



Qualitative and Quantitative Phytochemical analysis on *Ocimum basilicum* L. from Marathwada region

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ABSTRACT

Ocimum (Tulshi) is a fast-growing shrub belonging to the family Lamiaceae. The family is rich source of secondary metabolites and chemical nutrients. The various species of *Ocimum* are found in Indian tropical and subtropical regions. *Ocimum basilicum* L. is rich source of secondary metabolites, vitamins, essential oils and nutrients. Present study focus on Qualitative and Quantitative phytochemical analysis to determine the presence of phytochemical compounds such as Alkaloids, Flavonoids, Saponins, Tannins, Phenols, Proteins, Carbohydrates, by using standard methods in the different extracts of *Ocimum* leaves powder. The secondary metabolites obtained from these plants act as natural antioxidants which have medicinal values. In our present study, Qualitative and Quantitative phytochemical analysis has been done in the leaves of *Ocimum basilicum* L. plants which is potentially used in the Cosmetics, drug industry, pharmacology at greater scale.

Keywords- *Ocimum*, Phytochemical, Qualitative, Quantitative analysis, Metabolites etc.

1. INTRODUCTION

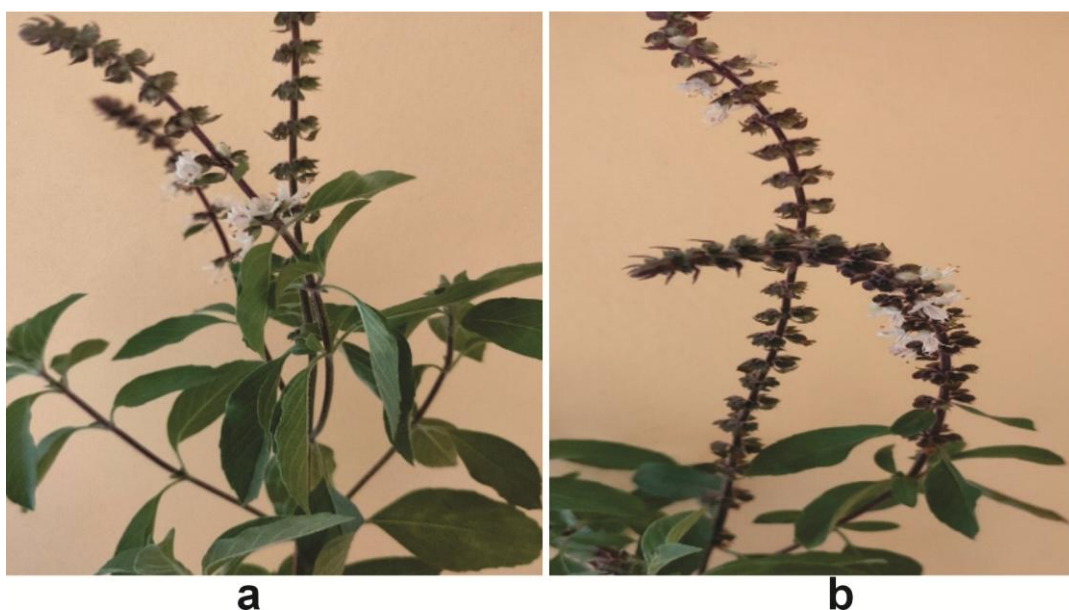
India has high diversity, especially in tropical/subtropical regions, with both wild and cultivated populations. The genus *Ocimum* (family Lamiaceae), commonly known as basil or tulsi, includes several species native or widely found/cultivated in India. India is a major centre of diversity for this genus, with both indigenous and introduced species. Estimates suggest around 9–10 or more species occur in the country (some sources cite up to ~68 historically reported, though many are synonyms or less common), but the most prominent and well-documented ones are listed below. These are frequently mentioned in Indian flora, ethnobotany, research, and cultivation contexts: *Ocimum tenuiflorum* L.- Holy Basil or Tulsi, *Ocimum basilicum* L. - Sweet Basil, *Ocimum gratissimum* L.- Clove Basil, *Ocimum americanum* L. -Hoary Basil or Kali Tulsi, *Ocimum africanum* L.-African Basil, *Ocimum kilimandscharicum* L. -Camphor Basil etc. Planted in most Hindu

