole and camphor were identified as the major components, confirming the richness of the essential oil in pharmacologically active terpenes. The presence of 1,8-cineole and camphor particularly supports the anti-inflammatory potential of O. kilimandscharicum through modulation of inflammatory mediators and reduction of oxidative stress.

Figure 4. GCMS Chromatogram of Ocimumkilimandus CharicumGuerke




**Thin-Layer Chromatography (TLC)**

TLC analysis showed an Rf value of 0.48 for the sample, closely matching the standard 1,8-cineole (Rf 0.50), indicating the presence of this marker compound.

Table 1. Observe Rf values for Standard and Sample

| Tra.ck | Compound | Distance (Spot) | Distance (Solv.ant) | Rf Val.ue |
|---|---|---|---|---|
| T | 1,8 Cine.ole (Stan.dard) | 4.5 | 9.0 | 0.50 |
| S | Sam.ple | 4.4 | 9.0 | 0.48 |

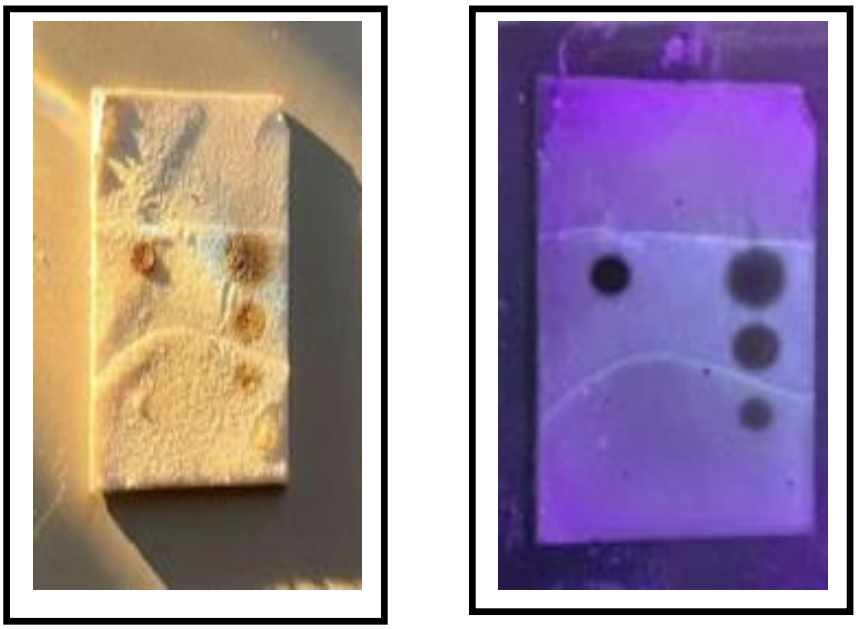


Figure 5. TLC plate

**UV Analysis**

UV spectroscopy exhibited maximum absorption in the range of 280–284 nm, confirming the presence of oxygenated monoterpenes.

