

Phytochemical analysis and antioxidant potential of *Ocimum micranthum* and *Ocimum basilicum* commonly consumed by Human in North India

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ABSTRACT

Ocimum species is a rapidly growing shrub that belongs to the Lamiaceae family. The pharmacological significance of various *Ocimum* species, such as *Ocimum micranthum*, *Ocimum micranthum*, *Ocimum kilimandscharicum*, *Ocimum sanctum* (green), and *Ocimum sanctum* (purple), has been well established. In order to determine the presence of phytochemical compounds including Alkaloids, Flavonoids, Saponins, Tannins, Phenols, and Quinones, qualitative and quantitative phytochemical analysis was conducted on different extracts of *Ocimum* leaf powder. The presence of these phytochemicals, which exhibit significant antiradical activity, further confirms the medicinal potential of *O. micranthum* and *O. basilicum*. These findings validate the traditional use of *O. micranthum* and *O. basilicum* in the study area, as the secondary metabolites derived from these plants possess natural antioxidant properties that hold medicinal value for humans.

Keywords: *Ocimum micranthum*, phytochemical, *Ocimum basilicum*, Antioxidants, Qualitative-Quantitative analysis.

Introduction

The family Lamiaceae comprises various species of *Ocimum*, including *Ocimum micranthum*, *Ocimum basilicum*, *Ocimum sanctum* (green), and *Ocimum sanctum* (purple). These species are renowned for their medicinal properties, such as antioxidant and antibacterial activity. In Turkey's East Anatolia region, *Ocimum micranthum*, commonly known as Basil, is prevalent¹ Essential oils extracted from *Ocimum micranthum* are used for flavoring meats and sausages. The leaves and flowers of *Ocimum micranthum* hold significant medical importance as they are used to treat fever, nausea, abdominal cramps, gastroenteritis, migraine, insomnia, depression, gonorrhoea, dysentery. Additionally, they possess other beneficial properties like being anti-spasmodic, aromatic, carminative, digestive, galactagogue, stomachic, and tonic agents.^{2,3}

Out of the 333 scientific plant names associated with *Ocimum*, 68 species names have been accepted, while the remaining are categorized as unassessed, unplaced, or synonyms.⁴ Nine species of *Ocimum*, primarily found in the tropical and peninsular region, represent India.⁵ The therapeutic properties of *Ocimum* have earned it significant recognition in Pharmacology.

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Our current study focuses on the phytochemical constituents present in the leaf extract of *Ocimum micranthum* and *Ocimum basilicum*, as well as their applications in the field of pharmacology. Phenolics, natural color pigments found in fruits of plants, are a type of chemical component that is widely distributed. These compounds are synthesized from phenylalanine through the activity of phenylalanine ammonia lyase (PAL). Phenolics play a crucial role in plant defense against pathogens and herbivores, and they also have potential applications in controlling human pathogenic infections.⁶ They can be categorized as (i) phenolic acids and (ii) flavonoid polyphenolics (such as flavonones, flavones, xanthenes, and catechins), and (iii) non-flavonoid polyphenols. Phenolics are natural antioxidants that are widely used as nutraceuticals. They are present in apples, green tea, and red wine, and are known for their strong anti-cancer properties.⁷ Additionally, they are believed to have a significant impact on preventing heart diseases and can also act as anti-inflammatory agents.⁸

Plants produce complex molecules called phytochemicals as part of their defense against diseases. These bioactive compounds include various substances such as alkaloids, tannins, flavonoids, and phenolic compounds. Historically, these compounds have been associated with medicinal properties in different plant parts like leaves, flowers, stems, roots, seeds, fruits, and bark. Research indicates that phytochemicals, particularly due to their antioxidant activity, have the potential to combat diseases.⁹

Antioxidant Activity:

