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Rapid Detection of Food Pathogens Via Volatile Organic Compounds (VOC) Emissions Using Plant-Based Natural Indicators

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Abstract: As foodborne illnesses increase, it is more important than ever to ensure food safety, but many detection techniques are still too costly or impractical for daily use. This project investigates a straightforward, environmentally friendly method that utilizes natural materials to swiftly and reasonably detect food spoilage. A common fungus, *Aspergillus Niger*, was used to create silver nanoparticles, and turmeric (*Curcuma longa*) and beetroot (*Beta vulgaris*), which are both known to be sensitive to chemical changes, were used to extract their colorful pigments. Cellulose was extracted from the invasive aquatic plant water hyacinth and transformed into nanocellulose to support these indicators. Without the use of laboratory equipment, these materials provided a clear indication of spoiling when applied to filter paper because they clearly changed color in response to bacterial activity and released volatile organic compounds.

Keywords: *VOC Sensing, Food Pathogens, Natural Indicators, Biosensor, Food Safety, Colourimetric Detection.*

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I. INTRODUCTION

Internationally, food safety is a major concern, particularly in areas where people are unable to access quick and inexpensive methods of contamination detection. Every year, millions of people fall ill from eating contaminated food, even after improvements are being made in food processing and hygiene. Almost 600 million people are impacted each year, and around 420,000 of them are children under five, who die, according to the World Health Organization (WHO). This emphasizes how urgency of the issue, especially in developing nations where it is difficult to locate sophisticated labs and cold storage. Thus, low-cost, quick, and environmentally friendly detection techniques are needed desperately to save lives.

Traditional techniques like culturing, PCR, or ELISA have a very high accuracy, but they are slow, costly, and require laboratory settings and skilled technicians. These can't be used in homes or marketplaces. Although biosensors are a clever substitute for these laboratory techniques, the majority of them still rely on expensive synthetic materials, sophisticated mechanisms or unstable chemicals, which prevent them from being widely used.

In this project, we concentrated on a straightforward concept: identifying food spoilage by detecting the volatile organic compounds (VOCs) released by bacteria during their growth in food, resulting in spoilage of the food. When these VOCs react with specific natural pigments, they undergo noticeable changes in their color. This enables the development of simple detection systems that don't require electricity or specialized tools and are simple to understand for common people.

We used natural pigments like beta lain from beetroot and curcumin from turmeric to develop such a sensor. Both of these pigments were used for VOC detection because they are safe, commonly used in food, and extremely sensitive to pH changes. For instance, when alkaline volatile organic compounds (VOCs) like ammonia are present, curcumin turns from yellow to orange or reddish-brown. Beta lain also changes color in response to varying pH levels, which may indicate microbial activity in spoiled food.

We also used silver nanoparticles (AgNPs), which are made in the lab with Aspergillus Niger, to increase the sensitivity of the sensor. Along with their well-known antimicrobial qualities, these nanoparticles aid in stabilizing and enhancing color changes. Rather than applying these ingredients to artificial materials, we used water hyacinth nanocellulose, which is regarded as a weed and is a major source of water clogging in many areas. We gave the project an additional environmental benefit by transforming this invasive plant into something beneficial. Through the combination of these natural pigments, AgNPs, and nanocellulose, we developed a biodegradable biosensor that uses color changes to detect food spoilage. It is easy to use, low-cost, and particularly beneficial in places without access to cold storage or laboratory facilities to prevent or detect

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food spoilage, such as rural homes and street food setups. All things considered, by utilizing common, plant-based ingredients, this project not only meets a practical need but also advances sustainability. It has a strong chance of becoming a smart packaging solution or even a tool for food safety at home, and it is easy to carry.

II. MATERIALS AND METHODS

➢ *Materials*

The materials used in this study included a combination of natural ingredients, laboratory glassware, equipment, chemicals, and biological cultures essential for the development of a biodegradable VOC-based biosensor. Plant materials comprised fresh water hyacinth (*Eichhornia crassipes*), beetroot (*Beta vulgaris*), and turmeric (*Curcuma longa*) as sources of nanocellulose and natural pigments (beta lain and curcumin). Glassware used during extraction and experimentation included beakers, Erlenmeyer flasks, funnels, test tubes, Petri plates, glass slides, 15 ml Falcon tubes, stir rods, and screw cap bottles. A variety of laboratory equipment was employed, including a spectrophotometer for pigment and nanoparticle analysis, centrifuge for sample separation, sonicator for nanocellulose preparation, hot water bath, thermometer, hot air oven, pH paper strips, zip lock bags for storage, and incubators for both bacterial and fungal cultures. Other essential tools included a laminar air flow chamber, autoclave for sterilization, and an Eppendorf system for precise handling. Chemical reagents, primarily sourced from HI Media Laboratories in Bengaluru, included sodium hydroxide, silver nitrate, nutrient broth, potato dextrose broth, hydrochloric acid, hydrogen peroxide, acetic acid, ethanol, yeast extract, malt extract, glucose, peptone, cupric sulfate, and distilled water. Additionally, *E. coli* culture was used as the test organism, and Whatman No. 1 filter paper served as the substrate for the biosensor.

