

QUALITATIVE AND QUANTITATIVE STUDY OF BIOACTIVE COMPOUND USING SPECTROSCOPIC AND CHROMATOGRAPHIC TECHNIQUES

Srashti Sahu*, Jyoti Ahirwar, Durga Kishora, Kuldeep Ganju
Sagar Institute of Pharmacy and Technology (SIPTec), Bhopal (M.P.)

*Corresponding Author's E mail: srashtisahu56@gmail.com

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ABSTRACT

The present study aimed to perform qualitative and quantitative analysis of bioactive compounds present in the hydroalcoholic extract of *Citrullus lanatus* using spectroscopic and chromatographic techniques. The extract was prepared by maceration and evaluated for its physicochemical properties, percentage yield, and phytochemical profile. Preliminary phytochemical screening revealed the presence of carbohydrates, flavonoids, phenols, proteins, and diterpenes. Quantitative estimation indicated total phenolic and flavonoid contents of 0.52 mg/100 mg and 0.87 mg/100 mg, respectively. High-Performance Liquid Chromatography (HPLC) analysis confirmed the presence of quercetin with a retention time of 2.369 minutes and a concentration of 0.211%. These findings demonstrate that *Citrullus lanatus* is a rich source of natural antioxidants and bioactive compounds, which could be utilized for pharmaceutical and nutraceutical applications.

Keywords: *Citrullus lanatus*, Phytochemical screening, Quercetin, Spectroscopic analysis, HPLC, Bioactive compounds, Antioxidant potential.

INTRODUCTION

Medicinal plants are a rich source of bioactive compounds that exhibit diverse pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, and anticancer effects^{1,2}. The identification and quantification of these phytoconstituents are crucial for understanding their therapeutic potential and ensuring the quality, safety, and efficacy of herbal formulations³.

Qualitative and quantitative evaluation of bioactive compounds is generally carried out using spectroscopic and chromatographic techniques. Spectroscopic methods such as UV-Visible, FTIR, and NMR provide valuable information on structural characteristics, functional groups, and purity of compounds^{4,5}. These methods are often used for preliminary screening and confirmation of phytochemical constituents present in plant extracts.

Chromatographic techniques, including Thin Layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPTLC), High Performance Liquid Chromatography (HPLC), and Gas Chromatography–Mass Spectrometry (GC-MS), are widely employed for qualitative fingerprinting and quantitative estimation of bioactive molecules^{6,7}. Among these, HPLC and HPTLC offer high sensitivity, reproducibility, and precision for detecting phytochemicals such as flavonoids, alkaloids, phenolics, and terpenoids^{8,9}.

The integration of both spectroscopic and chromatographic approaches provides a comprehensive understanding of the chemical profile of plant extracts, enabling reliable standardization and quality control of herbal medicines¹⁰. Hence, the present study focuses on the qualitative and quantitative determination of bioactive compounds using advanced spectroscopic and chromatographic methods for accurate phytochemical characterization.

Material and Methods

Material

The materials used for the investigation included fresh fruits of *Citrullus lanatus*, which were shade-dried, powdered, and extracted using a hydroalcoholic solvent (ethanol:water, 70:30 v/v). Analytical and HPLC-grade reagents such as methanol, ethanol, Folin–Ciocalteu reagent, aluminum chloride, sodium nitrite, and standard quercetin were used. Instruments like a rotary evaporator, UV–Visible spectrophotometer, and HPLC system equipped with a C18 column and UV detector were employed for qualitative and quantitative analysis of bioactive compounds.

Methods

Collection of plant material

Seeds of *Citrullus lanatus* were collected from local area of Bhopal in month of June 2025.

Extraction procedure

Defatting of plant material

50 gram of dried seeds of *Citrullus lanatus* was coarsely powdered and subjected to extraction with petroleum ether by maceration¹¹. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted seeds of *Citrullus lanatus* were extracted with hydroalcoholic solvent (Ethanol: Water; 75:25v/v) using maceration process (24hrs). The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extract.

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage¹². The percentage yields of each extract were calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powder drug taken}} \times 100$$

Qualitative evaluation

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical tests were done as per the methods given ¹³.

Quantitative studies of bioactive constituents

Estimation of total phenol content

Principle: The total phenol content of the extract was determined by the modified folin-ciocalteu method ¹⁴.

Preparation of Standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol

Preparation of Extract: 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol.

