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Evaluation of Natural Dye for DNA Visualization in Agarose Gel

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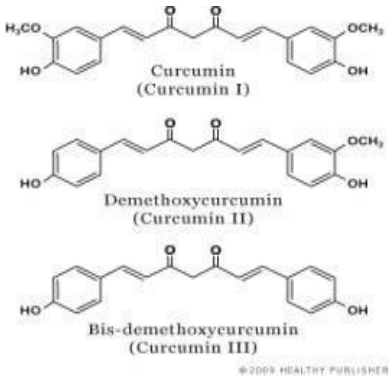
Abstract **- Public Curcumin, a natural polyphenol extracted from turmeric, has been explored as a novel DNA stain for molecular biology applications. This study aims to extract the curcumin from turmeric and the extracted curcumin is confirmed by analytical techniques. Further the curcumin is studied for its application as natural dye for staining the DNA in Agarose gel. A solvent extraction method using ethanol was employed, followed by purification using column chromatography. The purified curcumin was characterized using HPLC and spectroscopic techniques. The extracted curcumin was then evaluated as a DNA stain for agarose gel electrophoresis, demonstrating superior sensitivity and specificity compared to conventional DNA stains. This study provides a robust and efficient method for extracting and purifying curcumin for DNA visualization, paving the way for its potential applications in molecular biology and genomics.**

Keywords: Agarose gel, Ethidium bromide, Curcumin, natural dye, molecular biology.

I. INTRODUCTION

Turmeric is the native of tropical South Asia. It needs temperatures between 20º C and 30º C and a considerable amount of annual rainfall to thrive. As a dried rhizome of an herbaceous plant, turmeric is closely related to ginger. The spice is also sometimes called "Indian saffron" thanks to its yellow colour. Turmeric is a spice that comes from the root Curcumalonga L., a member of the ginger family (Zingaberaceae). Its bright yellow pigment is used as a food coloring agent. It has been used for centuries as a spice and a food preservative, and for its various medicinal properties 1.

The extract of turmeric has many medicinal properties including antioxidant, anti- inflammatory, antiviral, antibacterial, antifungal, and cancer chemo preventive actions. Curcumin, a yellow compound isolated from its rhizome, may be responsible for the bioactive effects. Recent research shows that curcumin may inhibit carcinogenesis and angiogenesis. They may have a potential to improve chronic inflammatory conditions in obesity. Curcumin is a liposoluble compound and can be easily dissolved into organic solvent such as methanol, ethanol, and acetone. However, poor water solubility often limits its biomedical uses using aqueous systems. This observation prompted us to examine turmeric extracts as a delivery system for curcumin and to examine the possibility of turmeric extract itself as candidate agent for pharmacologic evaluation.



In this preliminary study the total of curcuminoids which is about 4-6%, turmeric also contains 2-4% essential oil and 2-3% of fixed oil and various volatile oils, including turmerone, atlantoneand zingiberone. Other constituents include sugars, proteins and resins.

The choice of solvents for extraction is restricted to the few solvents of defined purity allowed by national and international food laws in the processing of food materials. Hexane, acetone, alcohol (ethanol, methanol), isopropanol and ethyl acetate are used in the extraction of oleoresins of spices. From consideration of solubility of active constituents, the curcuminoids are poorly soluble in the hydrocarbon solvents. Alcohol and acetone are good extractants and the yields can also be expected to be high because of extraction of non-flavor components. Soxhlet extraction of turmeric powder with acetone gave a yield of about 4.1% containing in 3 hours. Acetone as solvent was slightly superior to alcohol and ethyl acetate, the curcuminoids content also is on the high side, suggesting selective extraction. The results of extraction with acetone have, however been reported to give high yields of curcuminoids than alcoholic and remaining extraction The total of curcuminoids which is about 4-6%, turmeric also contains 2-4% essential oil and 2-3% of fixed oil and various volatile oils, including turmerone, atlantone, and zingiberone. Other constituents include sugars, proteins and resins.

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II. OBJECTIVES

- To extract curcumin from turmeric.
- To confirm the extracted curcumin by analytical methods(Thin layer chromatography,UV-Visible spectroscopy and HPLC).
- To check the efficiency of curcumin to stain DNA in agarose gel by agarose gel electrophoresis

III. METHODOLOGY

1) Extraction of Curcumin

A. Preparation of Turmeric Powder

- Turmeric roots
- Washed under tap water
- Washed with distilled water
- Air dry
- Powder

B. Solvent Extraction

- Powder
- Extract with ethanol(1:10)(turmeric:solvent)
- Collect the extract
- Distillation
- Drying
- Extraction: 10 g of turmeric powder was mixed with 100 mL of 95% ethanol. The mixture was stirred for 24 hours at room temperature.
- Filtration: The mixture was filtered to remove insoluble residues.
- Concentration: The filtrate was evaporated using a rotary evaporator to obtain a concentrated curcumin .
- Purification: The crude extract was purified using silica gel chromatography with a solvent system of chloroform:methanol (9:1).
- Characterization: The isolated curcumin was characterized using UV-Visible spectroscopy and TLC.