➢ *METHODS*

- *Synthesis of Silver Nanoparticles Using Aspergillus Niger*

The biosynthesis of silver nanoparticles (AgNPs) was performed by adapting the protocol outlined by [16], with slight modifications to suit laboratory conditions.

Initially, *Aspergillus niger* was isolated from soil through serial dilution and cultured in both MGPY and potato dextrose broth (PDB) media to promote robust fungal growth. The inoculated broths were incubated for 72 hours at 25°C with constant shaking at 120 rpm using an orbital shaker. Post-incubation, the fungal biomass was separated from the broth using Whatman filter paper No. 1 and suspended in 100 mL of sterile distilled water. The filtrate obtained after this process, which contained bioactive fungal metabolites, served as the reducing and stabilizing agent in the nanoparticle synthesis. To initiate AgNP formation, an equal volume (100 mL) of 1 mM silver nitrate ($AgNO_3$) solution was added to the fungal filtrate in a 250 mL Erlenmeyer flask. The mixture was kept under continuous shaking at 25°C and 120 rpm in the dark to prevent photoactivation. A control flask containing only the fungal filtrate (without $AgNO_3$) was maintained under identical conditions to confirm that any observed color change or nanoparticle formation was due solely to the silver ions. The synthesized nanoparticles were characterized using UV-Visible spectroscopy in the range of 300–500 nm to detect the characteristic Surface Plasmon Resonance (SPR) peak indicative of silver nanoparticle formation.

➢ *Pigment Extraction*

- *Extraction of Curcumin Pigment from Curcuma longa*

Curcumin was extracted from *Curcuma longa* following an ethanol-based protocol adapted from [12]. Dry turmeric rhizomes were purchased from a local grocery shop, thoroughly washed with tap water, and cleaned before being stored in sterile packaging. The cleaned turmeric was manually ground into a fine powder using a mortar and pestle. For extraction, 10 grams of this turmeric powder were mixed with 100 mL of 95% ethanol and stirred thoroughly to ensure proper dispersion. The mixture was incubated at room temperature for 24 hours to facilitate the release of curcumin into the solvent. After incubation, the extract was centrifuged at 3500 rpm for 10 minutes using a ROTEK laboratory centrifuge. The clear supernatant, containing the dissolved curcumin pigment, was collected and stored in sterile tubes at 4°C for further use.

- *Extraction of Betalain Pigment from Beetroot (Beta vulgaris)*

Betalain extraction from *Beta vulgaris* was carried out based on the methodology described by [11]. Fresh beetroot weighing approximately 50 grams was obtained from a hypermarket in Bangalore and cut into small pieces. These were blended with 250 mL of ethanol acidified with 2% citric acid, a solvent system chosen to stabilize and preserve the red betalain pigments. The extract was left to incubate at room temperature for 24 hours in a clean container. Following incubation, the mixture was filtered to remove solid residues, and the filtrate was concentrated using a water bath set at 40°C. This step reduced the ethanol content and yielded a denser pigment extract. For quantification, the concentrated extract was diluted with distilled water and analyzed spectrophotometrically at 535 nm, the absorbance peak for betalains. The total betalain content was calculated using the equation provided by [31]:

$$\text{Total betalains (mg/100g)} = A \times DF \times MW \times 1000 / \varepsilon L,$$

where the absorbance (A) was 0.74, dilution factor (DF) was 250, molecular weight (MW) was 550 g/mol, path length (L) was 1 cm, and the extinction coefficient (ε) was 60,000 L/mol·cm. This yielded a total betalain concentration of 1695.833 mg/100 g of beetroot, effectively quantifying the pigment content in the extract.

- *Extraction and Preparation of Nanocrystalline Cellulose From Water Hyacinth*

Nanocrystal line cellulose (NCC) was extracted from Eichhornia crassipes (water hyacinth) following a modified protocol based on [18]. Fresh samples were collected from Kithaganuru Lake, Bengaluru, and the stems were shade-dried

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for seven days before being chopped into small pieces. Alkalization involved soaking 10 g of dried stem in 15% NaOH for 48 hours at room temperature, followed by treatment with 1% NaOH at 60°C for 2 hours to remove non-cellulosic impurities. The bleached process used a 4:1 mixture of hydrogen peroxide and acetic acid at 60°C for 1 hour to remove lignin and pigments, followed by washing to neutral pH. Acid hydrolysis involved two sequential treatments with 5 M and 3.5 M HCl at 60°C for 30 minutes each to remove amorphous cellulose. After centrifugation at 2000 rpm for 30 minutes at 9°C, the pellet was neutralized using 0.5 M sodium bicarbonate. For NCC production, the cellulose was suspended at 0.5% (w/v) in 100 mL distilled water and sonicated using a PCi Analytics probe sonicator (130 W, 20 kHz) for 30 minutes in pulsed cycles (30 s on, 10 s off) in an ice bath. The resulting stable NCC suspension was stored at room temperature and characterized via UV-Vis spectroscopy, scanning wavelengths from 300 to 450 nm.

