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Biochemical Estimation of Different Parameters in *Catharanthus roseus*

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Abstract

Catharanthus roseus (L.) G. Don, a well-known medicinal plant, is widely recognised for its rich content of bioactive compounds and significant pharmacological properties. The present study focuses on the biochemical estimation of various primary and secondary metabolites in leaves of Catharanthus roseus. Key biochemical parameters such as total carbohydrates, alkaloids, lipids, carotenoids, phenols, flavonoids, and chlorophyll content were quantitatively analysed using standard spectrophotometric methods. Plant samples were collected, processed, and subjected to biochemical assays to determine the concentration of these metabolites. The results revealed considerable variation in the levels of biochemical constituents, indicating the plant's high metabolic activity and therapeutic potential. Elevated levels of phenolic and flavonoid compounds suggest strong antioxidant properties, while the presence of carbohydrates reflects their essential metabolic functions. This study highlights the importance of Catharanthus roseus as a valuable source of bioactive compounds and provides a scientific basis for its use in pharmaceutical and medicinal applications. The findings may further contribute to the development of plant-based drugs and promote the utilisation of this species in biochemical and pharmacological research.

Keywords: *Catharanthus roseus, biochemical estimation, Pharmaceutical, Antioxidant, spectrophotometry.*

Introduction

Catharanthus roseus (L.) G. Don, commonly known as Sadabahar or Madagascar periwinkle, is a perennial herb belonging to the family Apocynaceae. It is widely cultivated as an ornamental plant and is of great medicinal importance due to the presence of alkaloids such as vincristine and vinblastine. The plant exhibits typical dicotyledonous morphological features with well-developed vegetative and reproductive organs. The presence of numerous bioactive alkaloids and their pharmaceutical significance[1]. The biosynthesis of these alkaloids and emphasized the plant's medicinal value[2]. The significant changes in chlorophyll, proteins, carbohydrates, and antioxidant enzymes under different environmental conditions[3].

In addition, the plant exhibits antioxidant, antimicrobial, antidiabetic, and anti-inflammatory activities. Biochemical estimation of different parameters such as chlorophyll, carotenoids, proteins, carbohydrates, phenols, flavonoids, and antioxidant enzymes provides important insights into its physiological status, metabolic processes, and medicinal potential[4].

The analysis of these biochemical constituents helps in understanding plant growth, development, stress responses, and the accumulation of therapeutically important compounds. Therefore, the present study focuses on the biochemical estimation of various parameters in Catharanthus roseus to evaluate its physiological and medicinal significance and to contribute to its pharmaceutical and agricultural applications.

Materials and Methods Preparation of plant extract:

Fresh and healthy leaves of Catharanthus roseus (L.) G. Don were collected from the campus of Starex University, Bhora Kalan. The plant were identified based on standard taxonomic keys. The collected samples were washed thoroughly with distilled water to remove dust and contaminants.

The leaves were shade-dried at room temperature for several days and then ground into fine powder using a mortar and pestle. The powdered samples were stored in airtight containers for further biochemical analysis.

About 1 g of dried leaf powder was taken and homogenised with 10 ml of appropriate solvent (such as ethanol, methanol, or distilled water, depending on the test). The homogenate were centrifuged at 5000 rpm for 10 minutes, and the supernatant was collected. This extract were used for the estimation of various biochemical parameters.

Materials Required: Test tube, Cuvette, Filter paper, Conical flask, Lab dropper, Hand Gloves, Beaker, Spatula, Measuring cylinder, Conical funnel, Mortar and Pestle, UV-Visible spectrophotometer, Centrifuge Machine, Weighing Balance, Heating Mantle, Water bath, Hot air oven.

Chemicals Required: 80%Acetone, Aluminium Chloride, Benedict's Reagent, Distilled Water, Methanol, Anthrone Reagent, Calcium Carbonate, Sodium Carbonate, Dragendorff's Reagent, Petroleum Ether, Gallic Acid (Standard), Quercetin Hydrate 95%, Conc. Sulphuric acid, Acetic Acid, Potassium Acetate, 80% Ethanol, Glucose Standard Solution, Folin & Ciocalteu's Phenol Reagent.

