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Phytochemical Analysis of Curcumin from the rhizome of Curcuma Longa

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Abstract:

The Zingiberaceae family includes the commonly used and well-liked Indian medicinal herb known as turmeric (Curcuma longa). Indian turmeric is recommended because it contains more curcumin than turmeric from other nations. Small molecular weight, lipophilic curcumin is a polyphenolic molecule. This extracted turmeric active ingredient from Curcuma longa, and gives turmeric its colour. Curcumin exhibits anti-inflammatory, antioxidant, antibacterial, and anticancer effects, among other therapeutic benefits. It has been noted that the chemical makeup of the majority of herbs varies with geographic location, possibly as a result of climate factors and biochemical variances. The current study focuses on the phytochemical analysis of turmeric rhizomes collected from the Gwalior District and the quantification of its curcumin content. Using a known technique, curcumin was extracted from the rhizomes of turmeric. TLC, FTIR, DSC, and XRD were used to characterize the separated curcumin.

Keywords: Curcumin, Curcuma longa, Characterization, Isolation

Introduction:

Every Indian kitchen has a spice known as "haldi," without which a meal cannot be complete. Turmeric is the common name for this substance, or "haldi." This well-known spice's biological name is Curcuma longa, also known as turmeric, is a member of the ginger family, Zingiberaceae. In India, turmeric is utilized for a variety of therapeutic purposes in the form of Ayurvedic, Unani, and Siddha medications in addition to its usage in cuisine [1]. Both the largest producer and user of this therapeutic spice, turmeric, is said to be India. Turmeric has a widespread appeal as a culinary pigment, preservative, and flavor. Over 2400 metric tonnes of turmeric are imported each year into the USA for consumer usage, according to the Food and Agriculture Organisation of the United Nations [2]. Although India is the primary producer of turmeric, this spice is also grown in Bangladesh, Pakistan, Sri Lanka, Taiwan, China, Burma (Myanmar), and Indonesia in Asia. Jamaica, Haiti, Costa Rica, Peru, and Brazil are among the countries in the Caribbean and Latin America where turmeric is grown [3,4]. Curcuma longa's rhizomes are cooked, dried, cleaned, and polished to produce turmeric [5]. The whole rhizomes are gathered after harvesting [6]. These rhizomes are shipped in their whole state. They often resemble fingers,

measuring 2 to 8 cm long by 1 to 2 cm broad, with bulbs and cracks [6, 7]. To make turmeric powder, further processing is applied to the dried rhizomes.' Alleppey' and 'Madras' are two well-known varieties of turmeric that are produced in different parts of India. Alleppey is mostly imported from the United States and contains 3.5% to 5.5% volatile oils and 4.0% to 7.0% curcumin [4,6,8]. Only 2% of volatile oils and 2% of curcumin make up the Madras type, which is mostly preferred by Middle Eastern and British nations [6]. Both are employed in the food industry. India uses turmeric, which is made in Bengal, as a colour [3]. The United States does not support other producers of turmeric, such as those in the Caribbean, Central America, or South America, because their product has a lower percentage of curcumin and volatile oil than Indian kinds and is darker in colour [6,9]. The initial evaluation of turmeric's quality is based on the rhizome's outward appearance, consistency of colour, smoothness of coating, and audible breaking snap [4].

Curcumin is the primary ingredient in turmeric. Polyphenol curcumin gives turmeric its colour. As a polyphenol with a lipophilic nature, curcumin is soluble in ethanol, dimethylsulfoxide, and other organic solvents but insoluble in water and ether [10]. The stomach's acidic pH makes curcumin stable [11]. Volatile oils such as tumerone, atlantone, and zingiberone as well as carbohydrates, proteins, and resins are also found [12]. Turmeric is frequently used as a medication to treat a variety of illnesses because it contains volatile oils and the active ingredient curcumin. As an anti-inflammatory, it is widely used [13–16]. Thus, it also has antioxidant properties that are much greater than those of vitamins E and C [17]. The anticancer efficacy of the rhizome extract had been investigated [18]. Antimicrobial activity (against bacteria and fungus) is one of the other activities displayed [19,20]. Turmeric has beneficial cardiovascular benefits because it reduces cholesterol levels and prevents platelet aggregation [21,22]. Additionally, studies have shown that turmeric is useful in treating conditions including Alzheimer's, rheumatoid arthritis, and cancer [23,24]. The current study focuses on the phytochemical analysis of turmeric rhizomes collected from the Gwalior District and the quantification of its curcumin content. Using a known technique, curcumin was extracted from the rhizomes of turmeric. UV, FTIR, DSC, and XRD were used to characterize the separated curcumin.

