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Quantitative Analysis of Biochemical Constituents in Tulsi (*Ocimum sanctum* L.)

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Abstract

Tulsi (Ocimum sanctum L.), commonly known as Holy Basil, is an important medicinal plant widely used in traditional Indian medicine. The present study was conducted to evaluate the biochemical composition of Tulsi leaves and assess their medicinal significance. Fresh leaf samples were analysed for various biochemical parameters, including chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, lipids, phenols, reducing sugars, and alkaloids using standard laboratory methods. The results revealed the presence of appreciable amounts of these biochemical constituents, indicating the rich nutritional and medicinal value of Tulsi. Chlorophyll and carotenoid contents reflected the healthy physiological status of the plant, while phenols and alkaloids highlighted its strong antioxidant and therapeutic potential. The presence of lipids and reducing sugars further demonstrated its metabolic and biochemical importance. The study concludes that Tulsi is a biochemically rich medicinal plant with significant pharmaceutical and nutritional value. These findings support its traditional use in herbal medicine and provide a basis for future research on phytochemical analysis, drug development, and the formulation of herbal healthcare products.

Keywords: *Ocimum sanctum, Tulsi, antioxidant, pharmacological activities.*

Introduction

Tulsi (*Ocimum sanctum* L.), commonly known as Holy Basil, is one of the most important medicinal plants in India. The name *Tulsi* is derived from the Sanskrit word meaning "the matchless one" or "the incomparable," reflecting its esteemed status in traditional healthcare systems [1]. Besides its prominent role in Ayurveda, Tulsi has also been used in the Greek, Roman, and Unani systems of medicine for its wide range of therapeutic properties [2].

Tulsi is a perennial aromatic herb belonging to the family Lamiaceae and is widely cultivated in tropical and subtropical regions [3]. The plant grows erect with several branches and attains a height of approximately 30–60 cm at maturity. Its leaves are simple, opposite, elliptic to oblong in shape, with entire or slightly serrated margins. The leaves possess a characteristic aroma due to the presence of essential oils rich in bioactive compounds [4]. The flowers are small, purplish in colour, and borne on elongated racemes, while the fruits contain small reddish-yellow seeds [3].

Medicinal plants serve as a valuable source of bioactive compounds used in healthcare and drug development. Among them, species of the genus *Ocimum* are particularly important because of their antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and immunomodulatory activities [1]. *Ocimum sanctum* mainly occurs in two varieties: Krishna Tulsi, characterised by purple leaves, and Rama Tulsi, which possesses green leaves [3]. Owing to its rich phytochemical composition and therapeutic potential, Tulsi continues to be extensively studied for its medicinal and pharmaceutical applications [2].

Methodology

The present study was conducted on the campus of Starex University, Binola Village, Gurugram District, Haryana, India.

Plant Material

Fresh leaves of Tulsi (*Ocimum tenuiflorum* L.) were used for the biochemical analysis.

Selection and Collection of Leaf Samples

Healthy, mature leaves free from disease, insect damage, and discoloration were selected to ensure uniformity and reliability of the results. Leaves were collected from plants of the same variety to minimize variation.

Sampling was carried out during the early morning hours when metabolic activity and moisture content were relatively stable. The leaves were harvested using clean hands or sterilized scissors and immediately transferred to clean polythene bags for transportation to the laboratory. Wet leaves and leaves collected immediately after rainfall were avoided to prevent interference with biochemical estimations.

Biochemical Analysis

The collected leaf samples were analyzed under laboratory conditions for the estimation of important biochemical constituents. The parameters studied included:

- Chlorophyll (*a*, *b*, and total chlorophyll)
- Total sugars
- Reducing sugars
- Total carbohydrates
- Lipids
- Carotenoids
- Alkaloids
- Phenols

Standard biochemical methods were employed to determine the concentration of these constituents and to evaluate the nutritional and medicinal significance of Tulsi leaves.

Materials Required

Instruments

Test tubes, conical flasks, beakers, measuring cylinder, cuvettes, laboratory droppers, spatula, filter paper, hand gloves, face mask, mortar and pestle.

