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COMPREHENSIVE QUALITY ASSURANCE OF MEDICINAL *Curcuma longa*: PHYTOCHEMICAL, PHYSICOCHEMICAL, AND GC-MS ANALYSIS

D. Lashkari[1], T. Yadav[2], P. K. Sharma[1], S. K. Garg[3] and V. Kumar[4,✉]

[1]Department of Biotechnology, Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur, Rajasthan, India

[2]Department of Pharmaceutics, Amity Institute of Pharmacy, Amity University Rajasthan, Jaipur, Rajasthan, India

[3]Department of Pharmaceutics, Maharishi Arvind College of Pharmacy, Jaipur, Rajasthan, India

[4]Department of Pharmacology, Amity Institute of Pharmacy, Amity University Rajasthan, Jaipur, Rajasthan, India

✉Corresponding author: vikramyadav05@gmail.com

ABSTRACT

Curcuma longa Linn., commonly known as Haridra, is a widely used medicinal plant valued for its diverse pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant, antidiabetic, and anticancer activities. These therapeutic effects are primarily attributed to its rich phytochemical profile. This study aimed to perform a comprehensive quality assessment of *Curcuma longa* through phytochemical screening, physicochemical analysis, thin-layer chromatography (TLC), and gas chromatography–mass spectrometry (GC-MS). Standardization was conducted in accordance with the Ayurvedic Pharmacopoeia of India (API) guidelines. Organoleptic and powder microscopy confirmed authenticity, while physicochemical parameters such as moisture content (9.65%), total ash (4.9%), acid-insoluble ash (0.71%), and water-soluble ash (4.21%) were all within API limits. Extractive values for aqueous and alcoholic extracts were 17.23% and 9.98%, respectively, indicating the presence of significant bioactive compounds. Phytochemical analysis revealed the presence of carbohydrates, alkaloids, saponins, tannins, proteins, amino acids, and flavonoids in both extracts. TLC analysis showed the presence of curcumin, a key active compound, through distinct Rf values. GC-MS profiling identified 16 volatile compounds with high match factors (>92.0), confirming the presence of bioactive constituents such as o-Cymene, Linalool, Cyclohexene derivatives, and turmerone-related compounds. These findings support the therapeutic relevance of *Curcuma longa* and provide a validated framework for its quality control and standardization. The integration of multiple analytical methods ensures the reliability and consistency of this herbal drug for medicinal use.

Keywords: *Curcuma longa*; Medicinal Plants, Standardization, Phytochemicals, Physicochemical, GC-MS, Quality Control.

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INTRODUCTION

Medicinal plants have long been integral to global healthcare practices, forming the basis of numerous modern drugs. One such plant, *Curcuma longa* L., commonly referred to as turmeric, has attracted considerable attention due to its wide-ranging therapeutic properties. Native to regions of South and Southeast Asia, turmeric has been extensively utilized in traditional systems like Ayurveda, Chinese medicine, and local folk remedies to manage ailments such as inflammation, infections, and gastrointestinal disorders.[1,2] The primary bioactive constituent, curcumin, along with several other secondary metabolites, contributes significantly to its pharmacological potential.[3] However, ensuring the consistent quality and safety of *Curcuma longa* remains a challenge due to factors like geographic origin, cultivation practices, and processing methods.[4] Quality control is essential not only for therapeutic efficacy but also for consumer trust and regulatory compliance.[5] Traditional assessment methods, such as organoleptic evaluation and powder microscopy, help in the initial identification and detection of adulteration.[6,7] These are supported by physicochemical tests like ash values, moisture content, and extractive values, which provide deeper insights into purity and stability.[8] Phytochemical screening is

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pivotal for identifying key compound classes including flavonoids, tannins, and alkaloids, many of which underpin the plant's biological activity.[9] Advanced technologies like Gas Chromatography-Mass Spectrometry (GC-MS) enable the detailed profiling of volatile compounds such as turmerone and curlone, known for their bioactivity.[2] This study adopts a comprehensive approach combining traditional techniques and modern instrumentation to evaluate the quality of *Curcuma longa*, aiming to establish robust standards for its therapeutic use.[10]