Procedure: 2 ml of extract and each standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

6.8.2 Estimation of total flavonoids content

Principle: Determination of total flavonoids content was based on aluminium chloride method ¹⁵.

Preparation of Standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of Extract: 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.

Procedure: 1 ml of 2% AlCl₃ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Identification of marker compound (Quercetin) by HPLC

Quercetin was kindly provided by Scan Research Laboratories, Bhopal (India). Methanol and acetonitrile were of HPLC grade and purchased from Merck Ltd, New Delhi, India. Water used was of HPLC grade water from Merck Ltd, New Delhi, India. A thermospectronic model of Labindia 3000 + UV/VIS Spectrophotometer with 1cm. matched quartz cells was used for determination of $\lambda_{\max}$. The HPLC system (Waters) consisted of a pump, a U.V. Visible detector, a Thermo C₁₈ (250 X 4.6 mm, 5µm) column, a Data Ace software.

The chromatographic analysis was performed at ambient temperature on a RP-C18 analytical column with a mobile phase composed of Acetonitrile: Methanol (50:50 v/v) and was isocratically eluted at a flow rate of 1 mL min⁻¹. A small sample volume of 20 µL was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 256 nm ¹⁶.

Table 1: Selection of Separation Variable

Variable	Condition
Column	
- Dimension	250 mm × 4.6 mm
- Particle Size	5 µm
- Bonded Phase	Octadecylsilane (C18)
Mobile Phase	Acetonitrile : Methanol (50:50 v/v)
Flow Rate	1.0 ml/min
Column Temperature	Room Temperature
Sample Volume	20 µl
Detection Wavelength	256 nm
Retention Time	2.365 ± 0.5 min

Preparation of Standard Stock Solution

An accurately weighed 10 mg of quercetin was transferred into a 10 ml volumetric flask. The volume was made up to the mark with methanol to obtain a standard stock solution with a concentration of 1000 µg/ml (ppm).

Preparation of Working Standard Solutions

From the standard stock solution of quercetin (1000 µg/ml), 1 ml was pipetted into a 10 ml volumetric flask and diluted to volume with the mobile phase to obtain a working standard solution of 100 µg/ml. From this solution, aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, and 2.5 ml were each transferred into separate 10 ml volumetric flasks and diluted to volume with the mobile phase to prepare standard solutions of 5, 10, 15, 20, and 25 µg/ml, respectively.

Preparation and Analysis of Extract

A quantity of 10 mg of the plant extract was accurately weighed and transferred into a 10 ml volumetric flask. Methanol was added to dissolve the extract and make up the volume to 10 ml, yielding a solution with a concentration of 1000 µg/ml. The solution was first filtered through Whatman filter paper, followed by filtration using a 0.45 µm membrane filter. The filtered solution was then sonicated for 10 minutes to ensure complete dissolution and homogeneity before further analysis.

RESULTS AND DISCUSSION

The hydroalcoholic extract of *Citrullus lanatus* was obtained as a dark brown solid with a percentage yield of 10.5% (Table 2), indicating efficient extraction of polar and moderately non-polar phytoconstituents due to the solvent system's balanced polarity. The phytochemical screening (Table 3) revealed the presence of carbohydrates, flavonoids, phenols, proteins, and diterpenes, while alkaloids, glycosides, tannins, resins, steroids, and saponins were absent. These results suggest that *Citrullus lanatus* contains bioactive metabolites with potential pharmacological significance, particularly flavonoids and phenolic compounds known for their antioxidant and anti-inflammatory activities.

The total phenolic content and total flavonoid content were found to be 0.52 mg/100 mg and 0.87 mg/100 mg, respectively (Tables 4 and 5), confirming the presence of polyphenolic compounds that contribute to antioxidant potential. The HPLC chromatograms of standard quercetin (Figure 1) and hydroalcoholic extract of *Citrullus lanatus* (Figure 2) showed well-defined peaks, with the extract exhibiting a retention time (RT) of 2.369 minutes and quercetin content of 0.211% (Table 6). These findings confirm the presence of quercetin as a major flavonoid constituent, known for its antioxidant, anti-inflammatory, and hepatoprotective roles.

The chromatographic and spectroscopic results are in good agreement with qualitative phytochemical data, validating the analytical method for reliable quantification of bioactive components. The combined approach provides a comprehensive chemical profile that supports the therapeutic potential and standardization of *Citrullus lanatus* extract for further pharmacological evaluation.