Chemicals Required

Distilled water, calcium carbonate, petroleum ether, sulphuric acid, 80% acetone, 80% ethanol, methanol, gallic acid, glucose solution, acetic acid, Benedict's reagent, and Anthrone reagent.

Biochemical Estimation Methods

1. Estimation of Chlorophyll

Chlorophyll content was estimated by extracting fresh Tulsi leaves (0.5 g) with 80% acetone in the presence of calcium carbonate [5]. The extract was centrifuged, and the supernatant was collected and made up to a known volume. Absorbance was measured at 663 nm and 645 nm using a spectrophotometer. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents were calculated using standard equations.

2. Estimation of Carotenoids

Carotenoids were extracted from fresh Tulsi leaves using 80% acetone [5]. The extract was centrifuged to obtain a clear supernatant, and absorbance was recorded at 480 nm, 663 nm, and 645 nm. The carotenoid content was calculated using standard spectrophotometric formulae.

3. Estimation of Total Lipids

Total lipids were estimated by solvent extraction. Dried Tulsi leaf powder (0.5 g) was treated with petroleum ether and heated gently to facilitate lipid extraction [6]. The extract was filtered, and the solvent was evaporated on a water bath. The residue obtained after evaporation represented the lipid content of the sample.

4. Estimation of Total Sugars

Total sugar content was determined using Benedict's reagent [7]. Fresh leaf extract was prepared by grinding leaves in distilled water followed by centrifugation. The extract was mixed with Benedict's reagent and heated in a boiling water bath. The development of colour from blue to red indicated the presence of sugars, and the intensity of the colour corresponded to the sugar concentration.

5. Estimation of Total Carbohydrates

Total carbohydrate content was estimated by the Anthrone method [8]. Fresh leaf extract was reacted with Anthrone reagent in the presence of concentrated sulphuric acid and heated in a water bath. A green-coloured complex was formed, and its absorbance was measured spectrophotometrically. The carbohydrate concentration was determined using a glucose standard curve.

6. Estimation of Total Phenols

Total phenolic content was estimated using the Folin–Ciocalteu method [8]. Methanolic leaf extract was reacted with Folin–Ciocalteu reagent and sodium carbonate solution. After incubation, a blue-coloured complex developed, and absorbance was measured at 765 nm using a spectrophotometer. The phenolic content was quantified using gallic acid as the standard.

7. Estimation of Alkaloids

Alkaloid content was estimated by extracting dried Tulsi leaf powder with acidic ethanol [8]. The extract was filtered and concentrated, followed by precipitation of alkaloids using ammonium hydroxide. The precipitate was collected, dried, and weighed. The alkaloid content was expressed as a percentage of the dry weight of the sample.

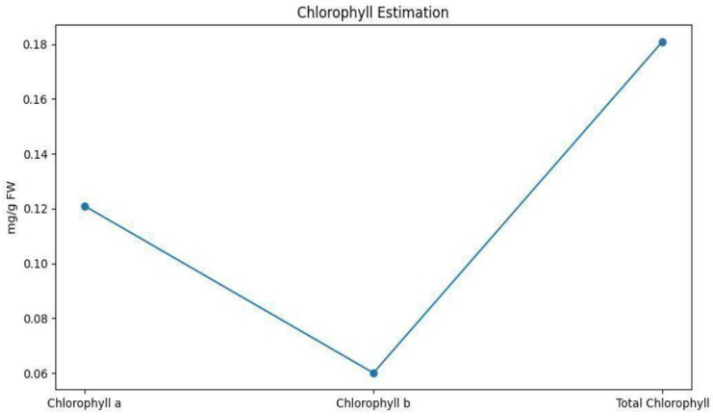
8. Estimation of Reducing Sugars

Reducing sugar content was estimated using the 3,5-dinitrosalicylic acid (DNS) method [7]. Fresh Tulsi leaves (1 g) were washed, homogenized with distilled water, and filtered to obtain a clear extract. The extract was reacted with DNS reagent and heated in a boiling water bath, resulting in the formation of an orange-red coloured complex. The intensity of the colour, measured spectrophotometrically at 540 nm, was directly proportional to the concentration of reducing sugars present in the sample. Glucose was used as the standard for quantification.