Biochemical Parameters Estimation:

Chlorophyll Estimation:

Chlorophyll pigments are extracted with 0.5g of fresh leaf in 80% acetone, add a pinch of calcium carbonate and quantified spectrophotometrically at 645 nm (Chl b) and 663 nm (Chl a). Total chlorophyll calculated using Arnon's equations based on specific absorbance coefficients.

$$\text{Total Chlorophyll (mg/g)} = \frac{20.2\,(A663) + 8.02\,(A645) \times \text{Total volume of extract}}{1000 \times \text{Sample weight (g)}}$$

Carotenoid Estimation:

Carotenoids are accessory pigments present in plant leaves[5]. They are extracted using organic solvents like acetone, and their absorbance is measured at 480 nm and 510 nm. The carotenoid content is calculated using standard equations based on absorbance values.

Lipid Estimation:

Lipids are non-polar compounds that are soluble in organic solvents such as petroleum ether. Fresh leaves of Catharanthus roseus were used for lipid extraction. The sample was groundand treated with petroleum ether, resulting in a light yellow extract, indicating the presence of lipids[6]. After filtration/centrifugation, a clear extract was obtained. Upon evaporation of petroleum ether, a greasy residue remained, confirming lipid extraction.

Reducing Sugar Estimation:

Benedict's reagent contains copper sulfate, sodium carbonate, and sodium citrate. Reducing sugars (such as glucose, fructose) reduce Cu^{2+} ions to Cu^{+} ions under alkaline conditions. This forms cuprous oxide (Cu_2O), which appears as a coloured precipitate.

After adding Benedict's reagent to the leaf extract of *Catharanthus roseus* and heating it in a boiling water bath, the solution turned from blue to green with slight turbidity.

The appearance of green colour indicating low concentration of reducing sugars in the leaf extract of *Catharanthus roseus*.

Total Sugar Estimation:

Total sugars were estimated by the Anthrone method, based on acid hydrolysis and dehydration of carbohydrates to form furfural derivatives, which react with Anthrone reagent to produce a stable green-colored complex. The appearance of green colour indicates the presence of total sugars (both reducing and non-reducing sugars) in the leaf extract of *Catharanthus roseus*[7].

Carbohydrate Estimation:

Fresh leaves of Catharanthus roseus were collected, washed thoroughly, and blot-dried before weighing 1g of the sample. The leaf material was homogenised in distilled water and centrifuged at 5000 rpm for 10 minutes to obtain a clear supernatant. Total carbohydrate content was determined by the Anthrone method[8]. A glucose stock solution (1 mg/ml) was used to prepare a series of standard concentrations. Both standard solutions and sample extracts were treated with Anthrone reagent and heated in a boiling water bath, leading to the formation of a bluish-green coloured complex. The intensity of the colour developed was measured spectrophotometrically at 620 nm. A standard calibration curve was plotted using glucose standards, and the carbohydrate content of the sample was calculated from this curve.

$$\text{Carbohydrate (mg/g)} = \frac{\text{Amount from graph} \times \text{Total volume}}{\text{Sample weight} \times \text{Aliquot}}$$

Total Phenol Content Estimation:

Total phenolic content in *Catharanthus roseus* leaves was determined using the Folin–Ciocalteu method. The extract was treated with Folin–Ciocalteu reagent and sodium carbonate to develop a blue-colored complex, and absorbance was measured at 765 nm using a spectrophotometer. Gallic acid was used as the standard for calibration by preparing a series of known concentrations to construct a standard curve. The results were expressed as mg of gallic acid equivalents (GAE) per gram of fresh weight[9].

$$\text{Total phenol (mg/g)} = \frac{\text{Concentration from standard curves} \times \text{volume of sample}}{\text{Sample weight}}$$

Alkaloids Estimation:

Total alkaloids in *Catharanthus roseus* were detected using Dragendorff's reagent, which produces an orange-brown precipitate with alkaloids. The formation of precipitate confirms the presence of alkaloids[10].

Flavonoids Estimation:

Total flavonoid content in *Catharanthus roseus* leaves was determined using the aluminium chloride colourimetric method. The prepared plant extract was treated with aluminium chloride reagent, resulting in the formation of a yellow-coloured flavonoid–aluminium complex. After proper incubation, the intensity of the developed colour was measured spectrophotometrically at 415 nm. A standard curve was constructed using quercetin as the reference compound, and the flavonoid content of the sample was calculated accordingly. The quercetin standard solutions showed a gradual increase in absorbance with increasing concentration, indicating a linear relationship.

