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Research Article

Extraction, Isolation, Identification and Characterization Method Development of Turmeric (*Curcuma longa*) Rhizomes by Column Chromatography

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Abstract

The title of this article is entitled as "Extraction, Isolation, Identification and Characterization Method Development of Turmeric (Curcuma Longa) by Column Chromatography". It is a research project.

Turmeric, curcuma longa L of Zingiberaceae family is a widely cultivated spice in India and other Asian countries. Curcumin is the main coloring substance in Curcuma longa and two related compounds, demethoxycurcumin and bisdemethoxycurcumin are altogether known as curcuminoid. Turmeric is rich in curcuminoids, and recognized for their broad spectrum of biological activities, varying in chemical structures, physico-chemical characteristics as well as the functional properties it posses varied factors for its characterization.

This research article basically involves to find a suitable organic solvents, and reliable method of extraction to get maximum yield and then Identification separation process of components by Column Chromatography, their phytochemical study and chemical components analysis by UV Spectrophotometer.

Key findings: Curcumin have been extracted and purified by extraction process that involves maceration and hydrodistillation of rhizomes.The use of column chromatography by 2 different Stationary phase is examples of traditional purification procedures and gives better understanding to identify a suitable method to relay on and get maximum of purified product, with its UV analysis.

Keywords: Turmeric root; Extraction; Column Chromatography; Silica Gel; Alumina; Phytochemical Test; UV Analysis

Introduction

Turmeric a herb native of tropical South Asia, as a dried rhizome of an herbaceous plant. Its bright yellow pigment is used as a food coloring agent and has been used as spice and food preservative for its various medicinal properties bearing anti-inflammatory, antibacterial, antidepressant, antioxidant, anticancer, depressive effects, diabetes, obesity, Alzheimer's disease, and stroke [1,2]. According to reviewed literature reported percentage composition of curcuminoids derivatives is around 3-5% comprising three derivatives including curcumin (75%), demethoxycurcumin (10-20%), and bisdemethoxycurcumin (5%) [3].

Curcumin is soluble in methanol, chloroform, ethanol, acetone, and practically insoluble in water [4]. The choice of an appropriate solvent is one of the factors that impact the extraction process and has a significant impact on the yield and composition of the extracts that are generated [5,6]. Chromatography has been employed for separation of mixtures, principle being differential partitioning between the mobile and stationary phases, being a preparative technique to purify compounds depending on their polarity or hydrophobicity [7]. Then utilizing UV spectroscopy, quantitative analysis technique to measures absorbance of light intensity and identification of bands obtained as a result of column, being cost-

effective, simple, versatile, non-destructive and suitable for a large spectrum of organic compounds [8]. Curcumin in ethanol has a broad UV-visible absorption at around 300-500 nm. The maximum absorption band is at 425 nm [2].

Experimental procedure

Material

Curcuma longa (Turmeric) rhizome were collected from narayana brand variety consisting of 6% of curcumin. Solvents(Ethanol, Silica gel, Alumina, Mayer regent, Ferric Chloride etc.) used in research were acquired from Merck and Lab chem.

Method: Extraction

To implement a conventional methods for extraction of Curcumin and is ecologically friendly green technology. The primary idea behind sample extraction was to identify a method which gives better extracts.

Table 1: Summary on Extractions of Curcuminoids.

Parameter	Maceration	Hydro Distillation
Temp	25 ± 5°C	50 ± 5°C
Duration	7 Days	12 Hours
Solvent	80% C_2H_5OH	80% C_2H_5OH
Turmeric quantity	20g	20g
Solvent	100ml	100ml

Hot Extraction - Hydro distillation

Dried rhizomes were milled and then soaked in 80% ethanol to make rhizome cell membranes permeable and then allowed for steam distillation boiling, liquefaction of essential oil in the solvent inside glands. Process involves solvent to permeate plants cell membranes via osmosis and transferred in to steam. Also speed of essential oil vaporization is affected by its degree of solubility in solvent and is not by oil components volatility. The time to distillate milled plant material is less than that for the non-milled plant.

Cold Extraction - Maceration

Dried rhizomes were grinding into powder, and then allowed for maceration in a beaker with solvents and kept at room temperature for 25 ± 5°C for 7 days. The contents are shaken every 24 hours for seven days.

Figure 1: Hydro distillation apparatus.

Figure 2: Maceration of rhizome with solvent.

Then Rotatory evaporator was utilized to remove solvent from a sample by evaporating it under reduced pressure.

Figure 3: Rotatory Evaporator assembly.

Percentage Yield- calculation of yield obtained by evaporation of solvent.