Results

1. Chlorophyll Content

The chlorophyll pigments of the Tulsi leaf sample were estimated spectrophotometrically. The chlorophyll *a* content (0.121 mg g^{-1} fresh weight [FW]) was higher than the chlorophyll *b* content (0.060 mg g^{-1} FW), while the total chlorophyll content was 0.181 mg g^{-1} FW. The higher concentration of chlorophyll *a* indicates that it is the primary photosynthetic pigment responsible for light absorption and the conversion of light energy into chemical energy during photosynthesis. Chlorophyll *b* functions as an accessory pigment by expanding the range of light wavelengths absorbed and transferring the captured energy to chlorophyll *a*, thereby enhancing photosynthetic efficiency.



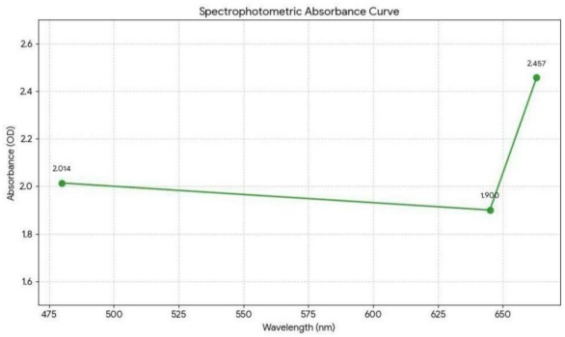
Graph 1: Estimation of Total chlorophyll of Ocimumsanctum

2. Carotenoid Content

The carotenoid content of the Tulsi leaf sample was estimated to be 0.0216 mg g^{-1} fresh weight (FW). Carotenoids are important accessory pigments that play a vital role in photosynthesis by absorbing light energy and transferring it to chlorophyll molecules. In addition, they protect the photosynthetic apparatus from oxidative damage by scavenging reactive oxygen species generated under environmental stress. The presence of carotenoids in the leaf sample indicates an efficient antioxidant defense system and contributes to the plant's physiological stability and stress tolerance.

Spectrophotometric Absorbance Values

Wavelength (nm)	Absorbance
480	2.014
663	2.457
645	1.900



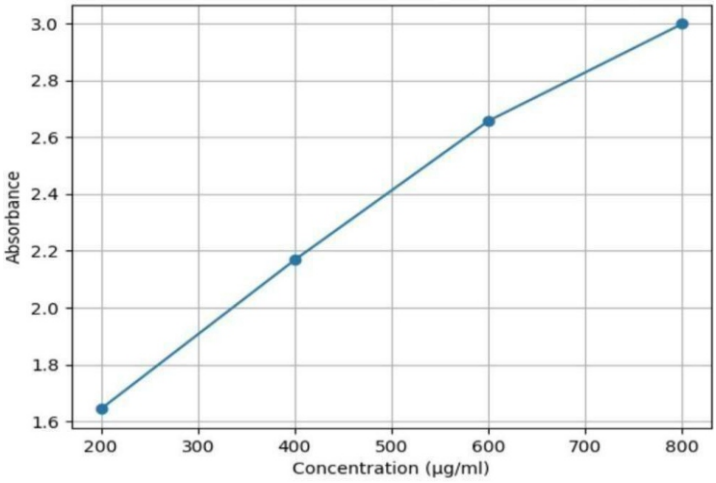
Graph 2: Estimation of Carotenoid Content in Ocimum sanctum

3. Total Carbohydrate Content

Carbohydrates are the primary products of photosynthesis and serve as the principal source of energy in living organisms. In the present study, the total carbohydrate content of the Tulsi leaf sample was found to be 0.8 g g^{-1}, indicating a relatively high carbohydrate concentration. This substantial carbohydrate content contributes to the nutritional value of the plant and reflects its active photosynthetic metabolism. Besides serving as energy reserves, carbohydrates also perform essential structural functions as constituents of cellulose, hemicellulose, and other polysaccharides that maintain cell wall integrity and support plant growth and development.