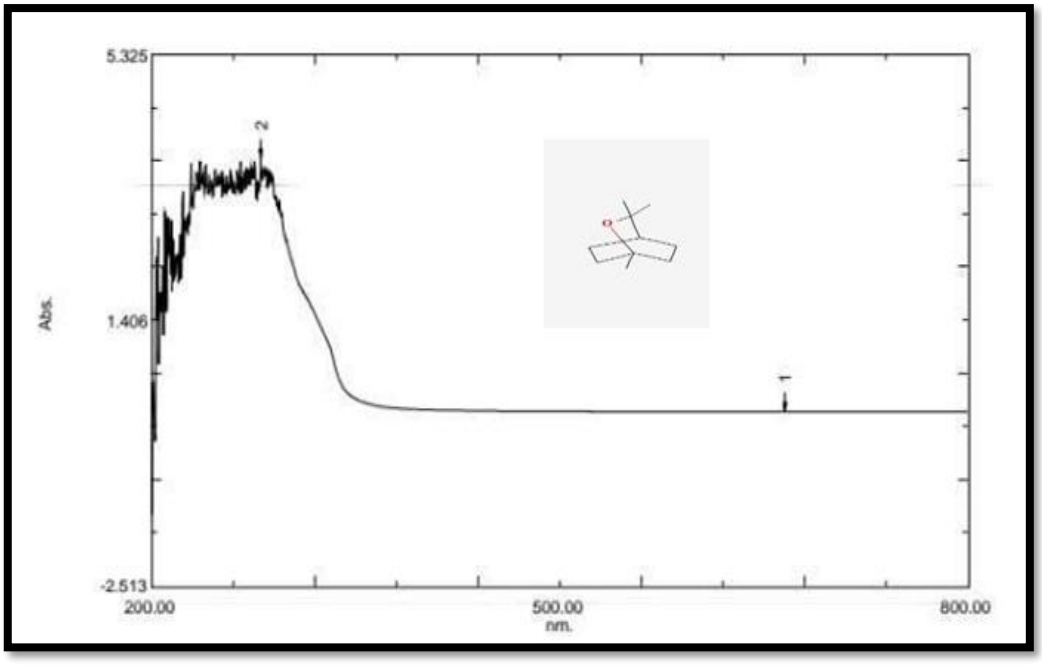



Figure 6. UV Spectra of Compound 1,8-Cineole

**FTIR Analysis**

FTIR spectra demonstrated characteristic absorption bands corresponding to O-H, C-H, C=C, and C-O-C functional groups.

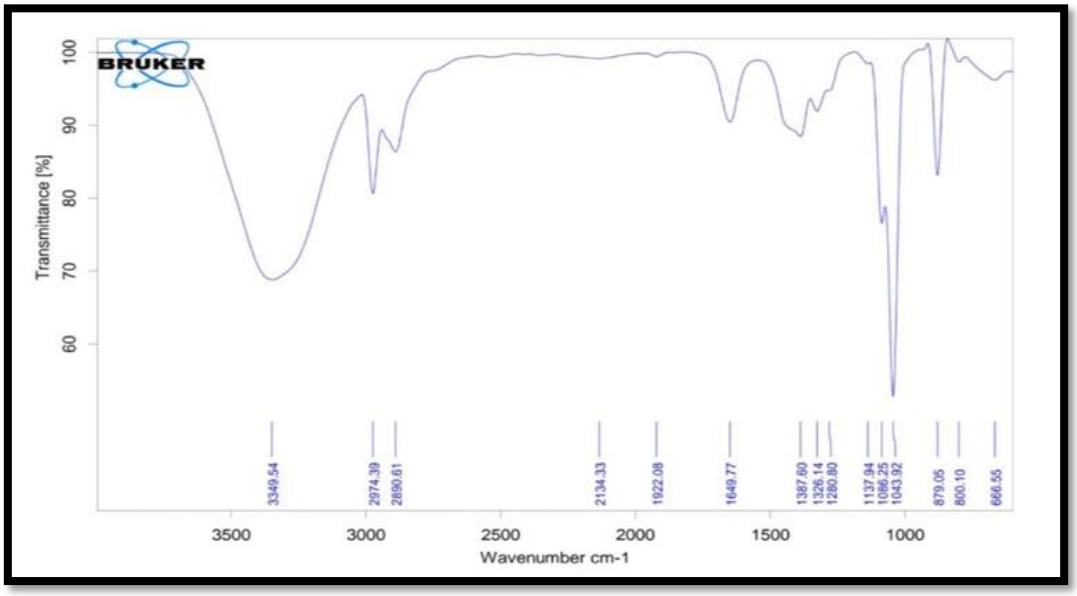



Figure 7. FTIR Spectra of Compound 1,8-Cineole

Table 2. FTIR Spectrum of Compound 1,8-Cineole

| **Peak wave number** | **(%) Transmittance** | **Intensity/Shape** | **Group** |
|---|---|---|---|
| 334.9.54 | 69% | Bro.ad | O-H |
| 297.4.39 | 81% | Str.ong | C-H |
| 289.0.61 | 87% | Med.ium | C-H |
| 164.977 | 91% | Weak | C=C |
| 138.7.6 | 89% | Med.ium | CH-3 |
| 108.6.25 | 53% | VeryStr.ong | C-O-C |
| 104.3.92 | 77% | Str.ong | C-O |
| 879..05 | 83% | Med.ium | Ring Skel.etal |

**Molecular docking studies**

The molecular docking study was performed to evaluate the anti-inflammatory potential of 1,8-Cineole against the target protein 5U7N. The docking analysis revealed a binding affinity of −4.3 kcal/mol, indicating favorable interaction between the ligand and the protein. The compound interacted with key amino acid residues including ALA68, ALA70, VAL71, PRO67, PRO92, PRO94, ILE95, and GLU96 through van der Waals, alkyl, and conventional hydrogen bond interactions. These interactions suggest stable binding of 1,8-cineole within the active site of the target protein, supporting its potential anti-inflammatory activity.