Table 2: % Yield of *Citrullus lanatus* extract

Extract	Consistency	Colour	% Yield
Hydroalcoholic	Solid	Dark brown	10.5%

Table 3: Phytochemical tests of extract of *Citrullus lanatus*

S.N.	Phytoconstituents	Test Name	Hydroalcoholic Extract
1	Alkaloids	Mayer's Test	-ve
		Dragendorff's Test	-ve
		Wagner's Test	-ve
		Hager's Test	-ve
		Raymond's Test	-ve
2	Glycosides	Killer Killani Test	-ve
		Legal Test	-ve
		Molisch's Test	-ve
3	Carbohydrates	Fehling's Test	+ve
		Benedict's Test	+ve
		Vanillin- HCl Test	-ve
4	Tannins	Gelatin Test	-ve
		Lead acetate Test	+ve
5	Flavonoids	Alkaline Reagent Test	+ve
		Turbidity Test	-ve
6	Resins	Libermann- Bur chard Test	-ve
7	Steroids	Salkowski Reaction	-ve
		Biuret Test	+ve
		Precipitation test	-ve
9.	Phenols	Ferric chloride test	+ve
10.	Saponins	Froth Test	-ve
11.	Diterpenes	Copper acetate Test	+ve

+ ve – Present, - ve – Absent

Table 4: Total phenolic content of hydroalcoholic extract of *Citrullus lanatus*

S. N.	Extract	Total phenol content (mg/100mg)
1	Hydroalcoholic extract	0.52

Table 5: Total flavonoid content of hydroalcoholic extract of *Citrullus lanatus*

S. N.	Extract	Total Flavonoid content (mg/100mg)
1	Hydroalcoholic extract	0.87

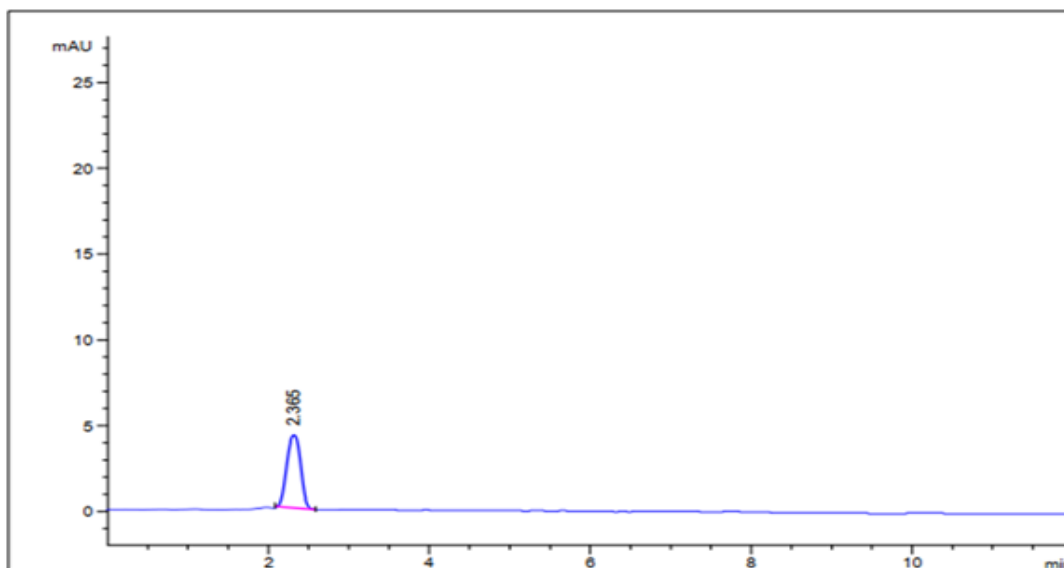


Figure 1: Chromatogram of standard Quercetin (5µg/ml)

