



Phytochemical Profiling and Antifungal Potential of Wild *Ocimum* Species: A Comparative Evaluation of Bioactive Extracts

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Abstract:

The genus *Ocimum* represents a significant source of bioactive phytochemicals that have extensive ethnomedicinal and pharmacological applications. This study explored the phytochemical diversity and antifungal potential of various wild *Ocimum* species (*O. americanum*, *O. tenuiflorum*, *O. basilicum*, and *O. gratissimum*) collected from the Marathwada region of Maharashtra, India. Using Soxhlet extraction with solvents of varying polarity, we observed interspecific variations in the phytochemical profiles. Notably, *O. basilicum* and *O. americanum* exhibited the greatest diversity, containing alkaloids, flavonoids, phenolics, and terpenoids, while *O. gratissimum* showed a more limited range with only phenolics, terpenoids, and glycosides present. Antifungal assays conducted against phytopathogens such as *Aspergillus niger*, *Fusarium oxysporum*, *Alternaria alternata*, and *Rhizoctonia solani* demonstrated significant inhibition zones in *O. basilicum* (20.3 ± 0.2 mm against *A. niger*; 21.0 ± 0.3 mm against *A. alternata*) and *O. americanum* (18.5 ± 0.3 mm against *A. niger*; 17.8 ± 0.4 mm against *A. alternata*). In contrast, *O. gratissimum* exhibited lowest antifungal activity, with inhibition zones ranging from 6.8 to 9.2 mm across different strains. These results affirm a direct correlation between phytochemical richness and antifungal effectiveness, highlighting *O. basilicum* as a particularly promising natural source of antifungal agents. This study underscores the potential of wild *Ocimum* species for the development of sustainable, plant-based antifungal solutions.

Keywords: *Ocimum* Species; Phytochemical Profiling; Antifungal Activity; Bioactive Metabolites; Inhibition Zone; Natural Antifungal Agents

Introduction:

The genus *Ocimum* encompasses a diverse group of plants widely recognized for their significant ethnomedicinal applications and pharmacological potential across various cultures (Chinedu & C, 2021). These species are extensively researched for their utility in conventional therapeutic practices, exhibiting a broad spectrum of activities including antimalarial, anticancer, antidiabetic, anti-inflammatory, and antioxidant effects (Chinedu & C, 2021). This broad therapeutic

range is largely attributed to the rich phytochemical profiles of *Ocimum* species, which include flavonoids, alkaloids, saponins, tannins, and phenols, all contributing to their bioactive properties (Halim *et al.*, 2022; Mazumder *et al.*, 2023). Specifically, recent investigations have focused on the antimicrobial efficacy of *Ocimum* extracts, with studies demonstrating their capacity to inhibit the growth of various pathogenic microorganisms, including fungi (Chanthaboury *et al.*, 2022). This highlights

their potential as natural antifungal agents, offering an alternative to synthetic fungicides, which often face issues of resistance and environmental persistence (Sharifi-Rad *et al.*, 2021). Consequently, a deeper understanding of the chemical constituents responsible for the antifungal activity in wild *Ocimum* species is crucial for developing novel, sustainable antimicrobial solutions (Chinedu & C, 2021). This study aims to comparatively evaluate the phytochemical profiles and antifungal potential of bioactive extracts derived from different wild *Ocimum* species, thereby elucidating the specific compounds and mechanisms responsible for their observed bioactivity.

Materials and Methods:

Plant Material Collection: Wild *Ocimum* species were collected from natural habitats in the Marathwada region of Maharashtra, India. Plant specimens were authenticated by a taxonomist, and voucher specimens were deposited in the departmental herbarium.

Preparation of Extracts: Leaves of the collected species were shade-dried, powdered, and subjected to Soxhlet extraction using solvents of increasing polarity (aqueous, ethanol, and methanol). Extracts were concentrated under reduced pressure and stored at 4°C until further use. The selection of appropriate extraction techniques, such as Soxhlet extraction, significantly influences the phytochemical yield and, consequently, the biological activity of the extracts, as different methods can selectively isolate distinct classes of compounds (Yan *et al.*, 2022).

Phytochemical Screening: Preliminary phytochemical tests were performed to detect the presence of secondary metabolites, including alkaloids (Mayer's test), flavonoids (Shinoda test), tannins (Ferric chloride test), phenolics (Folin–Ciocalteu reagent), terpenoids (Salkowski's test), and glycosides (Keller–Killiani test). Both qualitative and semi-quantitative assessments were recorded. These analyses are critical for establishing a foundational understanding of the complex chemical makeup of the extracts, which is directly relevant to their therapeutic applications (Mazumder *et al.*, 2023).