Table 3. Anti-Inflammatory activity result For1, 8-Cineole Against 5U7N

| **Sr. No.** | **PBD ID** | **Name of compound** | **Binding Affinity** | **Interaction Residue** | **Type of interaction** |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| 1. | 5U7N | - | -4.3 | ALAA:.70,ALAA:68,.PRO A:92 ,VAIA.:71,GLUA:96,.ILF A:95,PROA:.94,PRO A67 | Vander waa.ls Alk.yl Conven.tiona lhydr.ogen bond |

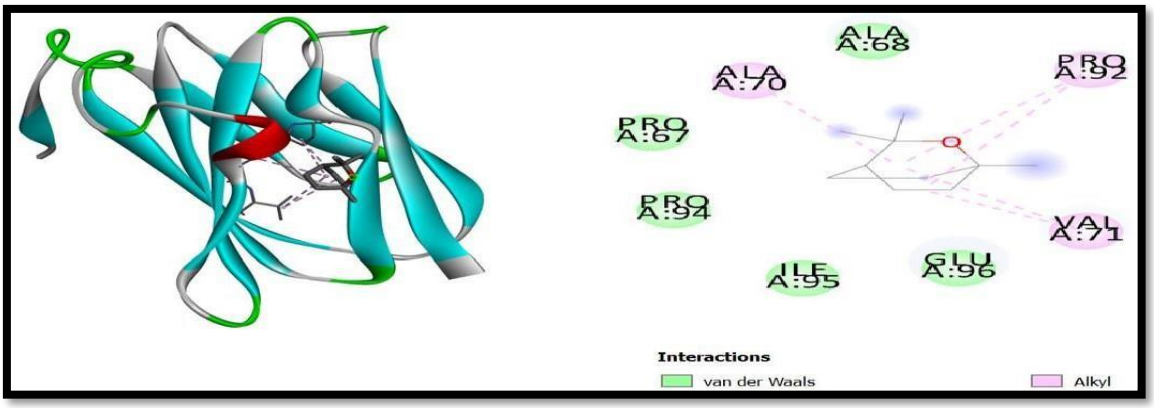



Figure 8. 2D 3D Structure

**Pharmacological Activity**

In-vitro anti-inflammatory activity by Protein denaturation method

Table 4. Anti-Inflammatory activity result For1, 8-Cineole wit STD

| | | | Protein denaturationassay | | | | | |
|---|---|---|---|---|---|---|---|---|
| **SR. NO** | **Sample code** | **Concentration (µg/ml)** | **Absorbance at 660 nm** | | | | | |
| | | | **Test1** | **Test2** | **Test3** | **Mean** | **% Inhibition** | **IC50 (µg/ml)** |
| **1** | **Control** | | 1.54 | 1.54 | 1.54 | **1.54** | - | |
| **2** | **Standard (Diclofenac Sodium)** | **20** | 1.43 | 1.44 | 1.43 | **1.43** | **7.14%** | |
| | | **40** | 1.20 | 1.20 | 1.22 | **1.20** | **22.07%** | **76.11** |
| | | **60** | 0.96 | 0.97 | 0.98 | **0.97** | **37.01%** | |
| | | **80** | 0.72 | 0.72 | 0.71 | **0.71** | **53.89%** | |
| | | **100** | 0.59 | 0.61 | 0.59 | **0.59** | **61.68%** | |
| | | | | | | | | |
| **3** | **CIN-L-01** | **20** | 1.43 | 1.47 | 1.44 | **1.44** | **6.49%** | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **40** | 1.24 | 1.29 | 1.26 | **1.26** | **18.18%** | |
| | | **60** | 1.06 | 1.02 | 1.07 | **1.05** | **31.82%** | |
| | | **80** | 0.86 | 0.89 | 0.91 | **0.88** | **42.86%** | **94.16** |
| | | **100** | 0.69 | 0.72 | 0.65 | **0.68** | **55.84%** | |
| | | | | | | | | |