Results:

Biochemical estimation of Quantitative parameters in Catharanthus roseus

S. No.	Parameters	Methods	Absorbance	Results
1	Total chlorophyll	Arnon's Method	645nm,663nm	0.455 mg/g
2	Carotenoids	Spectrophotometric Method	480nm,510nm	0.324 mg/g
3	Total Sugar	Anthrone Method	620nm	1.8 mg/g
4	Total Carbohydrate	Anthrone Method	620nm	1.64 mg/g
5	Phenol	Folin & Ciocalteu's Method	765nm	5.46 mg/g
6	Flavonoids	Alcl3 colourimetric	415nm	0.90 mg/g

Biochemical estimation of Qualtitative Test in Catharanthus roseus

S.No.	Parameters	Tests	Observation
1	Lipid	Solvent (Petroleum Ether)	Greasy (Yellowish)
2	Reducing Sugar	Benedict's Test	Green precipitate
3	Alkaloids	Dragendroff's Test	Dark Brown precipitate

This table shows the biochemical composition of Catharanthus roseus, indicating the presence of essential metabolites and secondary metabolites.

Chlorophyll Contents:

Image 1. Spectrophotometric absorbance of the sample at 663nm &645nm for chlorophyll estimation.

The spectrophotometric analysis of the sample showed an absorbance value of 0.649 at 645nm (A645) and 1.201 at 663nm (A663), which were utilised for the estimation of chlorophyll 'a', chlorophyll 'b', and total chlorophyll content in *Catharanthus roseus.*

Carotenoids Contents:

Image 2. Spectrophotometer absorbance of the sample at 480nm &510nm for carotenoid estimation

The total carotenoid content of *Catharanthus roseus* was 0.324 mg/g of fresh weight, indicating the presence of accessory photosynthetic pigments in moderate amounts.

Lipids Contents:

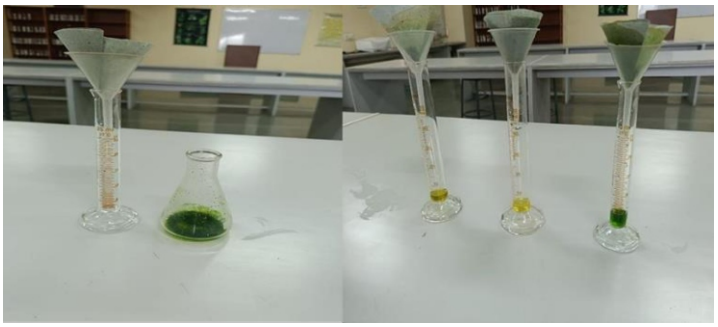


Image 3(a). Lipid extraction from Catharanthus roseus using petroleum ether, showing lipid residue

The lipid content of *Catharanthus roseus* was estimated by solvent extraction using petroleum ether. The extraction yielded 0.06g of lipid residue from 0.5g of sample, resulting in a total lipid content of 12% (w/w).

Image 3(b). Lipid extraction from C.roseus using petroleum ether, showing lipid residue

Reducing Sugar Contents:

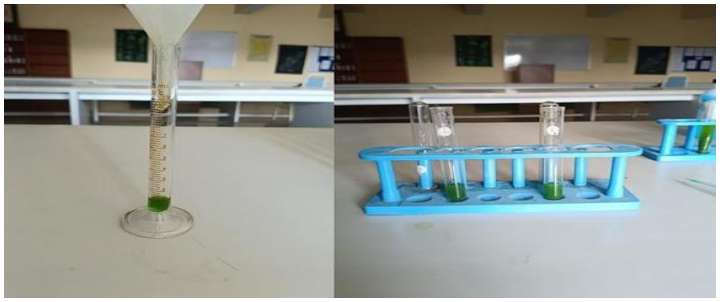


Image 4. Reducing sugar test of Catharanthus roseus showing green colouration, indicating low sugar content

The qualitative estimation of reducing sugars in *Catharanthus roseus* using Benedict's reagent showed a green colour, which confirms the presence of reducing sugars in low amounts. This indicated a partial reduction of Cu2+ ions to Cu+ due to a small quantity of reducing sugars in the sample.

Total Sugar Contents:

Image 5. Total sugar showing green colour in Catharanthus roseus leaf extract

The total sugar content in *Catharanthus roseus* leaf extract was determined to be 1.8mg/g fresh weight using spectrophotometric analysis. The development of green colour during the reaction confirmed the presence of total sugar in the sample.