EXPERIMENTAL

Sample Procurement and Preliminary Evaluation

Rhizomes of *Curcuma longa* were procured from Bilwal Medchem and Research Laboratory Pvt. Ltd., Reengus, Sikar, Rajasthan, which operates under Good Manufacturing Practices (GMP) certification. The collected material was authenticated and assigned the sample identification code 24/AD/10/014. Initial qualitative assessment included organoleptic characterization based on color, odor, and taste, aligned with the standards set by the Ayurvedic Pharmacopoeia of India (API). The sample exhibited a vibrant yellow color, aromatic scent, and a mildly bitter taste typical of turmeric.[11]

Powder Microscopy

A microscopic analysis was conducted to authenticate the powdered rhizomes. For this, 5–20 mg of the powdered sample was treated separately with standard reagents, including diluted ferric chloride, methylene blue, eosin, and safranin. Each prepared slide was briefly passed over a flame for 2–3 seconds to improve reagent adherence, mounted in glycerin, and examined under a compound microscope for distinguishing anatomical features such as starch grains, tracheids, and parenchyma cells. All microscopic evaluations adhered to CCRAS laboratory protocols.[11,12]

Physicochemical Analysis

Physicochemical parameters of *Curcuma longa* were evaluated in line with pharmacopoeial protocols.[13,14] Moisture content was determined by drying 5 g of air-dried powder at 105°C until a constant weight was achieved, then cooled in a desiccator and reweighed. For the alcohol-soluble extractive value, 5 g of powdered rhizome was macerated in 100 mL of ethanol for 24 hours with periodic agitation, filtered, and the filtrate was evaporated and dried at 105°C to calculate the yield. The water-soluble extractive followed the same method, replacing ethanol with distilled water. Total ash content was assessed by incinerating 5 g of the sample in a silica crucible at 450°C for six hours, measuring the residual ash. Acid-insoluble ash was derived by boiling the total ash with hydrochloric acid, filtering, and weighing the residue. Water-soluble ash was determined similarly, using water to dissolve soluble salts from the total ash.

Phytochemical Screening

Phytochemical analysis of *Curcuma longa* was performed using aqueous and ethanol extracts to detect the presence of key secondary metabolites, following standard qualitative protocols.[14,15] Carbohydrates were identified through Molisch's, Benedict's, and Fehling's tests, which yielded characteristic color reactions. Alkaloids were confirmed using Dragendorff's, Wagner's, and Hager's reagents, all of which produced visible precipitates. Protein and amino acid presence was demonstrated by positive Biuret, Ninhydrin, and Xanthoproteic tests. A foam test verified saponins based on stable froth formation. Glycosides were detected via Borntrager's test, where a reddish-pink hue appeared upon phase separation. Phenolic compounds and tannins were revealed using ferric chloride, potassium dichromate, and lead acetate, which gave specific color changes or precipitates. Steroids were confirmed through Salkowski's test, evidenced by a red coloration. These results suggest the sample contains diverse bioactive constituents with potential therapeutic relevance.

Thin Layer Chromatography (TLC)

TLC was performed using silica gel 60F254-coated plates as the stationary phase. A solvent system composed of formic acid, ethyl acetate, and ethanol served as the mobile phase. Developed plates were visualized using vanillin sulfuric acid as a detecting reagent. The retention factor (Rf) values of each spot were calculated by comparing the migration distance of the solute to the solvent front.[15]

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was conducted using a crude powdered sample of *Curcuma longa* to identify its volatile and semi-volatile components. The sample was injected into the GC-MS Agilent 5975C system equipped with a DB-5MS column (30 m × 0.25 mm, 0.25 µm film thickness). Helium was used as a carrier gas at a flow rate corresponding to 7.0–9.5 psi and a 1:5 split ratio. The column oven temperature was initially held at 80°C for three minutes and increased by 2.5°C per minute until reaching 250°C. Compounds were identified by comparing retention times and mass fragmentation patterns with standard entries in the NIST MS library.[16]