2) Analytical Techniques

A. Insolubility

A curcumin insolubility test essentially involves attempting to dissolve a sample of curcumin in water, where, due to its hydrophobic nature, it will largely remain undissolved, demonstrating its poor water solubility; this is considered a key characteristic of curcumin, indicating the need for special formulations to improve its bioavailability when used in medicinal applications.

B. Qualitative Test

A qualitative test for curcumin typically involves observing a bright red color in a solution when a sample containing curcumin (like turmeric extract) is dissolved in a suitable solvent, usually sulphuric acid due to curcumin's inherent yellow pigment.

C. Absorption Spectrum

A curcumin absorption spectrum test, typically performed using a UV-Visible spectrophotometer, measures the absorbance of curcumin at different wavelengths of light, revealing a characteristic broad peak with maximum absorption occurring around 425nm, which is attributed to the molecule's π-π^* electronic transitions; this peak is used to quantify the concentration of curcumin in a sample, as the absorbance at this wavelength is directly proportional to the curcumin content.

D. Thin layer chromatography

For checking the presence of curcumin through Thin-layer chromatography (TLC Method) method can be used.

3) Methodology of TLC

- Thin layer plate preparation
- Sample application
- Plate development
- Component detection

A. Thin layer plate preparation

- The stationary phase is prepared as a slurry with water or buffer at 1:2 and applied to a glass plate.
- Calcium sulphate CaSo4.1/H2O,(Gypsum) (10- 15%) is incorporated to the adsorbent as a binder, as it facilitates the adhesion of the adsorbent to the plate.
- After application of the adsorbent, the plates are air-dried for 10-15 min and then oven-dried for 10- 15 min at 50°C-60° C.
- This process is also known as activation of the adsorbent.

B. Sample application

- Draw a line lightly with a pencil about 1.5-2.0 cm from the bottom. The sample was spotted using micropipette tip.

C. Plate Development

- The chromatographic tank was filled with the developing solvent to a depth of ~1.5 cm and equilibrated for about 2 hrs.

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- The thin-layer plate is placed gently in the tank and allowed to stand for about 60 min.
- Make sure the spots do not touch the solvent directly.
- Capillary action causes the solvent to ascend as in paper chromatography and the separation of compounds takes place.
- As the solvent front reaches about 1-2 cm from the top of the plate, the plate is removed,solvent front is marked with a pencil immediately and allowed to air-dry placing the plate upside down.

D. TLC

1. Slurry- Silica gel + Distilled water Solvent system – Chloroform : methanol (9:1) Spraying reagent – H2SO4 (Sulphuric acid)
2. Slurry – Silica gel + Gypsum + Al2O3 (Aluminum nitrate) + Distilled water Solvent system – Chloroform:methanol (9:1)
3. Preparation of TLC plate.

Figure 1: Slurry preparation

Figure 2: Spotting of Sample

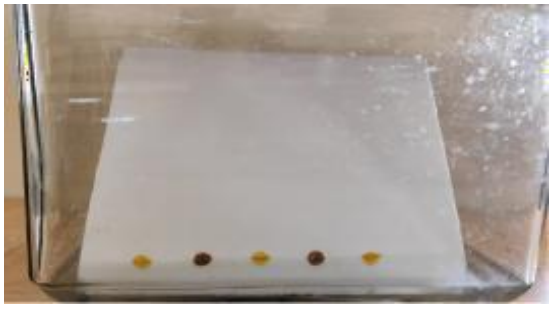

Figure 3: Saturation of tank with solvent

4) High Performance Liquid Chromatography

A. Sample Preparation

1. **Turmeric Powder:** Obtain high-quality turmeric powder From a reliable source.
2. **Solvent extraction:** Extract curcumin from turmeric powder using a solvent such as ethanol, methanol, or acetone.
3. **Filtration:** Filter the extract to remove any impurities or particulate matter.

B. HPLC Instrumentation

1. HPLC system: Use a reversed-phase HPLC system with a C18 column (250mm x 4.6 mm, 5 µm).
2. Mobile Phase: Use a mobile phase consisting of acetonitrile:water (50:50,v/v) with 1% trifluoroacetic acid (TFA).
3. Detection: Detect curcumin at a wavelength of 425 nm using a UV- Visdetector.