% yield = PY/TY x100

PY= Practical Yield

TY= Theoretical Yield

Identification test/preliminary phytochemical screening

Preparation of the extract

The rhizomes of *Curcuma longa* were grinded to get a fine powder, which were utilized for study.

Test for alkaloid

The extract was mixed with 3 ml of dilute hydrochloric acid and then filtered thoroughly. The filtrate was tested carefully with following test:

- **Mayer's Test**: To a 1 ml or 2 ml of filtrate, few drops of Mayer's reagent are added by the side of the test tube. The white or creamy precipitate indicated test as positive (presence of alkaloids).

Test for glycosides

To 2 ml test solution, added with equal quantity of Fehling's solution A and B and solution was heated gives the positive result of glycoside. A brick red precipitate was observed [8].

- **Keller-Killani Test:** To 2 ml glacial acetic acid containing a drop of $FeCl_3$ treated with extract. Formation of a brown colour ring indicates the presence of glycoside.

Test for flavonoids

- **Alkaline Reagent Test:** The test solution, was treated with sodium hydroxide solution, which gives a yellow or red colour.

Test for tannins

- **Ferric Chloride Test:** The extract solution mixed with drops of ferric chloride solution. Presence of gallic tannins, blue colour was observed and green black for catecholic tannins.

Test for triterpenoids

- **Salkowski Test:** The test solution was added with 2 ml chloroform and few drops of conc. Sulphuric acid (3 ml), and shaken well. Formation of reddish brown colour at lower layer indicates presence of steroids and yellow colour shows the presence of triterpenoids.

Test for phenol

- **Ferric Chloride Test:** 4 drops of Alcoholic $FeCl_3$ solution were added in the test extract. Appearance of bluish black colour indicates the presence of phenol.

Test for fats and fixed oils

Stain Test: Between the two filter papers small amount of the extract was pressed, the stain on the filter paper indicates the presence of fixed oils.

Test for proteins and amino acids

- **Ninhydrin Test:** To 2 ml test solution, ninhydrin solution was treated and then boiled. Formation of blue colour indicates the presence of amino acid. Again 2ml test solution, 0.2% ninhydrin solution was treated with amino acids and proteins, then boiled shows a violet colour.

Test for carbohydrates

The extract was dissolved in 5-10 ml of distilled water and filtered through Whatmann No.1 filter paper and the filtrate is used for the following test of carbohydrates.

- **Molish Test:** Firstly 2 ml solution was placed in a test tube then 1 drop of Molish Reagent was added. 2 ml of conc. HCl was added from the sides of the test tube. A violet ring was observed in the test tube. Formation of a violet ring at the junction of the two liquids indicates presence of carbohydrates.

Column Chromatography

Column adsorption chromatography is a useful technique for producing individual curcuminoids at a large scale with high purity. For instance, using a silica gel-based adsorption chromatography technique using ethanol as the mobile phase to separate curcumin, and its other components.

Column chromatography is a commonly used technique in chemistry to separate and purify compounds from a mixture. First of all Column was packed with glass wool at the bottom then sample (turmeric acid) was mixed with stationary phase (Silica Gel and Alumina) to make a slurry, then loaded the sample slurry in column, then left it to dry. (80:20) Ethanol: Water solvent was introduced based on the polarity of compounds and the stationary phase, monitored the elution process using a UV lamp [5].

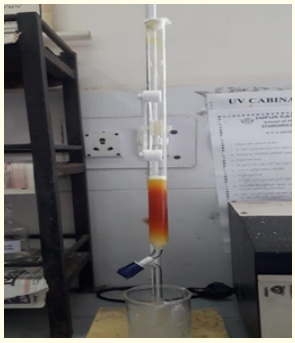

Figure 4: Column Chromatography Silica gel.

Figure 5: Column Chromatography Alumina.

UV

Ultraviolet-visible (UV-Vis) spectroscopy is a quantitative technique, used to measure absorbance, measuring the intensity of light that passes through a sample in comparison to a reference or blank sample. Being cost-effective, simple, versatile, non-destructive method is being widely used for large spectrum of organic compounds and some inorganic species quantification estimation, hence this approach was used to assess the purity of separated curcuminoids and other components [6].

Result and Discussion

Organoleptic properties

- **Description:** Externally yellowish to yellowish brown with root scars and annulations.
- **Odour:** Aromatic
- **Taste:** Warmly aromatic and bitter
- **Storage:** Protected from moisture.