RESULTS AND DISCUSSION

The standardization of *Curcuma longa* (turmeric) involved a multifaceted approach including microscopic evaluation, physicochemical and phytochemical assessments, chromatographic profiling via Thin Layer Chromatography (TLC), and identification of volatile constituents through Gas Chromatography–Mass Spectrometry (GC-MS). These analyses serve as essential tools for confirming the botanical identity, purity, and therapeutic potential of turmeric in pharmacognosy.

Powder Microscopy

Microscopic examination of *Curcuma longa* powder confirmed several diagnostic features consistent with the species' known morphology. Notably, starch granules, vascular elements such as vessels, calcium oxalate crystals, and cork cells were prominent, as shown in Fig.-1. These features are important in ensuring the material's authenticity and help distinguish genuine turmeric from potential adulterants.[14] The presence of these anatomical markers affirms the integrity of the sample and supports its use in herbal preparations.

Fig.-1: Powder Microscopy of *Curcuma longa*

Physicochemical Analysis

The physicochemical evaluation of *Curcuma longa* was conducted to assess its conformity with pharmacopoeial norms, as shown in Table-1. The moisture content (loss on drying) was 9.65%, within the allowable 12%, ensuring minimal microbial risk. Aqueous extractive value was 17.23%, and alcoholic extractive value was 9.98%, both exceeding standard limits, indicating a rich presence of water- and alcohol-soluble constituents beneficial for therapeutic formulations. Total ash content was measured at 4.9%, reflecting acceptable inorganic matter levels, while acid-insoluble ash was 0.71%, suggesting low siliceous contamination. The water-soluble ash was found to be 4.21%, representing a moderate quantity of soluble mineral salts.

Table-1: Physicochemical Analysis of *Curcuma longa*

S. No.	Tests	Value	
		Sample	API Reference Value
1	Loss on drying (%)	9.65	Not more than 12.0%
2	Aqueous Extractive Value (%)	17.23	Not less than 12.0%
3	Alcoholic Extractive Value(%)	9.98	Not less than 8.0%
4	Total Ash (%)	4.9	Not more than 9.0%
5	Acid Insoluble Ash (%)	0.71	Not more than 1.0%
6	Water Soluble Ash (%)	4.21	----------

These physicochemical findings demonstrate that the turmeric sample meets recognized quality parameters and supports its safe inclusion in herbal medicinal preparations.[17-19] These results collectively

confirm that the *Curcuma longa* sample adheres to quality standards, ensuring its suitability for medicinal use.

Phytochemical Screening

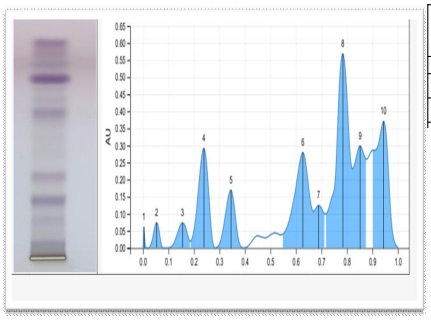
Preliminary phytochemical screening of *Curcuma longa* revealed diverse bioactive constituents in both aqueous and ethanol extracts, as depicted in Table-2. Carbohydrates were confirmed by Molisch's, Fehling's, and Benedict's tests, indicating the presence of reducing and non-reducing sugars, important for metabolic activity.[15] Alkaloids, detected using Dragendorff's and Wagner's reagents, are associated with antimicrobial and analgesic effects.[20] Proteins and amino acids tested positive with Biuret and Ninhydrin, highlighting nutritional relevance. Saponins and glycosides were found in the aqueous extract, suggesting water-solubility and roles in antimicrobial and anti-inflammatory responses. Phenolic compounds were present in the ethanol extract, supporting antioxidant activity.[21] Both extracts showed the presence of steroids and tannins, known for their anti-inflammatory and astringent properties. These results underscore how solvent choice influences the extraction of therapeutic compounds.

