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Comparative Analyses of Biochemical Parameters in Selected Citrus Species *(Citrus limon)*

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Abstract

Citrus limon leaves are a rich source of primary and secondary metabolites with important nutritional, medicinal, and antioxidant properties. The present study was carried out to comparatively analyze the biochemical parameters of Citrus limon leaves using standard biochemical methods. Chlorophyll (a, b, and total chlorophyll), carotenoids, lipids, reducing sugars, alkaloids, total flavonoids, total phenolic content, total carbohydrates, and total sugars were estimated using spectrophotometric and qualitative techniques. The analysis revealed considerable variation among the biochemical parameters. Total chlorophyll content was the highest (0.182 mg/g fresh weight), followed by total phenolic content (0.10 mg GAE/ml), total sugar (0.0614 mg/ml), total carbohydrate (0.053 mg/ml), flavonoid content (0.047 mg QE/ml), and carotenoid content (0.0216 mg/g fresh weight). Lipid content was recorded as 2% (dry weight), while reducing sugars and alkaloids were detected qualitatively. The findings are consistent with previous studies highlighting the antioxidant and physiological significance of citrus leaves. Overall, Citrus limon leaves possess a valuable biochemical profile and may serve as a promising natural source of bioactive compounds for pharmaceutical, nutraceutical, and agricultural applications.

Keywords: *Citrus limon, Biochemical parameters, Comparative analysis, Chlorophyll, Phenolics, Flavonoids.*

Introduction

Citrus limon (L.) Burm. f., commonly known as lemon, is an important fruit crop belonging to the family Rutaceae. It is widely cultivated in tropical and subtropical regions due to its nutritional, medicinal, and economic importance. Besides its fruit, the leaves of *Citrus limon* are rich in several bioactive compounds such as phenolics, flavonoids, carotenoids, alkaloids, carbohydrates, and sugars, which contribute to antioxidant, antimicrobial, anti-inflammatory, and therapeutic activities [1].

Recent studies have highlighted the growing importance of *Citrus limon* leaves as a valuable source of natural phytochemicals. The biochemical composition of plant leaves reflects their physiological and metabolic status. Primary metabolites, including chlorophyll, carbohydrates, sugars, and lipids, are essential for photosynthesis, energy production, and normal plant growth, whereas secondary metabolites such as phenolics, flavonoids, carotenoids, and alkaloids are involved in plant defense and antioxidant protection.

Although numerous studies have investigated the biochemical composition of citrus fruits and peels, comparatively limited information is available regarding the biochemical characteristics of *Citrus limon* leaves [2].

Therefore, the present study was undertaken to evaluate the biochemical attributes of Citrus limon leaves by estimating chlorophyll (a, b, and total chlorophyll), carotenoids, lipids, reducing sugars, alkaloids, flavonoids, total phenolic content, carbohydrates, and total sugars using standard biochemical methods. The findings of this study may contribute to a better understanding of the biochemical profile of *Citrus limon* leaves and support their potential applications in pharmaceutical, nutraceutical, agricultural, and herbal industries [3].

Methodology

Study Area

The present study was carried out in the Department of Botany, School of Life Sciences, Starex University, Binola Village, Gurugram District, Haryana, India.

Plant Material

Fresh leaves of Citrus limon (L.) Burm. f. were used for the biochemical analysis.

Collection of Leaf Samples

Healthy, mature, and disease-free leaves were collected from Citrus limon plants. The leaves were washed thoroughly with distilled water to remove dust and other impurities. After washing, the leaves were shade-dried at room temperature and ground into a fine powder using a clean mortar and pestle. The powdered samples were stored in airtight containers until further biochemical analysis.

Biochemical Analysis

The collected leaf samples were analyzed under laboratory conditions for the estimation of the following biochemical parameters:

- Chlorophyll (Chlorophyll a, Chlorophyll b and Total chlorophyll)
- Carotenoids
- Lipids
- Reducing sugars
- Alkaloids
- Total flavonoids
- Total phenolic content
- Total carbohydrates
- Total sugars

Standard biochemical methods were employed for the quantitative and qualitative estimation of these biochemical constituents.

Materials Required

Instruments

Spectrophotometer, Test tubes, Conical flasks, Beakers, Measuring cylinders, Pipettes, Micropipettes, Cuvettes, Mortar and pestle, Centrifuge, Water bath, Analytical balance, Filter paper, Glass rods and Funnels.

Chemicals Required

Acetone (80%), Methanol, Petroleum ether, Hydrochloric acid (HCl), Folin–Ciocalteu reagent, Gallic acid, Quercetin, Aluminium chloride, Potassium acetate, Anthrone reagent, Concentrated sulphuric acid (H_2SO_4), Sodium carbonate, Benedict's reagent, Dragendorff's reagent, Glucose, Distilled water and Calcium carbonate.