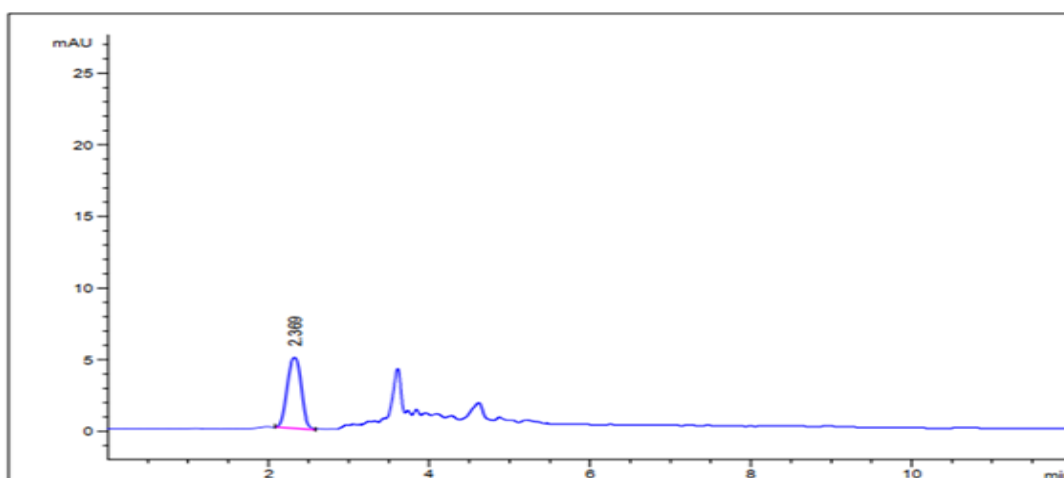


Figure 2: Chromatogram of hydroalcoholic extract of *Citrullus lanatus*

Table 6: Quantitative estimation of Quercetin in *Citrullus lanatus* extract

S. No.	Extract	RT	% Quercetin Content
1.	Hydroalcoholic extract	2.369	0.211%

CONCLUSION

The hydroalcoholic extract of *Citrullus lanatus* demonstrated the presence of several bioactive constituents, including carbohydrates, flavonoids, phenols, proteins, and diterpenes. Quantitative analysis confirmed notable levels of total phenolic and flavonoid content, suggesting significant antioxidant potential. HPLC profiling further verified the presence of quercetin as a key phytoconstituent, indicating its possible role in the therapeutic properties of the plant. Overall, the findings support that *Citrullus lanatus* can serve as a valuable natural source of phytochemicals with potential applications in pharmaceutical and nutraceutical formulations.

REFERENCES

1. Pandey A et al. Phytochemical screening of medicinal plants: a review. J Pharmacogn Phytochem. 2013;1(6):168–82.
2. Singh R et al. Evaluation of bioactive compounds and their therapeutic potential from medicinal plants. J Pharmacogn Phytochem. 2018;7(2):120–6.
3. Mukherjee PK et al. Quality control and evaluation of herbal drugs. Elsevier Health Sciences. 2019.
4. Silverstein RM et al. Spectrometric Identification of Organic Compounds. 8th ed. Wiley; 2014.
5. Pavia DL et al. Introduction to Spectroscopy. 5th ed. Cengage Learning; 2015.
6. Sasidharan S et al. Extraction, isolation and characterization of bioactive compounds from plants extracts. Afr J Tradit Complement Altern Med. 2011;8(1):1–10.
7. Srivastava M et al. Advances in chromatographic techniques for phytochemical analysis. J Liq Chromatogr Relat Technol. 2020;43(4):120–34.
8. Harborne JB et al. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. Chapman & Hall; 1998.
9. Khoddami A et al. Techniques for analysis of plant phenolic compounds. Molecules. 2013;18(2):2328–75.
10. Patel D et al. Recent advances in spectroscopic and chromatographic methods for phytochemical analysis. Curr Pharm Anal. 2021;17(3):195–204.
11. Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction technologies for medicinal and aromatic plants. 1st ed. Italy: United Nations Industrial Development Organization and International Centre for Science and High Technology; 2008. p. 66.

12. Arwande JO, Akinnusotu A, Alademeyin JO. Extractive value and phytochemical screening of ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) using different solvents. Int J Trad Nat Med. 2018;8(1):13-22.
13. Talukdar A, Chaudhary B. Phytochemical screening of ethanolic extracts of *Rubia cordifolia*. Pharm Biol Sci. 2010;1(4):530-6.
14. Javanmardi J, Stushnoff C, Locke E, Vivanco JM. Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. Food Chem. 2003;83:547-50.
15. Khan MS, Yusufzai SK, Rafatullah M, Sarjadi MS. Determination of total phenolic content, total flavonoid content and antioxidant activity of various organic crude extracts of *Licuala spinosa* leaves from Sabah, Malaysia. ASM Sci J. 2018;11(Special Issue 3):53-8.
16. Garg P. HPLC estimation of flavonoid (quercetin) of leaves and stem extracts of *Ocimum sanctum* and *Tinospora cordifolia*. J Phytopharmacol. 2021;10(4):220-4.