Table-2: Phytochemical Analysis of Both Aqueous and Ethanol Extracts of *Curcuma longa*

Test	Aqueous Extract	Ethanol Extract
Carbohydrates	Molish test (+ve), Fehling test (+ve), Benedict test (-ve)	Molish test (+ve), Benedict test (+ve), Fehling test (-ve)
Alkaloids	Dragendorff test (+ve), Hager's test (+ve), Wagner's test (-ve)	Dragendorff test (+ve), Hager's test (-ve), Wagner's test (+ve)
Amino acids	Ninhydrine test (+ve)	Ninhydrine test (+ve)
Proteins	Biuret test (+ve), Xanthoproteic test (+ve)	Biuret test (+ve), Millon test (+ve)
Saponins	Foam test (+ve)	Foam test (-ve)
Glycosides	Borntrager's test (+ve)	Borntrager's test (-ve)
Phenolic compounds	Phenolic test (-ve)	Phenolic test (+ve)
Steroids	Salkowski test (+ve)	Salkowski test (+ve)
Tannins	FeCl3 test (+ve), Potassium dichromate test (+ve)	FeCl3 test (+ve), Lead acetate test (+ve)

Thin Layer Chromatography (TLC)

TLC profiling of the ethanol extract was carried out using silica gel G60F254 as the stationary phase and a solvent system of chloroform: methanol (97:3). A total of 10 distinct spots were observed, with Rf values ranging from 0.003 to 0.944, as depicted in Fig.-2.



Test Method:	Sample: Ethanol Extract Stationary Phase: Silica gelG60F254 Mobile solution: Chloroform: Methanol (97:3)				
No of Spots	R_f	Hight	Area	%	Substance
1	0.003	0.0616	0.00032	0.26	Known
2	0.051	0.0733	0.00197	1.61	Known
3	0.154	0.0731	0.00321	2.63	Known
4	0.238	0.2915	0.01358	11.21	Curcumin
5	0.344	0.1691	0.00756	6.23	Curcumin
6	0.626	0.2791	0.01951	16.06	Curcumin
7	0.688	0.1244	0.00517	4.27	Known
8	0.785	0.5681	0.03291	27.14	Known
9	0.852	0.2983	0.01492	12.29	Known
10	0.944	0.3705	0.02214	18.26	Known

Fig.-2: Thin Layer Chromatography (TLC) Analysis of *Curcuma longa*

Among these, three spots corresponding to Rf values of 0.238, 0.344, and 0.626 were identified as curcumin, supported by their high area percentages. The most prominent spot, with an Rf of 0.626 and an area percentage of 16.06%, reinforces curcumin's status as a major bioactive marker in turmeric.[22] The TLC fingerprint not only confirms the presence of curcumin but also illustrates a complex phytochemical profile comprising phenolic and other active compounds. Such chromatographic data are essential for the standardization and quality assurance of herbal products.[23]

GC-MS Analysis

To characterize volatile and semi-volatile components, GC-MS was performed on the ethanol extract. Sixteen compounds were identified, as depicted in Fig.-3, each with match factors above 92%, indicating high confidence in identification.