Materials and Methods:

All the chemicals and reagents used were of analytical reagent grade. Pure Curcumin (Purity=95%) was obtained from Sigma Aldrich, Mumbai, India.

Collection and processing of plant material:

In the months of January and February, fresh turmeric rhizomes were harvested from the Gwalior District in the Indian state of M.P. The gathered rhizomes were cooked in water for around 30 minutes before being sun-dried. The dried rhizomes were shrunk in size and passed through a fine-mesh filter.

Extraction process:

95 percent ethanol was used to extract the coloring from powdered turmeric rhizome in a Soxhlet assembly. By evaporating ethanol, the resultant crude extract was concentrated into a semisolid brown mass.

Pre-phytochemical analysis of the crude extract:

Using numerous phytoconstituents, including proteins, carbohydrates, terpenes, steroids, flavonoids, tannins, and saponins, the crude extract was examined for their existence. Precipitation and coloring responses that are often used and recorded in standard reference books. Whatman No. 1 filter paper was used to filter the extract, which was then diluted in 10 ml of distilled water. The filtrate was then tested for the presence of carbohydrates.

Test for Carbohydrates:

a) The Molish test involved adding 2 ml of a solution to a test tube. Molish Reagent was added in a single drop. Conc. HCL in the amount of 2 ml was introduced from the test tube's sides. The test tube was observed to see if a violet ring formed. There are carbohydrates present, as evidenced by a Violet ring at the confluence of the two liquids [26].

Test for Proteins:

Protein analysis was performed on the filtrate after the extract was dissolved in 10 ml of distilled water and passed through Whatmann No. 1 filter paper. The filtrate was then tested for proteins and amino acids.

a) **Millon's Test:**

A few drops of Millon's reagent are added to 2 ml of filtrate for the Millons test. The outcome was seen. The presence of proteins was shown by a white precipitate.

b) **Biuret test:** A drop of 2% copper sulphate solution was added to a 2 ml sample of filtrate. This was followed by the addition of extra potassium hydroxide pellets and 1 cc of 95% ethanol. The ethanol layer's pink tint showed the presence of proteins.

Test for Alkaloid: Three milliliters of a 50 mg solvent-free extract were combined with ml of dilute hydrochloric acid and then filtered thoroughly. The filtrate was tested carefully with various alkaloid reagents as follows:

a) **Mayer's Test:** A few drops of Mayer's reagent are introduced by the side of the test tube to 1 ml of filtrate as part of the Mayer's test. A positive test result was indicated by the white or creamy precipitate.

b) **Wagner's test:** A few drops of Wagner's reagent are added by the side of the test tube to 1 ml of filtrate. The alteration in colour was seen. The presence of a reddish-brown precipitate verifies the test's success.

c) **The Dragendorff test**: which involves adding 2 ml of Dragendorff's reagent to 1 ml of filtrate and carefully observing the outcome. The presence of a noticeable yellow precipitate indicates a favourable result [27].

Glycosides

a) **The Borntrager Test:** Extract was heated in diluted sulfuric acid, filtered, and then chloroform was added and vigorously agitated. After separating the organic layer, ammonia is gradually added to it. The ammonical layer's pink to red colour indicates the presence of gylcoside.

b) **Legal Test:** The test is used to glycosides that include digitoxose. The extract was dissolved in pyridine, and then an alkaline solution of sodium nitroprusside was added. Gylcosides are present and are indicated by pink or red colour.

Terpenoid and steroid: 0.5 ml of acetic anhydride and 0.5 ml of chloroform were applied to four milligrammes of extract. After adding a progressively increasing concentration of sulphuric acid, red violet colour for terpenoids and green blue colour for steroids were seen [28]. Four millilitres of extract solution were treated with 1.5 millilitres of a 50% methanol solution to produce flavonoids. Metal magnesium was added after the solution had been warmed. Five to six drops of strong hydrochloric acid were added to this mixture, and the result was a red colour for flavonoids and an orange colour for flavones.

Tannins: 1 ml of water, 1 or 2 drops of ferric chloride solution, and 0.5 ml of extract solution were added. Gallic tannins were found to be blue in colour, whereas catecholic tannins were found to be green black [29].

Saponins: 0.2 g of the extract were brought to a boil after being agitated with 5 ml of distilled water. The presence of saponins is indicated by foaming, which has a creamy texture and tiny bubbles [30].