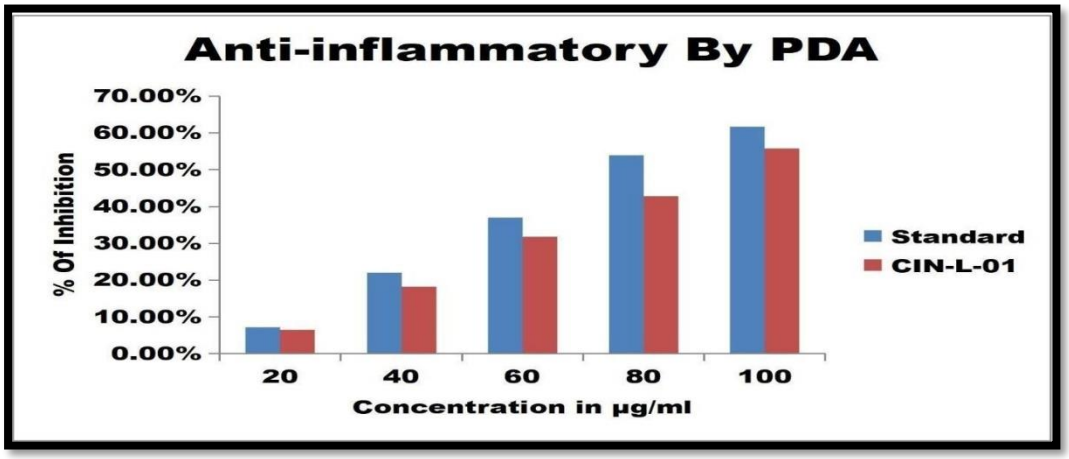



Figure 9. % of inhibition of anti inflammatory activity by PDA

## 4. Conclusion

The present study successfully established the pharmacognostic standards and phytochemical profile of Ocimum kilimandscharicum leaves. Macroscopic and microscopic evaluations confirmed the identity and authenticity of the plant material. Preliminary phytochemical screening demonstrated the presence of important secondary metabolites including alkaloids, flavonoids, tannins, terpenoids, saponins, and glycosides. Chromatographic and spectroscopic analyses confirmed the presence of 1,8-cineole as a major bioactive constituent. GC-MS analysis identified several pharmacologically important compounds such as camphor, limonene, linalool, and borneol. Molecular docking studies revealed favorable binding interactions of 1,8-cineole with the anti-inflammatory target protein 5U7N, supporting its therapeutic potential. Furthermore, the isolated compound exhibited significant concentration-dependent anti-inflammatory activity in the protein denaturation assay. Overall, the findings scientifically validate the traditional medicinal use of O. kilimandscharicum and indicate that the plant represents a promising natural source of anti-inflammatory agents. Further in vivo studies and clinical investigations are recommended to explore its full therapeutic potential and facilitate the development of safe and effective herbal formulations.

## References

1. World Health Organization. WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants. Geneva: World Health Organization; 2003.

2. Pandey AK, Singh P. The genus *Ocimum*: A review of ethnopharmacology, phytochemistry and pharmacology. Journal of Pharmacognosy and Phytochemistry. 2011;3(2):98–105.
3. Prakash P, Gupta N. Therapeutic uses of *Ocimum sanctum* Linn. (Tulsi) with a note on eugenol and its pharmacological actions: A short review. Indian Journal of Physiology and Pharmacology. 2005;49(2):125–131.
4. Koba K, Poutouli PW, Raynaud C, Chaumont JP, Sanda K. Chemical composition and antimicrobial properties of different basil essential oils from Togo. Fitoterapia. 2009;80(3):153–157.
5. Edris AE. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: A review. Phytotherapy Research. 2007;21(4):308–323.
6. Lawrence BM. Progress in essential oils: Camphor and cineole-rich basil oils. Perfumer and Flavorist. 2006;31:42–54.
7. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman and Hall; 1998.
8. Trease GE, Evans WC. Trease and Evans Pharmacognosy. 16th ed. London: Saunders Elsevier; 2009.
9. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 56th ed. Pune: Nirali Prakashan; 2023.
10. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. 3rd ed. Ibadan, Nigeria: Spectrum Books Ltd.; 2008.
11. Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd ed. Berlin: Springer-Verlag; 1996.
12. Sarker SD, Latif Z, Gray AI. Natural Products Isolation. 3rd ed. Totowa, New Jersey: Humana Press; 2006.
13. Silverstein RM, Webster FX, Kiemle DJ. Spectrometric Identification of Organic Compounds. 7th ed. Hoboken, New Jersey: John Wiley & Sons; 2005.
14. Stuart B. Infrared Spectroscopy: Fundamentals and Applications. Chichester, UK: John Wiley & Sons; 2004.
15. Pavia DL, Lampman GM, Kriz GS, Vyvyan JR. Introduction to Spectroscopy. 5th ed. Boston: Cengage Learning; 2015.
16. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. Journal of Computational Chemistry. 2009;30(16):2785–2791.
17. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. Journal of Computational Chemistry. 2010;31(2):455–461.
18. Anilakumar KR, Sahu A, Khanum F, Bawa AS. Anti-inflammatory effects of natural compounds isolated from medicinal plants: A review. Journal of Herbal Medicine and Toxicology. 2009;3(1):1–10.
19. Sakat S, Juvekar AR, Gambhire MN. In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. International Journal of Pharmacy and Pharmaceutical Sciences. 2010;2(1):146–155.

20. Umesh MK, Bairy KL, Devi PU. Evaluation of anti-inflammatory activity of medicinal plant extracts using protein denaturation assay. International Journal of Pharmaceutical Sciences Review and Research. 2011;7(2):115–118.