Spectrophotometric Measurement

- **Wavelength:** 620 nm
- **Blank absorbance:** 0.000



Graph 3: Estimation of Carbohydrate in Ocimumsanctum

Figure 2: Anthrone Test for Total Carbohydrate Estimation in Ocimum sanctum

The figure shows the Anthrone assay used for the estimation of total carbohydrates in *Ocimum sanctum* leaf extract. The green-coloured test tubes contain glucose, distilled water, and Anthrone reagent, representing the standard reaction. In contrast, the blackish-green coloured test tube contains glucose, distilled water, Anthrone reagent, and concentrated sulphuric acid, resulting in the development of the characteristic coloured complex used for spectrophotometric estimation of total carbohydrates. The intensity of the colour is directly proportional to the carbohydrate concentration in the sample.

Table 5: Absorbance of Carbohydrate

GLUCOSE	ABSORBANCE
0.2	1.643
0.4	2.168
0.6	2.658
0.8	3.000

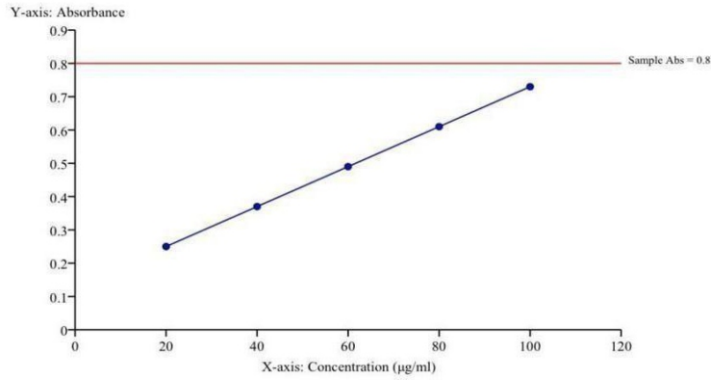
4. Total Phenol Content

The total phenolic content of the *Ocimum sanctum* leaf sample was estimated to be 2.24 mg g^{-1}. Phenolic compounds are important secondary metabolites that possess strong antioxidant, antimicrobial, anti-inflammatory, and therapeutic properties. The presence of an appreciable amount of phenols indicates the significant medicinal potential of Tulsi. These compounds play a crucial role in protecting plants against oxidative stress, microbial infections, ultraviolet radiation, and other environmental stresses. Furthermore, phenolic compounds contribute to the pharmacological activities of Tulsi and are largely responsible for its traditional use in herbal medicine due to their ability to neutralize free radicals and enhance the plant's natural defense mechanisms.

Table 6: Absorbance of Phenol

Concentration[ug\ml]	Absorbance
20	0.25
40	0.37
60	0.49
80	0.61
100	0.73

X-axis: Concentration of Gallic Acid Standard (µg/ml)
Y-axis: Absorbance at 765 nm



Graph 4: Estimation of Phenolin Ocimum sanctum

5. Reducing Sugar Content

Reducing sugars are simple carbohydrates that act as reducing agents because they possess free aldehyde or ketone functional groups. Common reducing sugars include glucose, fructose, lactose, and maltose. In the present investigation, the reducing sugar content of the *Ocimum sanctum* leaf sample was estimated to be 6.8 mg g^{-1}.

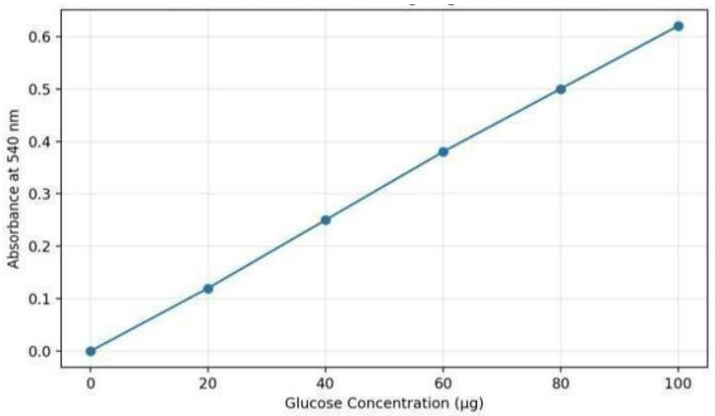
During the qualitative analysis, the development of a green colour following the reaction with the reagent indicated the presence of reducing sugars in low to moderate concentration. Reducing sugars are important intermediates in plant metabolism and contribute to cellular respiration, energy production, and various biosynthetic processes.