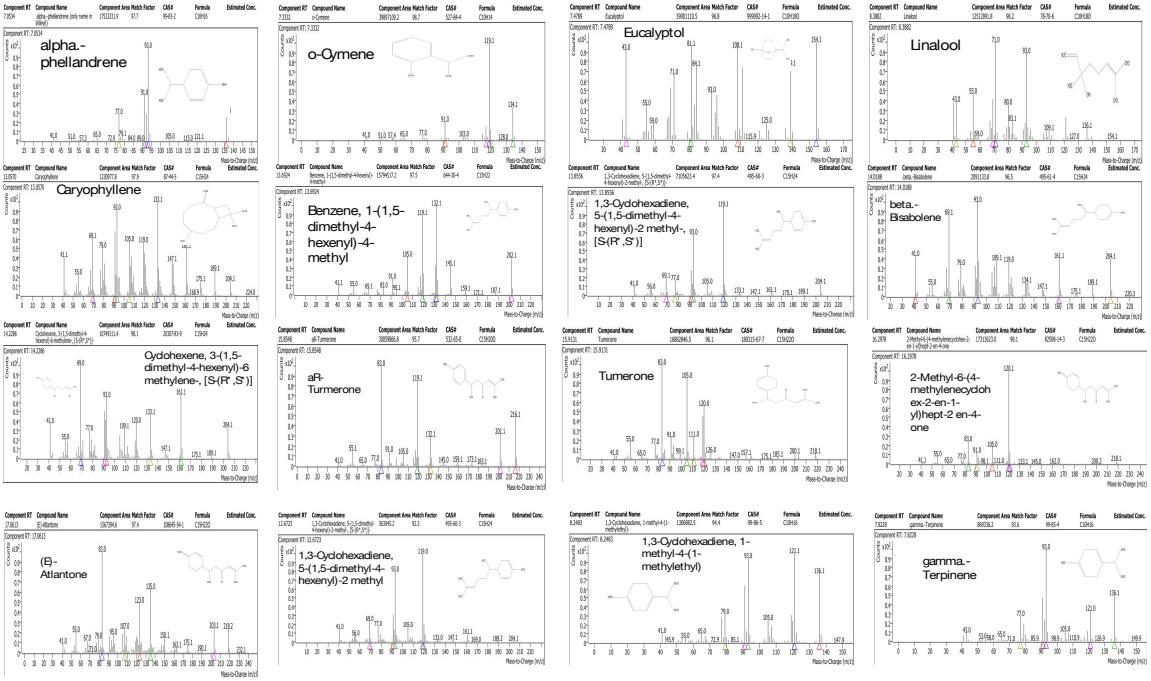


Fig.-3: GC-MS Analysis of the Ethanolic Extract of *Curcuma longa*

Prominent constituents included o-Cymene, Linalool, Eucalyptol, and Caryophyllene, all known for their aromatic and therapeutic properties.[24] Turmerone, ar-Turmerone, and (E)-Atlantone, three major sesquiterpenes specific to turmeric, were also identified. These compounds have been widely documented for their anti-inflammatory, neuroprotective, and antitumor potential.[25,26] Notably, *Curcuma longa* showed a rich presence of terpenoids and aromatic hydrocarbons, supporting its traditional use as an antimicrobial and anti-inflammatory agent. The GC-MS data establish a robust chemical fingerprint for turmeric's volatile profile, which is critical for quality control and therapeutic validation.

CONCLUSION

This study established a validated quality assessment protocol for *Curcuma longa* using integrated analytical techniques, including organoleptic analysis, microscopy, physicochemical evaluation, phytochemical screening, TLC, and GC-MS. The organoleptic and microscopic characteristics confirmed the sample's authenticity, while physicochemical tests complied with standards outlined in the Ayurvedic Pharmacopoeia of India, indicating good quality and stability. Phytochemical analysis identified essential bioactive groups linked to therapeutic activity. Thin-layer chromatography verified the presence of curcumin, and GC-MS revealed 16 volatile compounds such as turmerone and linalool, supporting the plant's pharmacological profile. The combination of these methods strengthens quality control and ensures consistency in herbal formulations. Overall, the findings support the medicinal value of *Curcuma longa* and provide a robust framework for its standardization, ensuring safe use in therapeutic and commercial applications. This comprehensive approach enhances the credibility and reproducibility of herbal medicine quality assurance practices.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-Being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

D. Lashkari https://orcid.org/0000-0003-1973-8075
T. Yadav https://orcid.org/0000-0001-7673-6127
P.K. Sharma https://orcid.org/0000-0003-0359-744X
S.K. Garg https://orcid.org/0000-0001-6834-3155
V. Kumar https://orcid.org/0000-0002-9994-8904

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[RJC-9364/2025]