Antioxidants are molecules that prevent other molecules from oxidizing. Oxidation is a chemical process where electrons are lost or oxidation state increases, potentially producing harmful free radicals. These free radicals can trigger chain reactions within cells, leading to damage or cell death. Antioxidants halt these chain reactions by neutralizing free radical intermediates and suppressing other oxidation reactions. They achieve this by being oxidized themselves, acting as reducing agents. Common antioxidants include thiols, vitamin C (ascorbic acid), and polyphenols.^{7, 10} Natural antioxidants are essential for maintaining good health and preventing chronic and degenerative diseases including cancer, coronary heart disease, and even altitude sickness. Antioxidants are also used extensively in industry, where they are used as gasoline and rubber preservatives, as well as preservatives in food and cosmetics.^{11, 12, 13}

Materials and Methods

Samples Collection: *Ocimum micranthum* and *Ocimum basilicum* samples were collected from various parts of the northern Indian states. After then, they were brought to Glocal University's Herbarium for identification.

Extract Preparation: The leaves were cleaned to get rid of any dirt, allowed to dry in the sun for seven days, then ground into a powder and kept in an airtight container in a cold, dry location. After that, another batch of leaves was gathered, rinsed, allowed to air dry, combined into a paste, packaged, and taken straight away for examination. The procedures described by Hernández et al¹⁴ were used to determine the organoleptic, phytochemical, and extraction of the active components. Vi 21 (Unican Ultra Violet Visible Spectrometry Vision 32 software) was used to characterize the extracts.

Solvent extract preparation: 50 ml of methanol is used to extract 5g of each powdered sample for 48 hours. After 48 hours the supernatant obtained was used to make the crude extract by the process of evaporation.

The preliminary phytochemical analysis was used to analyze the presence of compounds namely *Alkaloids, Flavonoids, Saponins, Tannins, Phenols* and *Quinones*.¹⁵

Quantitative Analysis for Phytochemical

Terpenoids determination: After weighing 5g of the material into 50ml of petroleum ether, it was extracted for 15 minutes, 420 nm wavelength absorbance was measured after filtering. The formation of a reddish brown precipitate allowed for the identification of terpenoids.

Flavonoids determination : 50 ml of 80% methanol was used to extract 5g, which was then filtered into a weighted Petri plate after standing for 4 hours. After being allowed to dry at 400 degrees Celsius in the oven, the Petri dish was weighed once it reached a stable weight. The development and disappearance of the yellow tint indicated the presence of flavonoids.

Alkaloids determination: A few drops of Wagner's reagent were added to 20 milliliters of 10% acetic acid in ethanol. After shaking, the sample was let to stand for four hours. After filtering, the filtrate evaporated to a volume that was around 25% of its initial volume. Concentrated ammonia was added with one drop. The precipitate was filtered through weighted paper and allowed to dry at 600 degrees Celsius in the oven. After drying to a steady weight, the filter paper was weighed (W2).

$$\% \text{Alkaloids} = \frac{W2 - W1}{W} \times 100$$

W

Where: W1 = initial weight of sample, W2 = weight of the ext (W1) filter paper. Leave the filter tract, W3 = final weight of the residue.

Saponins determination: 50 ml of 20% ethanol was added to 5g of sample, which was then weighed. The sample was then placed in a water bath at 60°C for six hours. After filtering, the residue underwent two washes with 20% ethanol, and the extract was baked down to around 5 milliliters. Within a separating funnel, 50ml of Petroleum Ether was added to the concentrated extract. Three milliliters of butanol were added to the pet ether layer, which was disposed away. Using 50 milliliters of 5% sodium chloride, the sample was cleaned. After then, the butanol was transferred to a petri dish that had been weighed, and it was baked until it evaporated completely and the residue was weighed.

Determination of Antioxidant Capacity

The DPPH scavenging was calculated according to the following equation:

$$\text{RSC \%} = (1 - \text{Asample}/\text{Ablank}) \times 100\%$$

Statistical Analysis: Microsoft Excel was used to perform the statistical analysis of the outcome. Using the non-linear regression curve, the FRAP method's antiradical activity has been estimated.