Biochemical Estimation Methods:

1. Estimation of Chlorophyll

Chlorophyll content was estimated by extracting fresh Citrus limon leaves (0.5 g) with 80% acetone in the presence of calcium carbonate. The extract was filtered, and absorbance was recorded at 663 nm and 645 nm using a UV–Visible spectrophotometer. Chlorophyll a, chlorophyll b, and total chlorophyll contents were calculated using standard equations [4].

2. Estimation of Carotenoids

Carotenoids were extracted using 80% acetone. The absorbance was measured at 480 nm, 663 nm, and 645 nm, and carotenoid content was calculated using standard formulae [5].

3. Estimation of Total Lipids

Total lipids were estimated by solvent extraction using petroleum ether. The solvent was evaporated, and the lipid residue obtained was weighed. Lipid content was expressed as percentage of dry weight [3].

4. Estimation of Reducing Sugars

Reducing sugars were determined using Benedict's reagent. The plant extract was heated with Benedict's reagent, and the development of green coloration indicated the presence of reducing sugars [6].

5. Estimation of Alkaloids

Alkaloids were qualitatively estimated using Dragendorff's reagent. Formation of a light orange precipitate confirmed the presence of alkaloids in the leaf extract [2].

6. Estimation of Total Flavonoids

Total flavonoid content was estimated by the Aluminium Chloride colorimetric method using Quercetin as the standard. Absorbance was measured at 415 nm, and the flavonoid content was expressed as Quercetin Equivalents (QE).

7. Estimation of Total Phenolic Content

Total phenolic content was determined by the Folin–Ciocalteu method using Gallic acid as the standard. Absorbance was recorded at 765 nm, and results were expressed as Gallic Acid Equivalents (GAE).

8. Estimation of Total Carbohydrates

Total carbohydrate content was estimated by the Anthrone method. The reaction mixture was heated in a boiling water bath, and absorbance was measured at 620 nm using a spectrophotometer [7].

9. Estimation of Total Sugars

Total sugar content was estimated by the Anthrone method using glucose as the standard. The absorbance was recorded at 620 nm, and the concentration was determined from the standard calibration curve.

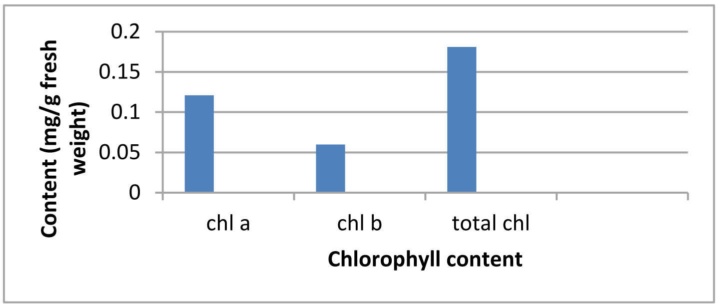
Result

The biochemical analysis of citrus leaves was carried out and the result obtained for various parameters are presented below.

Chlorophyll Content:

The chlorophyll content of citrus leaves was estimated spectrophotometrically using absorbance values recorded at 663 nm and 645 nm. The absorbance obtained at 663 nm was 0.529, while at 645 nm it was 0.239. Based on these observations, the chlorophyll content was calculated and found to be 0.121 mg/g fresh weight for chlorophyll a and 0.060 mg/g fresh weight for chlorophyll b. The total chlorophyll content of the citrus leaves was determined to be 0.182 mg/g fresh weight.

A higher amount of chlorophyll as compared to chlorophyll b indicates normal photosynthetic pigment composition in the leaves. The total chlorophyll content reflects the photosynthetic potential and physiological status of the citrus leaf sample.