households. Many species have essential oils rich in eugenol, linalool, camphor, etc., used in Ayurveda, aromatherapy, and as repellents/antimicrobials. *Ocimum basilicum* L., commonly known as Sweet Basil or simply Basil, is one of the most widely recognized and cultivated species in the genus *Ocimum*. It originated in regions including India, Persia (Iran), and tropical Asia, and has a long history in India. Native to India (especially lower hills of Punjab and surrounding areas) and widely naturalized/cultivated across the country. It's both wild and grown commercially. *Ocimum basilicum* L. is annual or short-lived perennial herb, growing 30–60 cm tall. Leaves are ovate-oblong, 3–6 cm long, glossy green (sometimes purple in varieties), with a characteristic sweet, clove-like aroma due to compounds like linalool and eugenol. It is cultivated in India mainly in states like Uttar Pradesh, Punjab, Haryana, and parts of Maharashtra. Thrives in warm, humid climates with well-drained loamy soil (pH 6–7.5). Sown from August–October (post-monsoon) or spring; direct seeding or transplanting. Commercial for essential oil production (rich in linalool), dried leaves, or fresh culinary use. India produces significant quantities for domestic and export markets. Sometimes co-cultivated with food crops like maize, pulses, or vegetables to boost farm income and efficient land use. Medicinal/Ayurvedic: Used for headaches, coughs, digestion, antimicrobial properties. Essential oil has applications in perfumes, cosmetics, and as a natural repellent. Other: Ornamental in gardens; some traditional beliefs link it to spiritual uses. *Ocimum basilicum* L. is rich in

secondary metabolites, which are non-essential compounds produced by the plant that contribute to its aroma, flavor, medicinal properties, defence against pests/pathogens, and antioxidant activity. These metabolites vary by chemotype (genetic variety), growing conditions (soil, climate, light, elicitors), phenological stage, and extraction method.

Major Classes of Secondary Metabolites in *O. basilicum*

L.- Essential Oil Components (Volatile terpenoids and phenylpropanoids). These make up the bulk of basil's characteristic scent and bioactivity. The essential oil yield is typically 0.2–1.5% (dry weight), higher in leaves. Common major compounds (varying by chemotype): Linalool (often 30–77% in sweet European/Indian types; floral, fresh scent) Methyl chavicol (estragole; 20–50% in some varieties; anise-like), Methyl eugenol (up to 39% in some Indian samples), Eugenol (clove-like; 5–15%, common in purple-leaved or spicy types), 1,8-Cineole (eucalyptol; 5–20%), Others: β -caryophyllene, α -pinene, β -pinene, ocimene, geraniol, nerol, limonene, camphor (in camphor types). In Indian contexts (e.g., Western Ghats/North India), methyl eugenol and methyl chavicol often dominate, while linalool is prominent in many cultivated varieties. **Phenolic Compounds (Non-volatile, antioxidant-rich),** Rosmarinic acid -one of the most abundant and bioactive; responsible for much of basil's strong antioxidant capacity (higher than many herbs). Levels increase under stress or elicitors. Caffeic acid derivatives, flavonoids (e.g., quercetin, kaempferol glycosides), tannins, and phenolic acids.



Photoplate 1: a-*Ocimum basilicum* L. plant, b-*Ocimum basilicum* L. inflorescence

Other Phytochemicals, Flavonoids (e.g., orientin, vicenin), Alkaloids (minor), Saponins, tannins, cardiac glycosides (reported in roots/leaves/seeds in some studies), Terpenoids beyond volatiles (e.g., triterpenes), Factors Influencing Secondary Metabolites in India- Chemotypes vary regionally; Indian-grown basil often shows higher methyl chavicol/eugenol in some areas (e.g., Karnataka, North India), while linalool dominates in others. Elicitors (e.g., methyl jasmonate, salicylic acid, yeast extract) or stresses (UV, plasma treatment, light quality) boost production - studies show increases in rosmarinic acid, eugenol, and volatiles. In Maharashtra, basil is grown commercially, with potential for high metabolite content in warm, semi-arid conditions with proper management.