Results and Discussion

Qualitative Analysis: The numerous species of *Ocimum micranthum* and *Ocimum basilicum* plants have undergone phytochemical study by methanol extract-based phytochemical evaluation. Table 1 displays the findings of the phytochemical screening process used to look for different chemical ingredients using methanol extract. The table below displays the various constituents' presence or absence in relation to methanolic extract. All phytochemicals were discovered to be present in the methanolic extract of the *Ocimum micranthum* and *Ocimum basilicum* sample that is being studied here, with the exception of phenols. While tannins are lacking from *Ocimum micranthum* and *Ocimum basilicum*, phenols are absent from both species. However, all other components are found in both species.

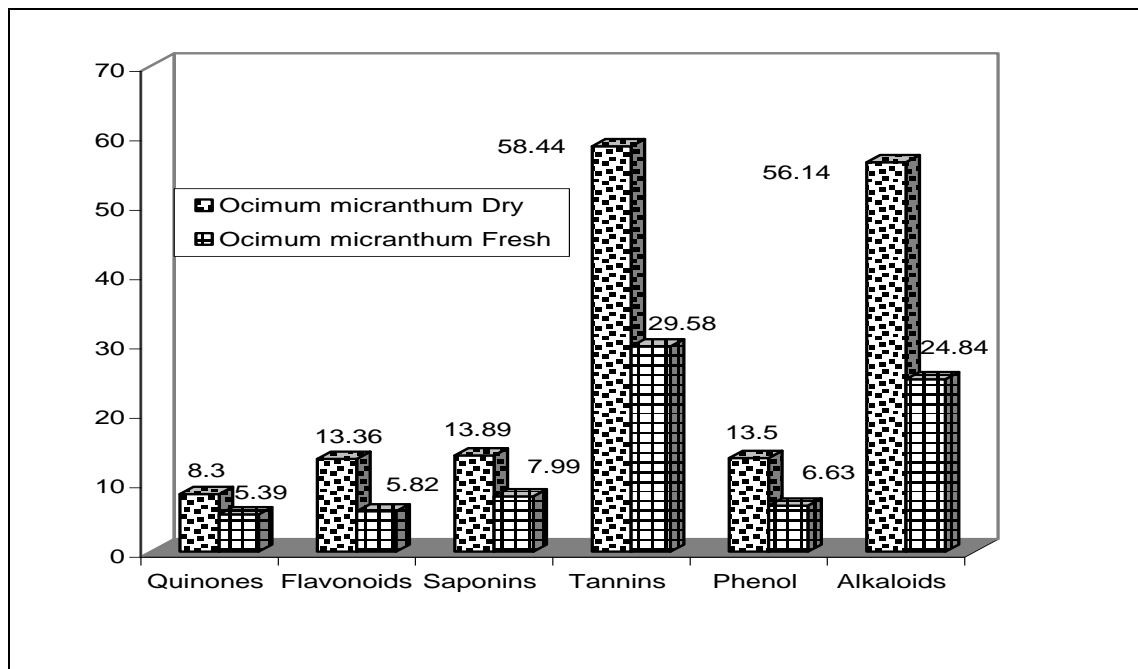
With the exception of phenols, *Ocimum micranthum* and *Ocimum basilicum* species contain significant phytochemical contents. The significant antioxidant activity of *Ocimum micranthum* and *Ocimum*

basillicum species is shown by the presence of phytochemicals such as flavonoids and saponins. These naturally occurring oxidants in phytochemicals shield essential oils from oxidative damage. Other literary works state that studies have been done on the presence of phytochemicals such as alkaloids, tannins, flavonoids, and phenolic substances.¹⁶ Several studies have demonstrated the increased antioxidant activity of these phytochemicals.^{17, 18} Our current research makes it abundantly evident that every phytochemical isolated from the methanolic extract of *Ocimum micranthum* and *Ocimum basillicum* leaves exhibited increased antioxidant activity.