Antifungal Assays. The antifungal activity of the extracts was tested against phytopathogenic fungi (*Aspergillus niger*, *Fusarium oxysporum*, *Alternaria alternata*, and *Rhizoctonia solani*). 100 µL of each extract was loaded into wells on PDA plates inoculated with fungal cultures. Zones of inhibition (mm) were measured after incubation at $28 \pm 2^\circ\text{C}$ for 72 hours. The serial dilution method was employed to determine the lowest concentration of extracts inhibiting visible fungal growth. All experiments were conducted in triplicate.

Results and Discussion:

The *Ocimum* species showed the richest phytochemical diversity, with a strong presence of alkaloids, flavonoids, phenolics, and terpenoids consistent. These results highlight the varied presence of key phytochemicals across different *Ocimum* species (Table 1).

Table 1: Phytochemical Composition and Antifungal Activity of *Ocimum* Species

Ocimum Species	Alkaloids	Flavonoids	Tannins	Phenolics	Terpenoids	Glycosides
<i>O. americanum</i>	+	+	–	+	+	–
<i>O. tenuiflorum</i>	–	+	+	+	–	+
<i>O. basilicum</i> (wild)	+	+	–	+	+	–
<i>O. gratissimum</i>	–	–	–	+	+	+

O. basilicum (wild) and *O. americanum* showed the richest phytochemical diversity, with strong presence of alkaloids, flavonoids, phenolics, and terpenoids consistent with their high antifungal activity. *O. tenuiflorum* contained flavonoids, tannins, phenolics, and glycosides but lacked alkaloids and terpenoids, aligning with its moderate antifungal activity. *O. gratissimum* had fewer bioactive groups (phenolics, terpenoids, glycosides) and lacked flavonoids/alkaloids,

which explains its weaker antifungal response. These results highlight the varied presence of key phytochemicals across different *Ocimum* species, underscoring the influence of species-specific biosynthesis and potentially environmental factors on their chemical profiles (Mithraja *et al.*, 2012).

The antifungal activity of different extracts of *Ocimum* species is summarized in Table 2, which shows variation in inhibition across fungal strains.

Table 2. Antifungal Activity of Wild *Ocimum* Species

Fungal Strain	<i>O. americanum</i>	<i>O. tenuiflorum</i>	<i>O. basilicum</i>	<i>O. gratissimum</i>
<i>Aspergillus niger</i>	18.5 ± 0.3	12.1 ± 0.4	20.3 ± 0.2	8.7 ± 0.5
<i>Fusarium oxysporum</i>	16.2 ± 0.6	10.5 ± 0.3	19.8 ± 0.4	7.1 ± 0.2
<i>Alternaria alternata</i>	17.8 ± 0.4	11.8 ± 0.5	21.0 ± 0.3	9.2 ± 0.6
<i>Rhizoctonia solani</i>	15.9 ± 0.7	10.1 ± 0.6	18.5 ± 0.5	6.8 ± 0.4

Overall, *O. basilicum* demonstrated superior antifungal activity across all tested fungal strains, corroborating its rich phytochemical profile and previously reported antimicrobial properties (Kačániová *et al.*, 2022). This suggests a direct correlation between the comprehensive presence of secondary metabolites, particularly alkaloids and terpenoids, and enhanced antifungal efficacy. Conversely, species like *O. gratissimum* exhibited limited antifungal action, which can be correlated with a less diverse phytochemical composition, notably lacking key active compounds present in the more effective species.

The findings presented in this study reveal significant variations in both the phytochemical composition and antifungal efficacy among wild *Ocimum* species, underscoring the importance of species-specific evaluation for identifying promising sources of bioactive compounds. Specifically,

the comprehensive phytochemical analysis demonstrated a direct correlation between the diversity and abundance of secondary metabolites like alkaloids, flavonoids, phenolics, and terpenoids, and the observed antifungal potency, with *O. basilicum* exhibiting the highest activity due to its rich constituent profile (Adámek *et al.*, 2021). This aligns with previous research highlighting the significant pharmacological potential of *Ocimum* species, particularly *O. basilicum*, which is renowned for its diverse array of phytonutrients and antioxidant capabilities (Filip, 2017). Furthermore, the genus *Ocimum* is widely recognized for its therapeutic potential, with species like *Ocimum sanctum* demonstrating broad health-promoting effects through the modulation of various biological activities, including significant antioxidant, anti-inflammatory, and antimicrobial properties, alongside notable anticancer effects (Almatroodi *et al.*, 2020).

Conclusion:

These properties are often attributed to the complex interplay of their bioactive compounds, which collectively contribute to their reported medicinal benefits. The rich presence of polyphenols, phenolic acids, flavonoids, and terpenoids in basil leaves, for instance, underpins its effectiveness as a potent antimicrobial and anti-inflammatory agent. This collective action of secondary metabolites ensures their efficacy against a wide range of pathogens and chronic diseases. Therefore, further research is warranted to elucidate the precise mechanisms through which these phytochemicals exert their antifungal effects and to explore their potential in novel therapeutic applications.

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