Graph 1: chlorophyll content in citrus leaves

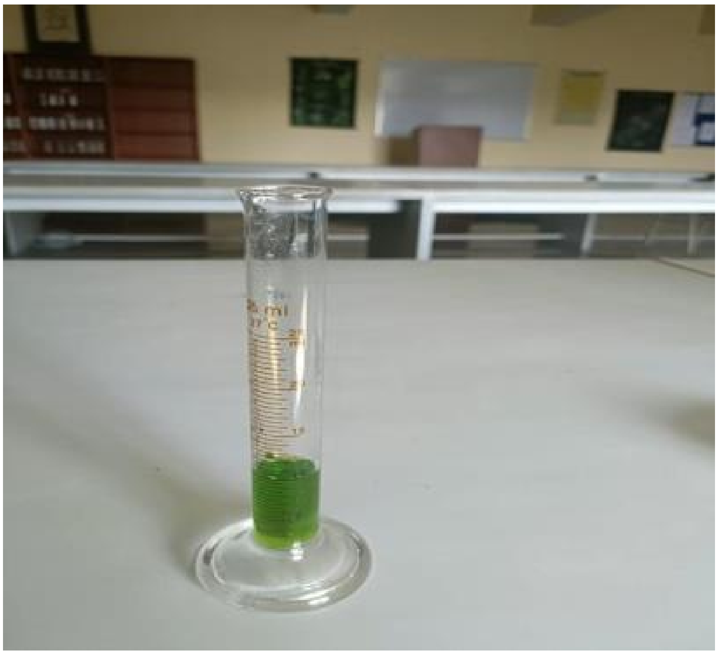


Image 1: chlorophyll extract

Carotenoid content:

The carotenoid content of citrus leaves was estimated spectrophotometrically using absorbance values recorded at different wavelengths. The absorbance values obtained were 2.014 at 480 nm, 2.457 at 663 nm, and 1.900 at 645 nm. Based on these observations, the carotenoid content of the citrus leaves was calculated and found to be 0.0216 mg/g fresh weight.

The presence of carotenoids indicates their role as accessory photosynthetic pigments and antioxidants, contributing to light harvesting and protection of the photosynthetic apparatus against oxidative damage. The measured carotenoid content reflects the physiological condition and photosynthetic efficiency of the citrus leaf sample.

Image 2: Carotenoid extract

Lipid content:

The lipid content of citrus leaves was estimated by the gravimetric method. During the analysis, the weight of the empty beaker was recorded as 52.37 g, while the weight of the beaker containing the extracted lipid was 52.38 g. A sample weight of 0.5 g was used for the estimation. The difference between the two weights indicated a lipid residue of **0.01 g**. Based on these observations, the lipid content of the citrus leaves was calculated to be 2% on a dry weight basis.

The measured lipid content reflects the presence of structural and storage lipids in the leaf tissue, which play important roles in membrane formation, energy storage, and various physiological processes in the plant

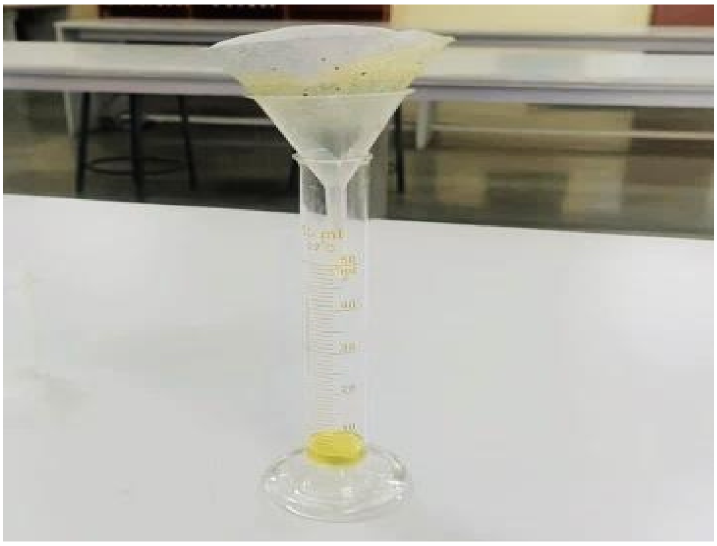


Image 3: Lipid extract before heating

Image 4: lipid residue after heating

Reducing sugar content:

The reducing sugar content of citrus leaves was estimated using Benedict's reagent. Upon heating the leaf extract with Benedict's reagent, a green coloration was observed, indicating the presence of reducing sugars in low concentration. The green color development suggests that citrus leaves contain a relatively small amount of reducing sugars, which are important metabolites involved in energy production and various physiological processes in plants.

Image 5: Benedict's reagent

Image 6: Reducing sugar solution before heating

Image 7: sample under heating

Image 8: Reducing sugar after heating

Alkaloid Content:

The alkaloid content of citrus leaves was qualitatively analyzed using Dragendorff's reagent. Upon addition of the reagent to the leaf extract, a light orange coloration was observed, indicating the presence of alkaloids in the sample. The development of the light orange color confirms that citrus leaves contain alkaloid compounds, although in relatively low concentration. Alkaloids are important secondary metabolites that play significant roles in plant defense mechanisms and possess various biological and pharmacological properties.