Isolation of curcumin from Curcuma Longa:

Alcohol was used to dissolve the crude extract before filtering it. Concentrated filtrate was produced. The resulting concentration was dissolved in benzene. Benzene solution received a 0.1% w/v addition of sodium hydroxide. The two layers of curcumin were divided using a sprinkling funnel. Curcumin was precipitated by applying a diluted hydrochloric acid solution to a sodium hydroxide layer. The resulting precipitate was dried after being vacuum filtered. Further analysis was conducted using the extracted curcumin.

Evaluation of Isolated curcumin by thin-layer chromatography (TLC):

On precoated silica gel G plates (Stationary Phase), TLC of the extracted curcumin was carried out with n-hexane and ethyl acetate in a 7:3 solvent system. As a benchmark, curcumin was used. The plate was sprayed with vanillin-sulphuric acid reagent to perform the detection. Calculated Rf values for the separated spots were compared to values found in the literature as well as the Rf value of pure curcumin.

Fourier transform infrared spectroscopy (FTIR) of isolated curcumin:

Using an FTIR Spectrophotometer (IR Affinity-1, Shimadzu, Japan), a curcumin spectrum in isolation was captured in the infrared spectrum. Using the potassium bromide disc technique, the IR spectra of the materials were acquired across the scanning range of 500 to 4000 cm-1.

Differential Scanning Colorimetry of isolated curcumin:

Thermal characteristics of CU were analyzed using DSC (**DSC 821e, Mettler-Toledo International Inc., and Switzerland**). Each example (3-5 mg) was warmed in a creased aluminum skillet somewhere in the range of 25±0.5 °C and 300±0.5 °C at a filtering pace of 20±0.5 °C/min and under a nitrogen stream of 40 ml/min. Instrument was recently adjusted with void search for gold and indium for heat rate [24].

X-ray Diffractometry (XRD) of isolated curcumin:

The X-ray diffraction was recorded using D8 ADVANCE diffractometer (Bruker, Germany), operated at a voltage of 40 KV and a current of 40 mA. The samples were investigated in the 2θ angle range of 5–55° and the process parameters were set as: scan step size of 0.02°, scan step time of 17.7 s.

Result and Discussion:

Extraction and pre-phytochemical analysis of curcumin:

Extraction of curcumin 95 percent ethanol was used to extract the coloring from powdered turmeric rhizome in a Soxhlet assembly. By evaporating ethanol, the resultant crude extract was concentrated into a semisolid brown mass. Extraction by Soxhlet apparatus of curcumin depicted in figure-1.

Figure: Extraction of Turmeric by Soxhlet extraction process

The results of the pre-phytochemical analysis of curcumin are depicted in table-1 and figure-2. The results indicated the presence of Amino acids, Proteins, Alkaloids, Carbohydrates, Flavonoids, and Terpenoids.

Table-1 Pre-Phytochemical Evaluation of curcumin

Sr. No.	Test	Observation
1	Carbohydrates	+ve
2	Proteins and Amino-acids	+ve
3	Alkaloids	+ve

4	Glycosides	-ve
5	Terpenoids and Steroids	+ve (Terpenoid)
6	Flavonoids	+ve
7	Tannins	-ve
8	Saponins	-ve

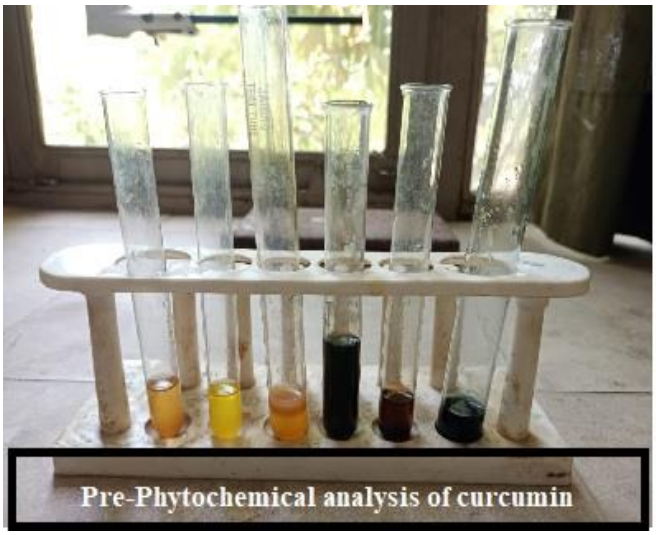

Figure-2 Pre-phytochemical analysis of curcumin

Thin Layer Chromatography of curcumin:

Bright yellow characterised the curcumin powder that had been isolated. Table 2 displays the outcomes of TLC. The Rf value of the test solution's intense spot matched the Rf value of the standard curcumin. Due to the test solution, two or more dimmer spots were seen. The existence of additional curcuminoids in the separated curcumin may be the cause of these spots.