C. HPLC Method

1. Injection Volume

Figure 4: Injecting Curcumin sample to HPLC system

- Inject 10 µL of the filtered extract into the HPLC system.
- Flow rate: Set the flow rate to 1 mL/min.
- Run time: Set the run time to 30 minutes.
- Column temperature: Maintain the column temperature at 30°C.

D. Preparation of Agarose Gel

1. Agarose gel 1(1%) was prepared by dissolving 0.5 g of agarose powder in 50mL of 50X TAE buffer.
2. Agarose gel 2 (1%) was prepared by dissolving 0.5 g of agarose powder in 50mL of 50X TAE buffer.
3. Then add 2µL of Ethidium Bromide (EtBr) to the gel 1 solution when agarose dissolved in 50xTAE buffer.

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4. The solution was heated until clear, cooled slightly, and poured into a gel casting tray with a comb to create wells.
5. The gel was allowed to solidify and placed in an electrophoresis tank filled with TAE buffer.

E. DNA Staining with Curcumin

1. The prepared DNA sample with brormophenol blue was loaded into the wells of the agarose gel1.
2. The Incubated Sample of DNA sample with curcumin of 1:1,1:5,1;10 with brormophenol blue was loaded into the wells of the agarose gel 2.
3. Electrophoresis was performed at 80 V for 20 min.
4. Electrophoresis was performed at 100 V for 40 min.
5. The gel was visualized under a UV transilluminator, and fluorescence was compared to a gel stained with ethidium bromide.

IV. RESULTS AND DISCUSSION

A. Results

Methodology Results

Figure 5: Turmeric

Figure 6: Solution

Figure 7: Turmeric dissolved in solvent

B. Analysis of extracted curcumin

1. Insolubility

Figure 8: Insolubility of Curcumin

- It is insoluble with water.

2. Curcumin turns red when reacts with sulphuric acid

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Figure 9: Curcumin turning into red colour

- 2ml of Curcumin turns red when 2mlof sulpharic acid

3. Absorption Spectrum

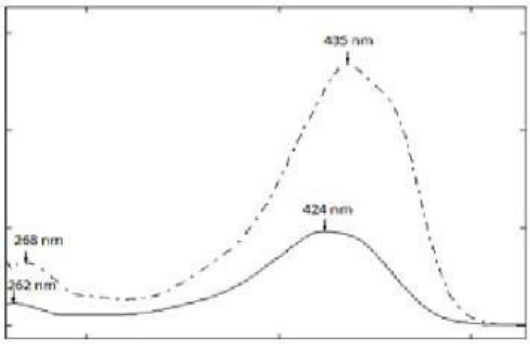

Figure 10: Absorption Spectrum

- The UV–Vis spectrum of curcumin is a broad band with a maximum absorbance peak at a wavelength of around 425 nanometers.

4. Thin layered chromatography

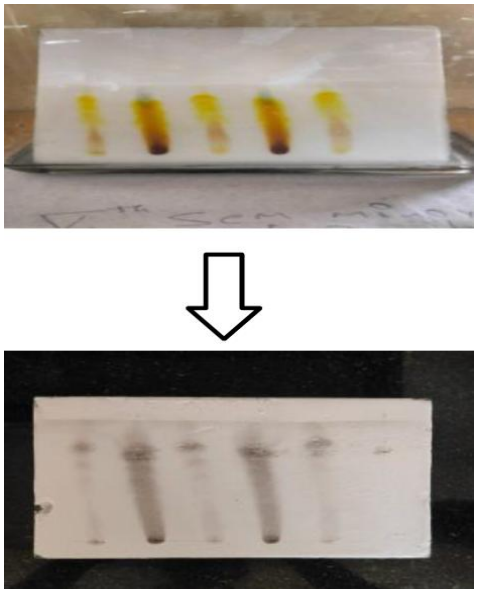

Figure 11: Curcumin presence after TLC process RF (retardation factor) Value

The Rf value is the ratio of the solute's distance travelled to the solvent's distance travelled.

From the below reading we can find the Rf value

Figure 12: Solvent and solute front

Solute Frount	Solvent Front	Solute Frount/Solvent frount
10.9 cm	14.5 cm	10.9/14.5 = 0.75 cm

Interpretation:

The Rf value is 0.75 cm, which indicates the presence of curcumin.