Spectrophotometric Reading

- Sample absorbance: 0.43

Table 7: Absorbance of Reducing sugar

SUGAR[ug]	ABSORBANCE
0	0.00
20	0.12
40	0.25
60	0.38
80	0.50
100	0.62



Graph 5: Estimation of Reducing sugar in Ocimum sanctum

6. Alkaloid Content

Qualitative analysis of alkaloids in the *Ocimum sanctum* leaf extract was carried out using Mayer's test, Wagner's test, and Dragendorff's test. The observations confirmed the presence of alkaloids in the leaf sample, indicating that Tulsi contains important nitrogen-containing secondary metabolites. Alkaloids are biologically active compounds known for their diverse pharmacological properties, including antimicrobial, anti-inflammatory, analgesic, antioxidant, antipyretic, and immunomodulatory activities. The presence of alkaloids contributes significantly to the medicinal value of *Ocimum sanctum* and supports its widespread use in traditional herbal medicine for the prevention and treatment of various diseases.

Table 8: Observations of Mayer's, Wagner's, and Dragendorff's Tests for Alkaloids in Ocimum sanctum

Testname	Observation	Inference
Mayer'stest	Cream ppt	Positive
Wagner's test	Reddishbrownppt	Positive
Drafendorffstest	Orangeppt	Positive

Test	Observation	Inference
Mayer's Test	Cream or pale yellow precipitate observed	Alkaloids present (+)
Wagner's Test	Reddish-brown precipitate observed	Alkaloids present (+)
Dragendorff's Test	Orange to reddish-orange precipitate observed	Alkaloids present (+)

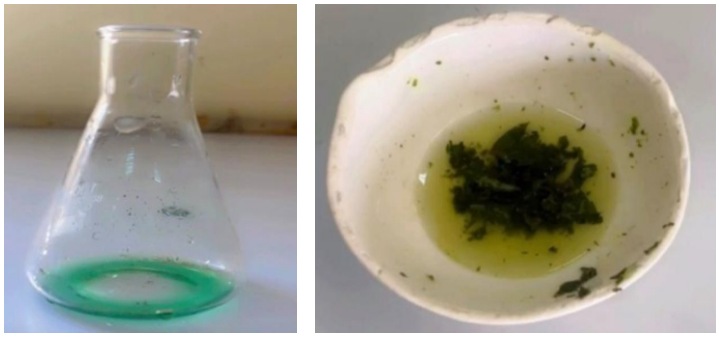
7. Estimation of Total Sugar

The total sugar content of the Tulsi (*Ocimum sanctum*) leaf was analyzed using a qualitative biochemical test. During the experiment, the development of a green colour was observed in the reaction mixture. The appearance of the green colour indicates the presence of a low concentration of total sugars in the leaf sample.

This result confirms that Tulsi leaves contain soluble sugars, although in comparatively small amounts. Sugars are important primary metabolites that serve as an immediate source of energy and participate in several physiological processes, including respiration, growth, photosynthesis, and the biosynthesis of various organic compounds. The presence of total sugars also reflects the metabolic activity and nutritional value of the plant.

Observation: Development of green colour.

Inference: The green colour indicates the presence of a low concentration of total sugars in the Tulsi leaf sample.



A] Green colour observed in sample B]distilled water solution

8. Lipid Content

The total lipid content of the *Ocimum sanctum* leaf sample was estimated to be **0.01 mg g^{-1} fresh weight (FW)**, indicating a comparatively low lipid concentration. Although Tulsi is not considered a major source of lipids, the presence of small amounts of these compounds is biologically important. Lipids are essential constituents of cellular membranes and play critical roles in maintaining membrane integrity, energy storage, signal transduction, and various metabolic processes. They also serve as precursors for several bioactive molecules involved in plant growth, development, and responses to environmental stress. Thus, even at low concentrations, lipids contribute significantly to the physiological and biochemical functions of the plant.