Image 9: Light orange colour indicating presence of alkaloids

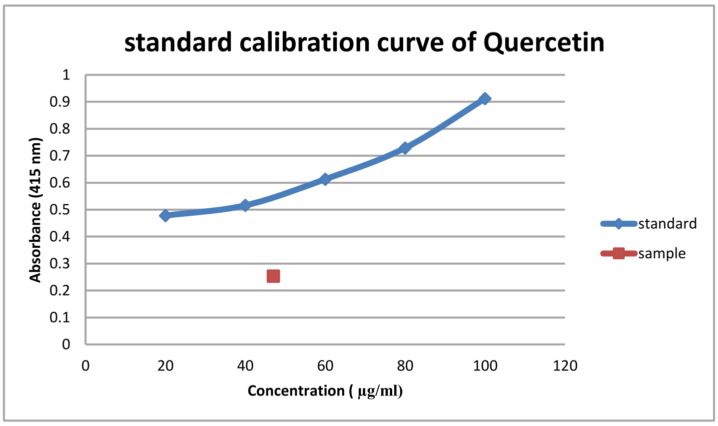
Flavonoid Content:

The total flavonoid content of *Citrus limon* leaves was estimated using the Aluminium Chloride colorimetric method with Quercetin as the standard. The concentration was calculated from the standard calibration curve and was found to be 0.047 mg QE/ml. The result confirms the presence of flavonoids, indicating the antioxidant and medicinal potential of the leaf extract. The standard calibration curve is presented in graph 2.

Quercetin standards used in flavonoid estimation are presented in Table1.

Table 1: Standard Observation table of flavonoids
(T1 sample absorbance = 0.254)

Tube	Concentration (µg/ml)	Absorbance (415 nm)
S1	20	0.478
S2	40	0.516
S3	60	0.613
S4	80	0.729
S5	100	0.912



Graph 2: Standard calibration curve of Quercetin

Total phenolic content:

The total phenolic content of citrus leaf extract was estimated by the Folin–Ciocalteu method using Gallic acid as the standard. Based on the absorbance value of the sample and the Gallic acid standard calibration curve, the total phenolic content was calculated to be 0.1 mg GAE/ml (Gallic Acid Equivalent per milliliter of extract).

Phenolic compounds are important plant secondary metabolites that contribute significantly to antioxidant activity. The standard observations are presented in Table 2, sample observations in Table 3, and the Gallic acid standard calibration curve is shown in Graph 3.

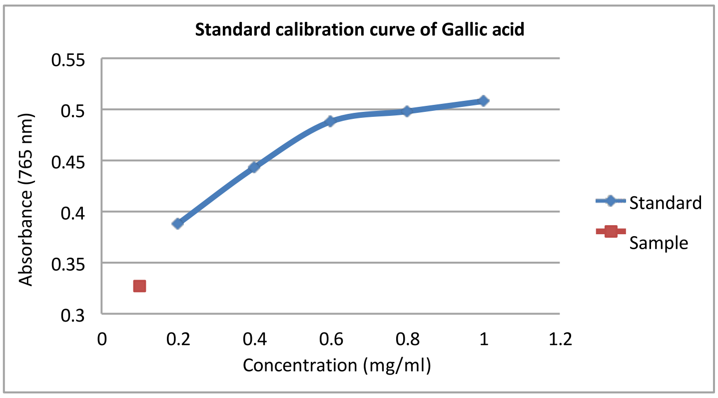
Table 2: standard observation table of phenol

S.NO.	Concentration (mg/ml)	Absorbance (765nm)
S1	0.2	0.388
S2	0.4	0.443
S3	0.6	0.488
S4	0.8	0.498
S5	1.0	0.508

Table 3: sample observation table of phenol

Sample	Wavelength (nm)	Absorbance
T1	765	0.327

Image 10: standard solution used for phenol analyses



Graph 3: standard calibration curve of Gallic acid

Total carbohydrate content:

The total carbohydrate content of *Citrus limon* leaves was estimated using the Anthrone method. The concentration was determined from the glucose standard calibration curve and was found to be 0.053 mg/ml. The result indicates the presence of carbohydrates, which play an important role in plant metabolism and energy storage.

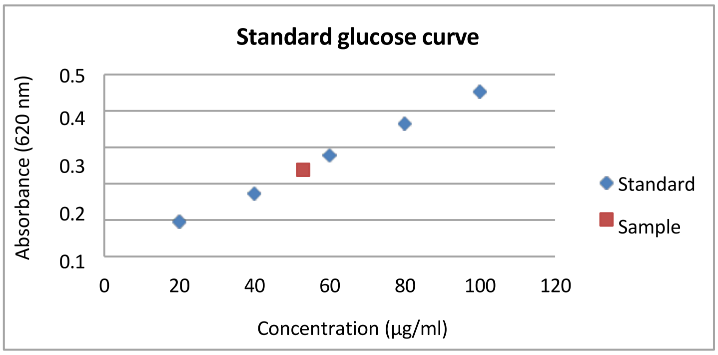
"The standard and sample observations for carbohydrate estimation at 620 nm are presented in Tables 4 and 5, respectively, while the corresponding calibration curve is shown in Graph 4."