Total Carbohydrates Contents:

Image 6. Estimation of carbohydrate showing moderate intensity in plants

Spectrophotometric estimation of carbohydrate content of *Catharanthus roseus* leaf extract showing a value of 1.64mg/g fresh weight at 620nm, indicating a moderately high level with characteristic green colour development.

Table 1. Standard reading of Carbohydrates

S. No.	Glucose Solution(mg/ml)	Distilled Water (ml)	Anthrone Reagent(ml)	Absorbance (620 nm)
Blank	-	1	4ml	0.000
1	0.2	0.8	4ml	0.210
2	0.4	0.6	4ml	0.380
3	0.6	0.4	4ml	0.580
4	0.8	0.2	4ml	0.790
5	1.0	0	4ml	1.001

Phenol Contents:

Image 7. Spectrophotometric estimation of total phenolic content at 765nm.

The total phenolic content was quantitatively estimated as 5.46mg/g fresh weight using spectrophotometric analysis at 765nm, confirming the appreciable accumulation of phenolic compounds in the sample.

Table 2: Standard readings of Phenol

Test tube	Stock Solution (ml) Gallic acid	Distilled Water	Phenol Final Conc.	Absorbance(765)
1	0.2	9.8	20ug	0.429
2	0.4	9.6	40ug	0.691
3	0.6	9.4	60ug	0.855
4	0.8	9.2	80ug	0.980
5	1.0	9.0	100ug	1.241
Blank	0.5(Folin reagent)	5	2ml(Na2co3)	0.000

Alkaloids Contents:

Image 8. Qualitative detection of alkaloids by Dragondroff's Reagent

The presence of alkaloids was confirmed by the formation of a strong dark brown precipitate, indicating a high concentration of alkaloid compounds in the sample.

Flavonoids Contents:

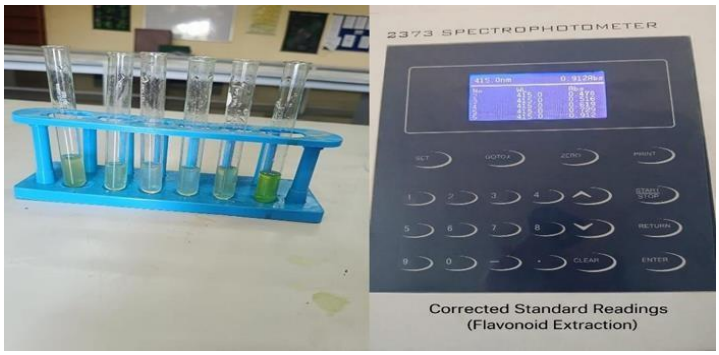


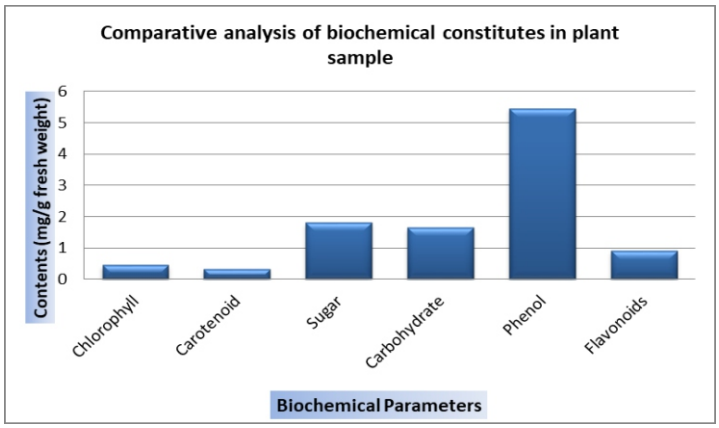
Image 9. Estimation of total flavonoid content of the sample by the spectrophotometric method.

The flavonoid content was quantitatively estimated as 0.90mg/g fresh weight using spectrophotometric analysis, confirming the presence of flavonoids in the sample.