Image 4: Comparison of Tulsi extract with citrus and Sadabhar extract

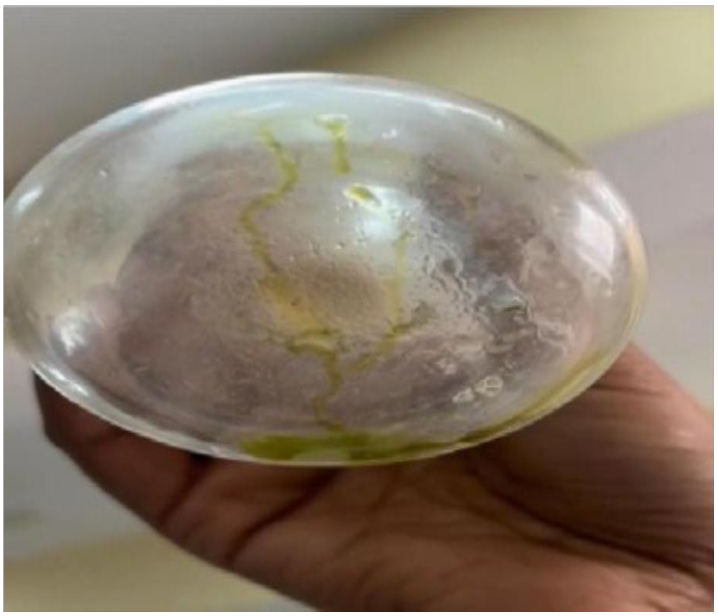
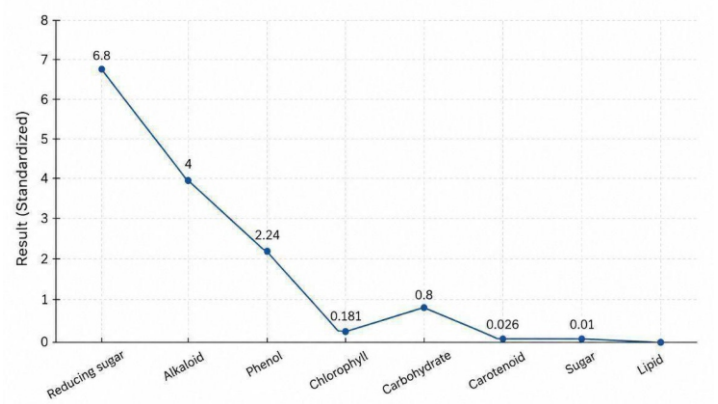


Image 5: Extracted lipid [oil] after solvent evaporation

Image 6: grinded Tulsi powder



Graph 6: Comparative Analysis of Biochemical Constituents in Ocimum sanctum

Graph 6 presents the comparative distribution of the major biochemical constituents analyzed in the *Ocimum sanctum* leaf sample. The estimated parameters include chlorophyll, carotenoids, carbohydrates, total sugars, reducing sugars, lipids, phenols, and alkaloids. The graph illustrates the variation in the concentration of these primary and secondary metabolites, reflecting the biochemical composition and metabolic status of the plant.

The observed differences in the concentrations of these constituents indicate their diverse physiological roles in plant growth, photosynthesis, energy metabolism, stress tolerance, and defense mechanisms. Primary metabolites, such as carbohydrates and sugars, are essential for energy production and cellular metabolism, whereas secondary metabolites, including phenols and alkaloids, contribute significantly to the antioxidant, antimicrobial, and therapeutic properties of Tulsi.

The inclusion of standard deviation (SD) values in the graph demonstrates the precision and reproducibility of the experimental measurements. The relatively small variation among replicates indicates the reliability of the analytical methods and supports the accuracy of the biochemical estimations. Overall, the graphical representation provides a comprehensive overview of the biochemical profile of *Ocimum sanctum*, highlighting its nutritional and medicinal significance.

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