Table-2 Results of TLC study of curcumin

Sample	**Rf value**
Standard curcumin	Spot = 0.457
Test sample (Isolated curcumin)	Spot 1 = 0.064
	Spot 2 = 0.244
	Spot 3 = 0.468 (Intense spot)

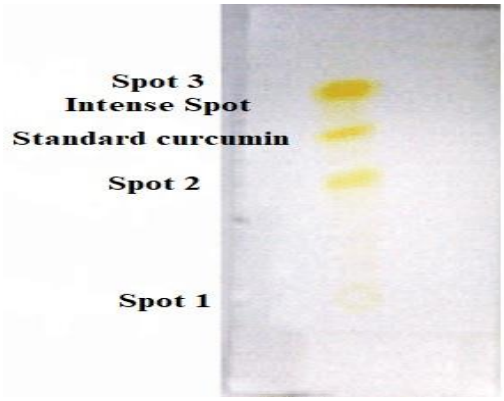

Figure-2 TLC spots of curcumin

FTIR Analysis of Curcumin:

Curcumin's chemical structure is shown in Figure 3, which provides information on the numerous functional groups the substance has. FTIR provided further evidence supporting the separated curcumin's identification. Curcumin's FTIR spectrum revealed a recognizable stretching band of O-H at 3512 cm-1. O-H. C-H stretching is shown by the peak at 3014 cm-1, while C=C symmetric aromatic ring stretching is represented by the peak at 1602 cm-1. C=O is represented by the peak at 1506 cm-1, whereas the enol C–O peak was found at 1280 cm-1 and the benzoate trans–C–H vibration was at 962 cm-1. The curcumin's FTIR spectrum matched the FTIR spectrum described in the literature [31]. Figure 4 shows the FTIR spectrum of extracted curcumin from turmeric rhizome harvested in the Gwalior district. The identified ingredient is curcumin, according to the findings of a TLC and FTIR investigation.

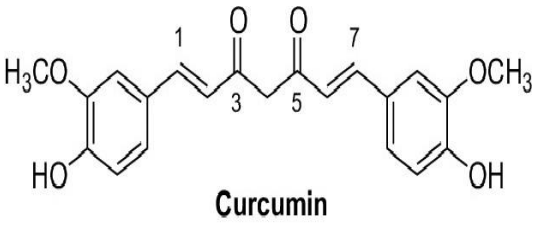

Figure-3 Chemical Structure of Curcumin

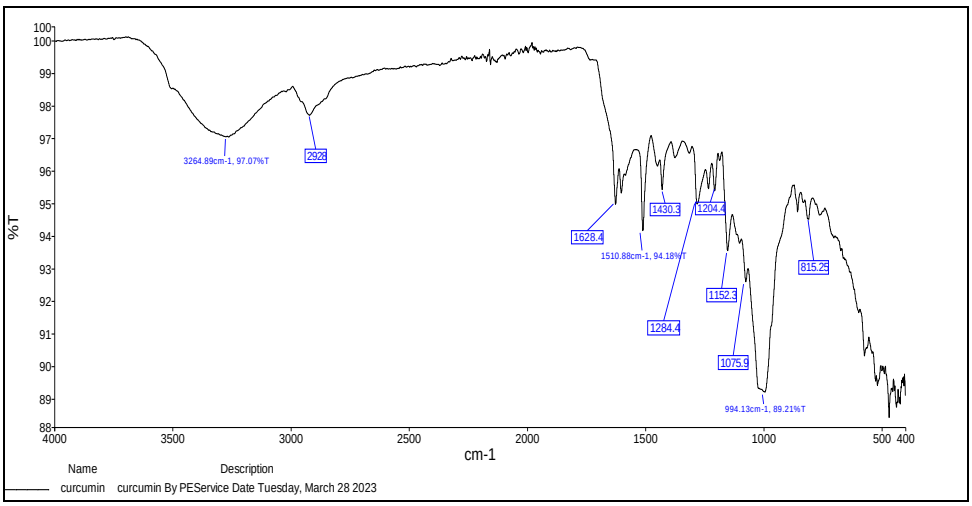

Figure-4 FTIR Of Curcumin

DSC Analysis:

Curcumin DSC curves were displayed in Figure 5. At 104 °C, curcumin displayed a pronounced endothermic peak.