MATERIALS AND METHODS

1. Qualitative Phytochemicals Analysis

The plant of *Ocimum basilicum* L. species samples were obtained from Dr. Babasaheb Ambedkar Marathwada University Chhatrapati Sambhajinagar, Maharashtra. The Plant identified authentically and voucher specie's deposited at Department of Botany. Fresh leaves were cleaned with distilled water, dried in shade and made fine powder with mixer and put in air tight bottle for further use.

Solvent Extract preparation- 50 ml Ethanol is used to extract 10 gm of powdered sample by soxhlet method for 3 to 4 hours. After that supernatant was used to make crude extract by rotary evaporator process. The preliminary phytochemicals analysis was used to analyse presence of phytochemicals such as Phenols, Proteins, Alkaloids, Flavonoids, Tannins, Carbohydrates, Saponins, Glycosides, Terpenoids etc.

Determination of phenols 1ml of 5% aqueous ferric chloride was added to 1ml of ethanol extract, Phenols were determined by the appearance of blue colour in the extract.

Determination of proteins 1ml of 40% NaoH and a few drops of 1% copper sulphate was added to 2ml of ethanol extract. The peptide linkage molecule in the extracts were determined by the appearance of violet colour.

Determination of alkaloids Wagner's reagent were added in few drops to 2ml of ethanol extracts, the

reddish brown precipitates observed the presence of alkaloids.

Determination of flavonoids: The yellowish colour was appears when 20% of NaOH was added in few drops to 2ml of ethanol extract. To this, 70% dilute HCl was added in few drops and the yellow colour disappeared. The flavonoids presence were determined by the formation and disappearance of yellowish colour.

Determination of tannins Alcoholic Ferric chloride was added in the concentration of about 10% to 1ml of ethanol extract, Tannins were determined by the appearance of blue or black colour.

Determination of carbohydrates Molisch's reagent were added in few drops and 1ml of concentrated sulphuric acid was added along the sides of the test tubes to 1ml of extract. The mixture was allowed to stand for 2-3minutes. The appearance of red or dull violet colour determined the presence of carbohydrates in the extract.

Determination of Saponins 2ml of ethanol extract was mixed with 6ml of distilled determined by the appearance of yellow precipitate. water and shaken vigorously. Saponins were determined by the appearance of bubbles or foam.

Determination of cardiac glycosides 0.5ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride was added to 1ml of ethanol extract. The cardiac glycosides in the extract were determined by the appearance of brown ring at the interface.

Determination of Terpenoids 0.5ml of chloroform and a few drops of concentrated sulphuric acid was added to 1ml of ethanol extract. The terpenoids were determined by the appearance of reddish brown precipitate.

Result and Discussion of Qualitative Analysis:

The phytochemical analysis of species *Ocimum basilicum* L. plants by subjecting to various phytochemical evaluation using ethanol extract. The results of the phytochemical screening carried out for various chemical constituents with ethanol extract is shown in Table 1. The presence or absence of different constituents with respect to ethanollic extract is shown in the below table. All phytochemicals were

found to be present in ethanolic extract of *O. basilicum* L. species sample which is selected to study here. *O. basilicum* L. species have high phytochemicals content. Presence of phytochemicals like flavonoids, saponins, tannins in *O. basilicum* L. species indicates the high antioxidant activity. These phytochemicals are natural oxidants which prevent essential oils from oxidative stress. According to other works of literature, the presence of phytochemicals like alkaloids, tannins, flavonoids, and phenolic compounds has been studied (Hill, 1952). and it has been shown that these phytochemicals have higher antioxidant activity (Farombi et al, 1998; Halliwell et al., 1992). From our present work, it is clear that all the phytochemicals extracted from ethanolic extract of leaves of *O. basilicum* L. species showed higher antioxidant activity.