Extraction

- The outcomes demonstrated that 80% ethanol extraction at 50°C for 6 h generated the highest yield. In accordance with it, ethanol was the most favored organic solvent for extracting curcumin.
- Another extraction technique that was adopted for bioactive substances and essential oils is hydro distillation. To obtain flavor-free curcumin, it has been used to extract the essential oil from raw turmeric powder. A good yield of deodorized turmeric was produced following hydro-distillation, deodorized turmeric contained the same amount of curcuminoids as the control sample and a sample that had undergone extraction using hexane for deodorization. These outcomes demonstrated the efficacy of the hydro-distillation technique with negligible turmeric taste and color.

Percentage yield

Extraction with Hydro distillation (Hot Process)
Percentage Yield = 4.15/100 x 100 = 4.1%

Extraction with Maceration (Cold process)
Percentage Yield = 8.35/100 x 100 = 8.3%

Identification test/preliminary phytochemical screening

Test for Alkaloid

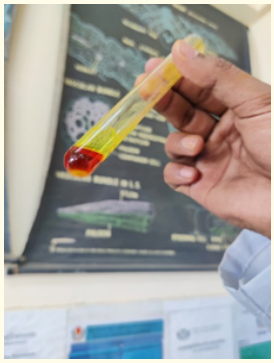

Figure 6: Test for alkaloid- white/creamy precipitate at bottom.

Test for Glycoside

Figure 7: Test for glycocides- formation of brown colour ring.

Test for Flavonoids

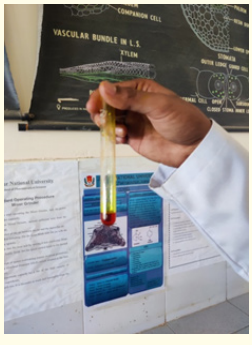

Figure 8: Test for flavanoids- yellowish red precipitate at bottom.

Test for Triterpnoids

Figure 9: Test for terpenoids- bluish green precipitate at bottom.

Test for Phenol

Figure 10: Test for phenol- bluish black colour.

Test for Fixed oil/ Fats

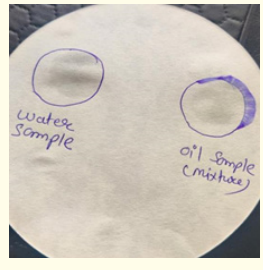

Figure 11: Test for fixed oil- oil spot on filter paper.

Test for Protein and Amino acids

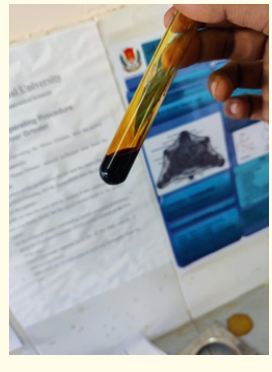

Figure 12: Test for protein- blue voilet colour.

Test for Carbohydrates

Figure 13: Test for carbohydrates- voilet ring at junction.

UV Spectroscopy

UV absorbance (Column chromatography)	Alumina	Silica Gel
1st band	2.778	0.380
2nd band	0.431	0.283
3rd band	-0.034	0.293

Table 2

Conclusion

Over the past few decades, a large amount of research on the extraction and purification of curcumin from plant sources has been published. This study's main objective is to give researchers and industry experts a comprehensive understanding of the various processes for extracting and purifying curcumin from plant sources so that they may obtain effective curcumin. Curcumin extraction techniques should use safe solvents, generate a high yield, and be energy efficient. Although traditional extraction methods have been demonstrated to be time-consuming, notably with Soxhlet, it has been established in several studies that they compare favorably to more modern extraction methods. Some of the main advantages of the conventional extraction method are its ease of use, minimal operational expenses, and, therefore, reasonable price. The results also demonstrate that the temperature and pressure of the extraction had the greatest effects on the curcuminoid extraction.

The purifying procedure is essential for the generation of curcumin. Both conventional (such as column chromatography and HPLC) and advanced techniques (such as counter-current chromatography and thin-layer column chromatography, crystallization, etc.) have been thoroughly studied. The extra innovative methods have exceptional accurate and provide high-quality yields quickly and affordably. Numerous research on the extracting and purifying of curcumin has focused on the possible implementation of curcumin in various industries, including pharmaceuticals, cosmetics, and food. The biological effects of free and encapsulated curcumin as well as its potential industrial applications have been extensively studied in the literature.

Acknowledgements

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Authors' Contributions

Ankita Raikwar designed the study, along with Nikhil Saini and Nitesh Sahu carried out the laboratory work. Ankita Raikwar collected and analyzed the data. Ankita Raikwar and Ashwini Kumar wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing Interests

There are no competing interests for this research project.

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