Table 1: Results of the Phytochemical contents of fresh and dry leaves of *Ocimum micranthum* and *Ocimum basillicum*

Test	<i>Ocimum micranthum</i>		<i>Ocimum basillicum</i>	
	Dry	Fresh	Dry	Fresh
Quinones	8.3	5.39	10.66	7.82
Flavonoids	13.36	5.82	20.46	9.89
Saponins	13.89	7.99	16.42	10.67
Tannins	58.44	29.58	48.5	23.58
Phenol	13.5	6.63	9.7	4.38
Alkaloids	56.14	24.84	60.54	29.43

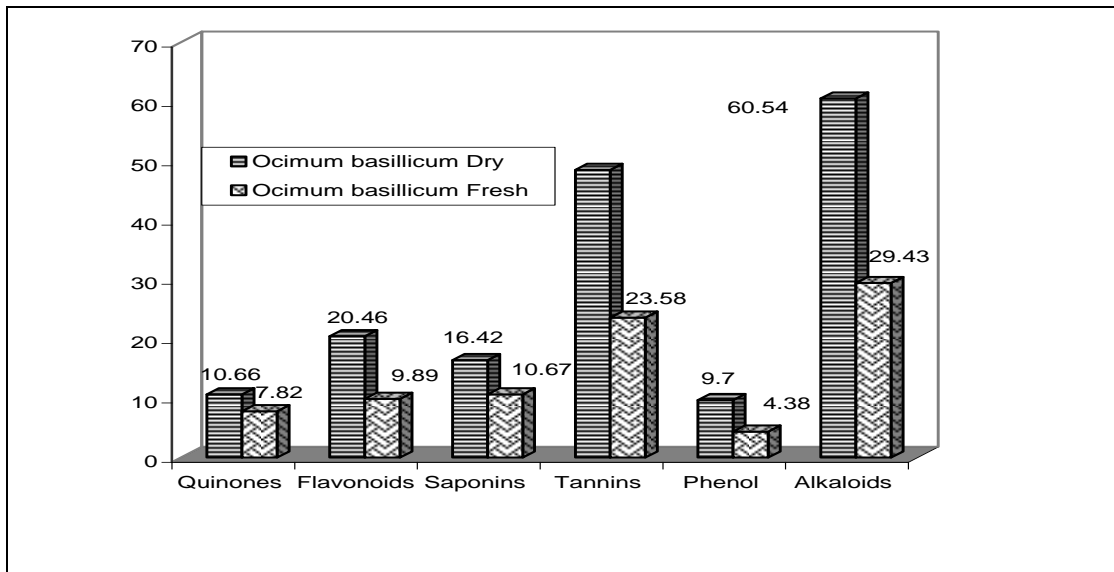
Figure- 1: Photochemical contents of fresh and Dry Leaves of *Ocimum micranthum* spp.



The findings displayed in above table demonstrated that *Ocimum micranthum* and *Ocimum basillicum* dry leaf phytochemical concentration and antioxidant activity were substantially higher than those found in their fresh leaves at $p < 0.05$ (Figure 1). The results presented in (Table-1) showed that the phytochemical concentration and antioxidant