Table 4: standard observation table of carbohydrates

Tube	Glucose conc. (µg/ml)	Absorbance (620 nm)
S1	20	0.095
S2	40	0.172
S3	60	0.277
S4	80	0.365
S5	100	0.452

Table 5: sample observation table of carbohydrates

Sample	Absorbance (620 nm)
Test sample (T1)	0.238



Graph 4: Standard glucose curve

Total sugar content:

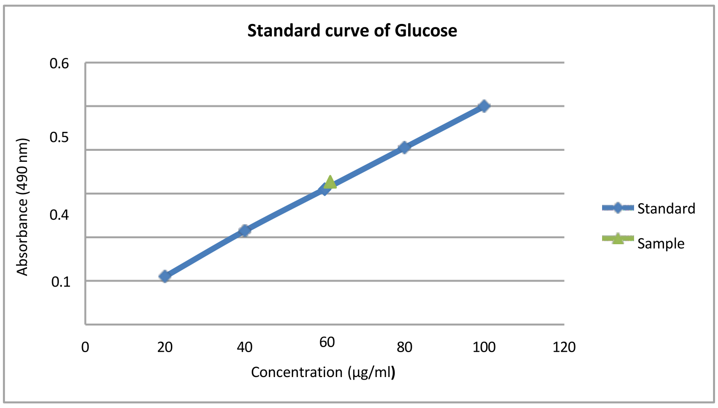
The total carbohydrate content of *Citrus limon* leaves was estimated using the Anthrone method. The concentration was determined from the glucose standard calibration curve and was found to be 0.053 mg/ml. The result indicates the presence of carbohydrates, which play an important role in plant metabolism and energy storage. "The standard observations and sample absorbance values for total carbohydrate estimation at 490 nm are presented in Tables 6 and 7, respectively, whereas the corresponding standard calibration curve is shown in Graph 5."

Table 6: standard observation table of total sugar

S.NO.	Standard Concentration (µg/ml)	Absorbance at (490 nm)
S1	20	0.125
S2	40	0.21
S3	60	0.295
S4	80	0.38
S5	100	0.465

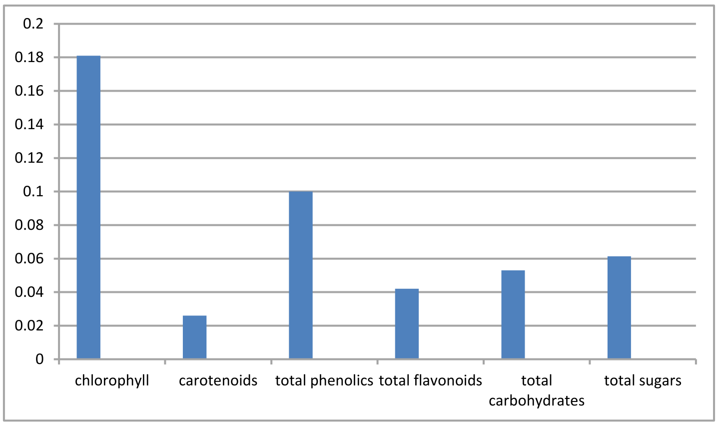
Table 7: sample observation table of total sugar

Sample	Absorbance at (490 nm)
Leaf extract	0.327



Graph 5: standard curve of glucose

Comparative Analysis of Biochemical Parameters in Citrus Leaves Using Bar Graph



Graph 6: comparative analyses of biochemical parameters

CONCLUSION

The present study demonstrated that Citrus limon leaves contain several important biochemical constituents, including chlorophyll, carotenoids, lipids, reducing sugars, carbohydrates, and alkaloids. The biochemical analysis confirmed that these metabolites play significant roles in plant growth, photosynthesis, metabolism, and defense mechanisms. The presence of these compounds indicates the nutritional and medicinal potential of Citrus leaves

The findings suggest that Citrus limon leaves are a valuable source of bioactive compounds with antioxidant and therapeutic properties. Therefore, the plant may have promising applications in pharmaceutical, nutraceutical, and herbal industries. Further studies involving detailed phytochemical characterization and biological activity are recommended to explore its full medicine

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