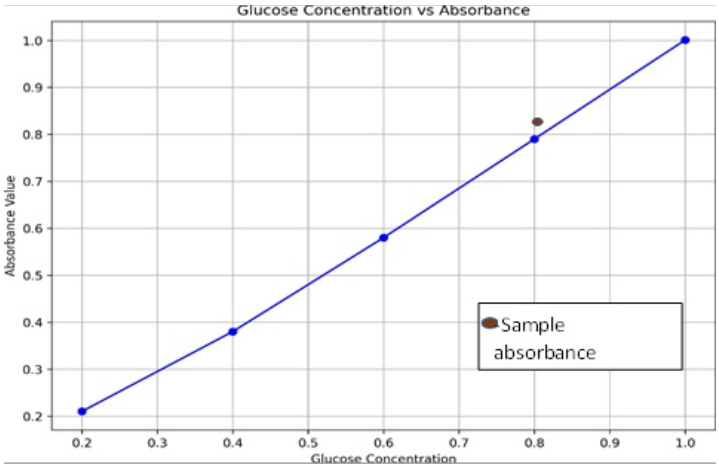
Table 3: Standard readings of Flavonoid

Test tubes	Stock Solution(ml) (Quercetin)	Methanol (ml)	Concentration (ug/ml)	Absorbance (415nm)
1	0.2	9.8	20	0.478
2	0.4	9.6	40	0.516
3	0.6	9.4	60	0.619
4	0.8	9.2	80	0.729
5	1.0	9.0	100	0.912

Blank	1 (Potassium acetate)	2(Al2cl3)	2ml (Distilled water)	0.000

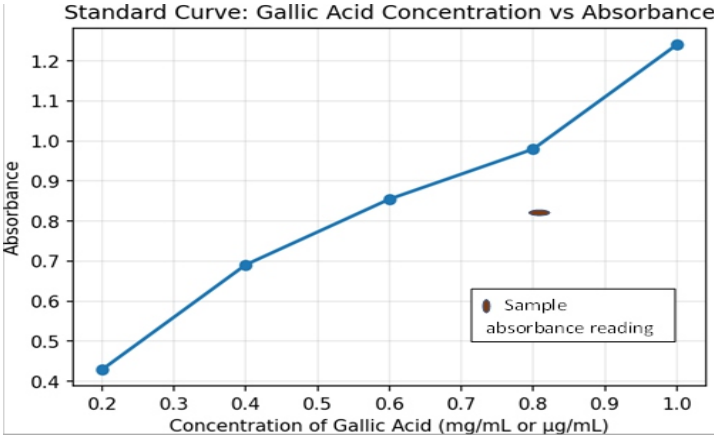


Graph 1: Biochemical Parameters of Catharanthus roseus



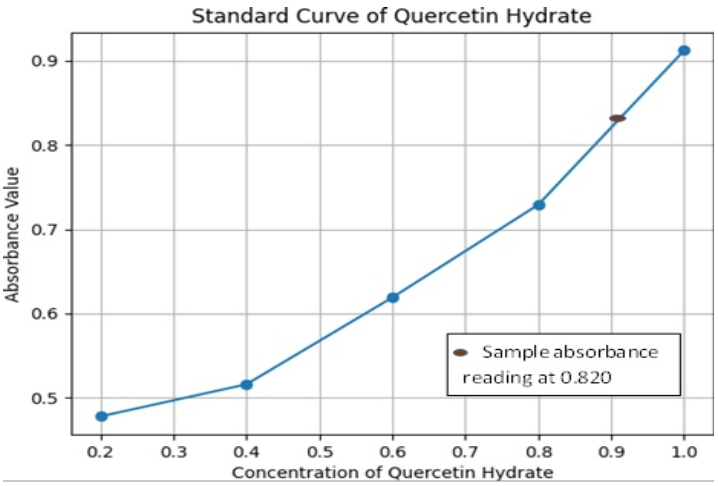
Graph 2: Standard Curves for Carbohydrates

The concentration was calculated using standard calibration curve plotted between absorbance and concentration.



Graph 3: Standard Curves for Phenols

The concentration was calculated using standard calibration curve plotted between absorbance and concentration.



Graph 4: Standard Curves for Flavonoids

The concentration was calculated using standard calibration curve plotted between absorbance and concentration.

Conclusion:

The biochemical estimation of different parameters in *Catharanthus roseus* demonstrated the presence and relative concentration of important biomolecules such as chlorophyll, proteins, carbohydrates, phenolic compounds, and other primary and secondary metabolites. These biochemical constituents play vital roles in the plant's growth, metabolism, and defense mechanisms. The results indicate that *Catharanthus roseus* possesses significant biochemical richness, which supports its well-known medicinal value and its use in pharmaceutical applications. The study also highlights that variations in these biochemical parameters may occur due to environmental conditions, plant age, and physiological status. Overall, the biochemical analysis provides valuable information on the metabolic profile of *Catharanthus roseus* and emphasizes its importance as a medicinal plant with potential therapeutic and research applications.

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