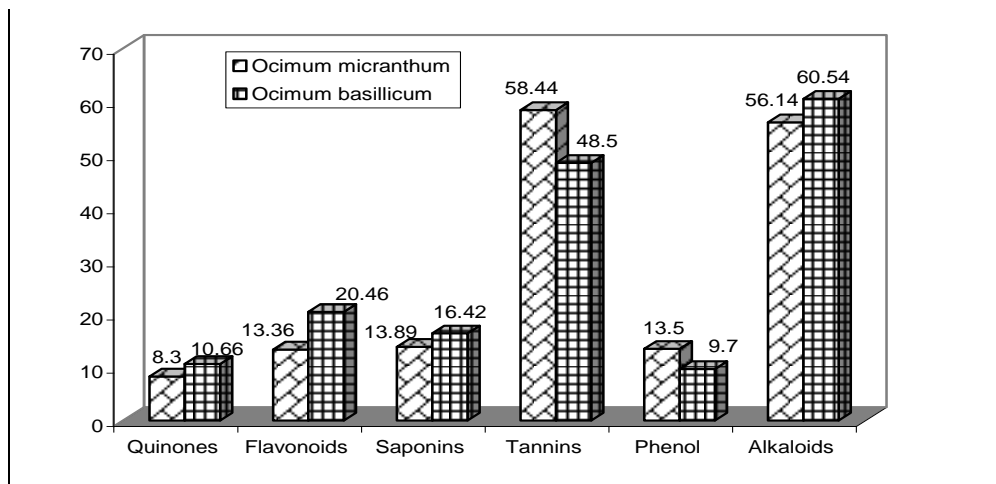
activity in the dry leaves of *Ocimum micranthum* and *Ocimum basilicum* were significantly higher than those obtained in their fresh leaves at $p < 0.05$. (Figure 1).

Figure- 2: Phytochemical Contents of Fresh and Dry Leaves of *Ocimum basilicum* spp.



This may be explained by the fact that the dry leaf extract is more concentrated per gram of sample than the fresh leaf extract due to the fresh samples' comparatively larger moisture content (Figure. 2).

Figure-3: Quantitative comparison of phytochemical contents of *Ocimum micranthum* and *Ocimum basilicum* (Dry leaves).



Additionally, compared to *Ocimum micranthum*, the dried and fresh leaf extracts from *Ocimum basilicum* have substantially larger phytochemical contents in terms of flavonoids, saponins, and alkaloids. On the other hand, *O. micranthum*'s phenolic and tannin content levels were noticeably greater. Furthermore, compared to *O. micranthum*, *P. basilicum* has greater antioxidant activity (Figure 3).

Conclusions

The phytochemical analysis conducted in this study has demonstrated the existence of biologically active substances, such as phenols, flavonoids, saponins, tannins, and alkaloids, which are known to have antioxidant activity,

in all species of *Ocimum micranthum* and *Ocimum basillicum*. The *Ocimum basillicum* has a higher quantity of phytochemicals, particularly flavonoids. The components found in ocimum plants have demonstrated a better benefit in the management of a number of illnesses. All of the ocimum's sources contain phytochemicals, and these have demonstrated increased antioxidant activity. However, due to the saponin and alkaloid content of the leaf extracts, which may produce certain adverse effects, care should be taken when administering and consuming them. Thus, it can be inferred from our current research that phytochemical components are the abundant sources of antioxidants which has a more beneficial role in the pharmacology.

The analysis of phytochemicals in the present study has proved that in all the *Ocimum micranthum* and *Ocimum basillicum* species, the presence of the phytochemicals which are known as biologically active compounds such as phenols, flavonoids, saponins, tannins, alkaloids has antioxidant activity. A higher concentration of phytochemicals is found in *Ocimum basillicum* especially flavonoids. The constituents of Ocimum plants have shown a greater advantage in the treatment of various diseases. The phytochemicals present in all the sources of Ocimum has shown higher antioxidant activity. However, caution should be taken in the intake and administration of the leaf extracts because of the saponin and alkaloid content which may induce some side effects. Therefore from our present work, it can be concluded that phytochemical components are the rich sources of antioxidants which has a more beneficial role in the pharmacology.

Recommendations: To determine the extent or degree of adverse effects related to these plants, toxicology studies should be conducted on both fresh and dry samples of their leaves. Additionally, additional research on the other plant components of these two plant species may be conducted.

Competing Interests: The authors declare that they have no competing interests.

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