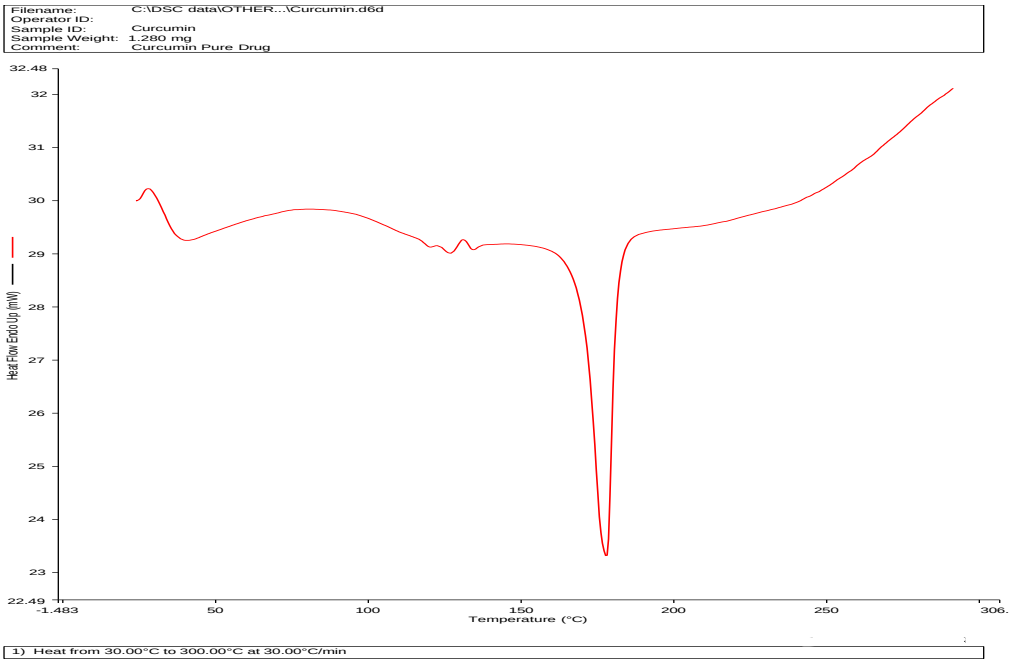

Figure-5 DSC of Curcumin

XRD Analysis:

The curcumin XRD spectrum depicted in figure 6 indicates that the powder is semi-crystallized.

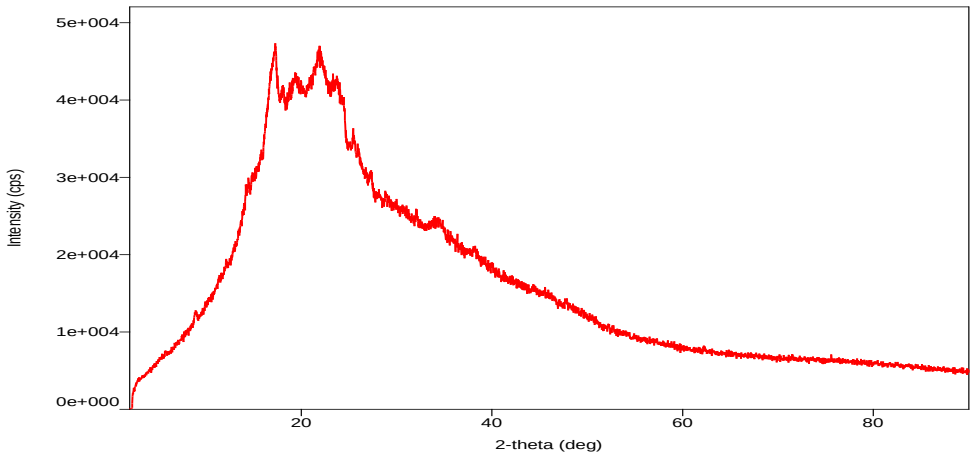

Figure-6 XRD of powder curcumin

Conclusion:

In this work, the pre-phytochemical analysis of curcumin from Curcuma longa rhizomes, followed by the extraction of turmeric, the phytochemical analysis of curcumin by TLC, and the successful determination of the physical-chemical properties of curcumin by XRD, FTIR, and DSC. The amount of curcumin in turmeric

determines how therapeutic it is. Turmeric's colour, quality, and medicinal value are all influenced by the amount of curcumin it contains. As a result, turmeric's curcumin component is crucial both commercially and medically. It is necessary to do more research to examine the formulation of curcumin-loaded nanoparticles for targeted distribution.

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