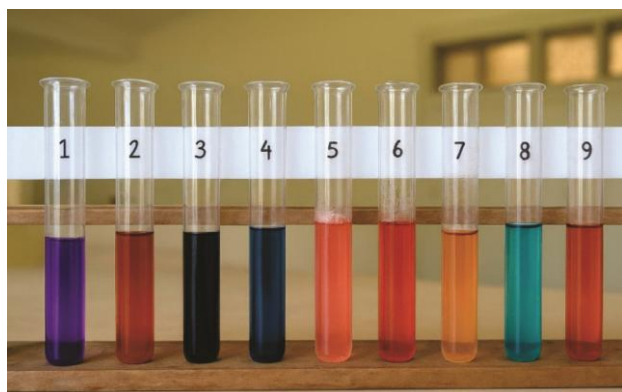


Photo2: Qualitative Phytochemicals screening test of *Ocimum basilicum* L.

Table 1. Qualitative Phytochemicals analysis of *Ocimum basilicum* L.

Sr. No.	Test	Observation	Results
1.	Alkaloids	Red or dull violet color	++++
2.	Carbohydrates	Blue/green	++
3.	Flavonoids	Purple/Violet color	+++
4.	Phenols	Blue color	+++
5.	Protein	Pink or red color	++
6.	Saponins	Light orange/Amber color	+
7.	Steroids	Reddish brown ppt	++
8.	Tannin	Blue or black color	+++
9.	Terpenoids	Reddish Brown color	++

2. Quantitative Phytochemicals Analysis

Materials and Methods:

The quantitative assay was done based on the results of qualitative method for the determination of Alkaloids, Tannins, Phenols, Flavonoids, Saponins, Proteins and Carbohydrates.

Total Phenol content Determination:

The most common and widely accepted method for determining total phenolic content (TPC) in plant extracts is the Folin-Ciocalteu (F-C) colorimetric method. In a test tube, added 20 μ L sample (standard or blank = water) 1.5 mL distilled water, 100 μ L Folin-Ciocalteu reagent mixed properly and incubated for 8 min at room temperature. Added 300 μ L 7.5–10% Na_2CO_3 solution. Mixed and incubated in the dark for 2 hours at room temperature ($\sim 20\text{--}25^\circ\text{C}$). After that measured absorbance at 765 nm against blank. Repeated triplicates for all standards and samples. Results are typically expressed as mg gallic acid equivalents (GAE) per gram or per liter of sample.

Total Alkaloid content determination: 1gm of a powdered sample, 40ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. The filtrate is concentrated to 1/4th of its original volume by using the water bath. The concentrated ammonium hydroxide is added drop wise to the extract until the precipitation is completed. The solution is allowed to settle. The dilute ammonium hydroxide is used to wash the collected precipitate and then filtered. The residue was dried and weighed.

Total Flavonoids Content Estimation: 5ml of 2% AlCl_3 prepared in ethanol was mixed with the same volume and mixed with the same volume of extract solution. After 10 min, absorbance was taken at 415nm against blank. Blank was prepared as 5 ml of extract mixed with 5 ml of ethanol without AlCl_3 . Catechin was used to prepare a standard graph.

Total Tannins Content Determination: Folin and Ciocalteu method was slightly modified for the determination of Tannins. 7.5ml of distilled water, 0.5ml of Folin Ciocalteu reagent, 1ml of 35% sodium carbonate solution was added to 1ml of sample extract. The absorbance was read by 725nm. 0 to 0.5mg/ml of Tannic acid dilutions were used as standard solutions. The Tannic acid is expressed in

terms of mg/ml of extract to determine the results of tannin.

Total Saponins Content Estimation: 20ml of 20% ethanol was used to disperse 2g of each sample. The suspension is heated in the hot water bath for 4h at about 55°C with continuous stirring. The mixture is filtered and 20ml of 20% ethanol is used to re-extract the residue. The water bath at about 90°C was employed to reduce the combined extracts to 40ml. To the 250 ml separatory funnel, the concentrate was transferred and 2ml of diethyl ether was added and shaken vigorously. The purification process is repeated after recovering the aqueous layer. 6ml of n-butanol was added and the combined n-butanol extracts were washed twice by using 1ml of 5% aqueous sodium chloride. The water bath was used to heat the remaining solution. The samples were dried in the oven to a constant weight by the process of evaporation.