5. High Performance Liquid Chromatography

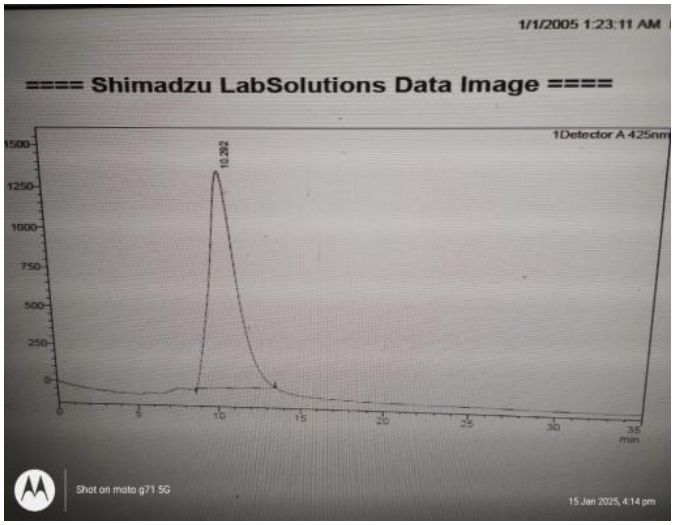

Figure 13: Graphical Representation of Presence of Curcumin after HPLC Process

Interpretation:

After Running HPLC process the presence of curcumin is observed in the taken sample at 425 nm wavelength.

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6. Solvent Extraction

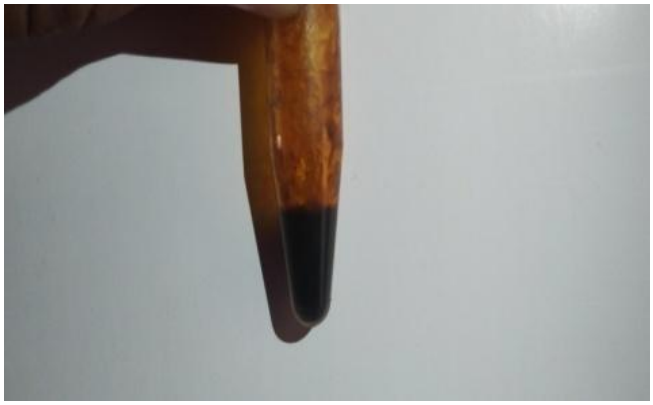

Figure 14: Curcumin Solution After Extraction Process

7. Results of agarose gel electrophoresis to detect the efficiency of curcumin to bind DNA

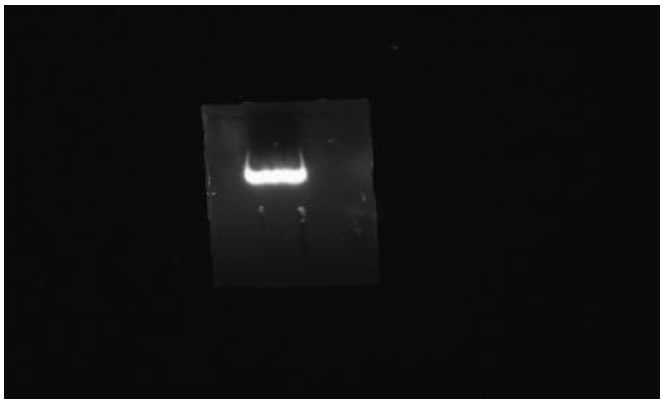

Figure 15: Agarose gel with EtBr

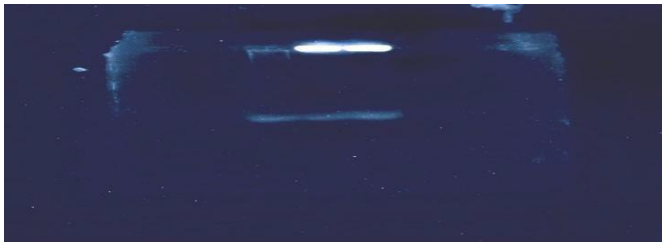

Figure 16: Agarose gel with Curcumin

C. Discussion

The results demonstrate that curcumin is a viable alternative to ethidium bromide for DNA detection in agarose gel electrophoresis.

Its low toxicity and cost-effectiveness make it particularly suitable for educational and research settings in resource- limited environments. However, further optimization is needed to enhance its fluorescence intensity and reduce background staining.

V. CONCLUSION

- The curcumin was successfully extracted using solvent extraction process
- The extracted curcumin shows the UV absorbance at 425nm
- The TLC of curcumin shows Rf value of 0.75
- The HPLC of curcumin demonstrated and peak at 425nm
- The curcumin shows effectiveness in binding to DNA in agarose gel.

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