RESULTS AND DISCUSSION

Phenols: Phenols are the organic compounds consisting of Hydroxyl (OH) group attached to a carbon atom that is part of an aromatic ring. They are used as intermediates in household products and for industrial synthesis. Phenols are used in the manufacture of plastics, explosives such as picric acid, drugs such as aspirin in the industry as a raw material. The essential oils of plants consists of complex phenols which are used as flavourings and aromas. In this study, the phenol content of *Ocimum basilicum* sample extracts was in the range of 33 to 37mgGAE/g.

Alkaloids: They are naturally occurring organic compounds containing basic nitrogen atoms. It exhibits medicinal properties including antimalarial, antiasthma anticancer. Most alkaloids have a bitter taste when ingested. In the present study, *Ocimum basilicum* have higher concentration is 16mg/g.

Flavonoids: The plant-derived products consists of naturally occurring polyphenolic compounds known as Flavonoids. The antioxidant effects associated with various diseases such as cancer, Alzheimer's disease, atherosclerosis are present in flavonoids (Lee et al., 2009; Ovando et al., 2009; Burak & Imen, 1999). The attractive colors of flowers, fruits, and leaves are due the presence of flavonoids. A variety of fruits and vegetables, including tea, coffee, and other grains

consists of flavonoids like Quercetin and rutin. It is reported that flavonoids have a preventive effect on cancer, anti-inflammatory, antiviral activities. In the present study, *Ocimum basilicum* showed the highest flavonoid content is 14mgQE/g

Tannins: Many species of plants consists of polyphenolic biomolecules such as Tannins which play a important role in protecting from predation and regulates plant growth (Katie E. Ferrell et al., 2006). Martin and Synge discovered the paper chromatography methods for the first time for determining the phenolic constituents a highest tannins content was found in *O. basilicum* L.. The leaf, bud, seed, root and stem tissues such as secondary phloem and xylem contains Tannins which regulate the growth of these tissues. The present study revealed that *O. basilicum* L. has the concentration 7mgTAE/g of tannins, Therefore *O. basilicum* L. showed a higher concentration of tannins, saponins, and flavonoids compared to other species of *Ocimum*. In other research studies, it has been reported that age, geographical conditions are the factors to determine the phytochemical contents.

Saponin: Saponins are present in the various parts of the plant leaves, stems, roots, bulbs, blossom, and fruit. Saponins resulting in highly astringent properties. Saponins contains nitrogen-free glycosides such as a sapogenin and a sugar. The saponin may be a steroid or a triterpene and the sugar moiety is glucose, galactose, pentose or methyl pentose (Stecher et al., 1960). In our study Saponins are found in a lower concentration of 0.46mg/g in *O. basilicum* L..

Table2. Total Phenol, Alkaloid, Flavonoid, Tannin and Saponin Content in Ethanolic Extract:

Sr. No.	Ethanolic Extract	Conc. Per 100mg of extract
1.	Total Phenol Content	33 to 37 mgGAE/g.
2.	Total Alkaloids content	16mg/g.
3.	Total Flavonoid Content	14mg QE/g
4.	Total Tannin Content	7mg TAE/g
5.	Total Saponin Content	0.46mg/g

CONCLUSIONS

Phytochemical analysis revealed the presence of alkaloids, phenols, flavonoids, saponins, and tannins in

O. basilicum L. with notable concentrations of phenolics and flavonoids contributing to its strong antioxidant activity. The quantitative analysis of *O. basilicum* L. shows the revealed that presence of total phenolic and flavonoids contents, also total tannins and alkaloids content contributes the antioxidant activities. These compounds support various effects, including anti-inflammatory, antimicrobial, hepatoprotective, and antitumor activities.

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Conflict of interest

The authors declare no competing interests.

Data Availability Statement: Not applicable.

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