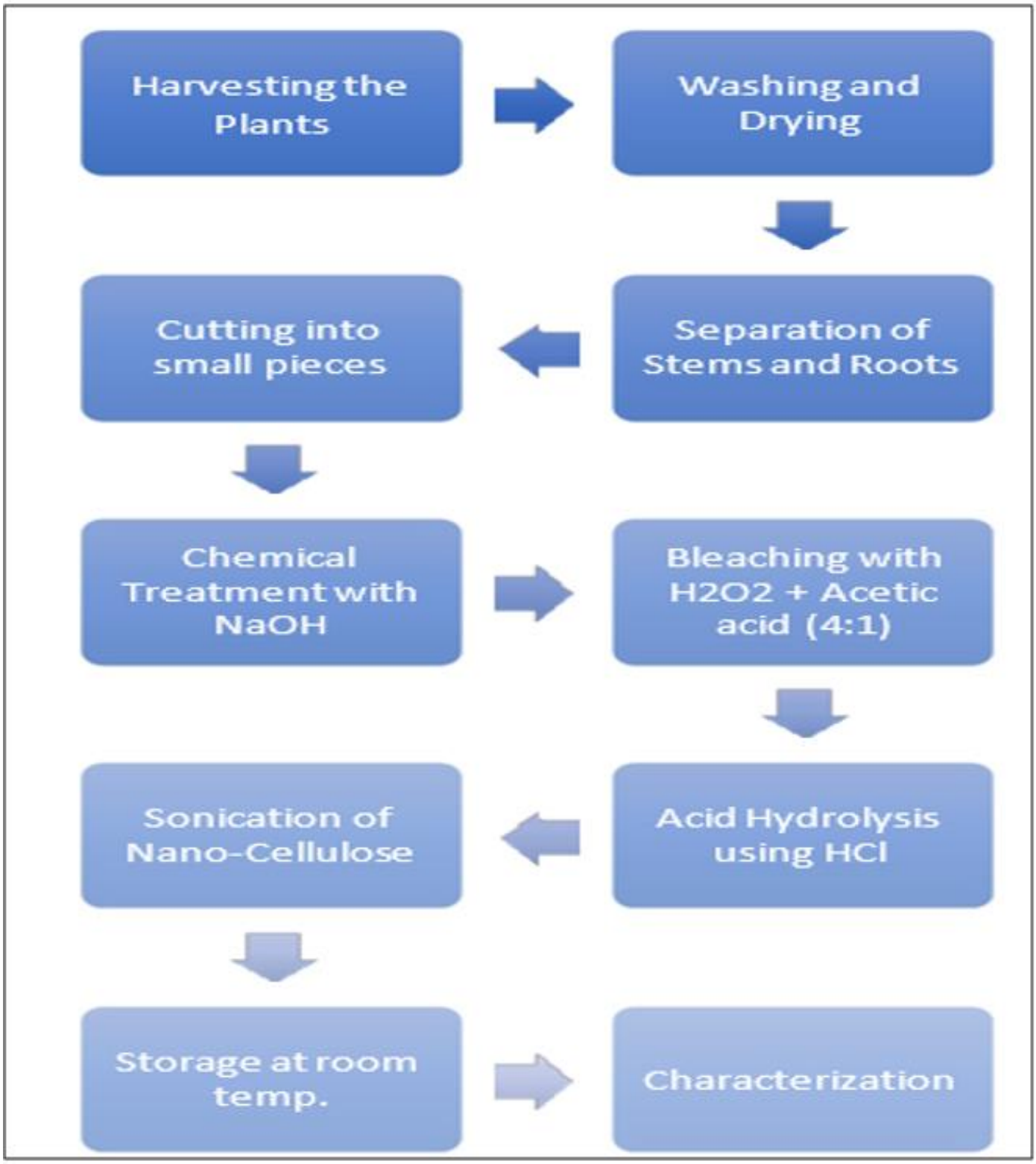

Fig 1 Extraction of Nanocrystal line Cellulose (NCC)

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- *Evaluation of Microbial Load via Color Shift using Curcumin-Coated Filter Paper Squares*

To evaluate curcumin's responsiveness to bacterial presence, 21 square Whatman filter papers were prepared, with one paper coated in curcumin solution and allowed to dry under sterile conditions. Two papers served as positive controls. A serial dilution of *E. coli* cultures (10^{-1} to 10^{-6}) was prepared and applied to separate curcumin-coated papers using a micropipette. Following a 10-minute incubation at room temperature, the papers were observed for color changes. Initially yellow, the curcumin shifted to orange in the presence of higher bacterial concentrations, indicating a potential interaction or degradation by bacterial metabolic activity.

➢ *Total Work Flow*

There are four main stages in the experimental workflow: During the extraction step, Aspergillus niger is used to isolate silver nanoparticles (AgNPs), turmeric is used to extract curcumin, beetroot is used to extract betalain (red pigment), and water hyacinth is used to extract nanocellulose. Filter paper strips are made from the extracted nanocellulose during the preparation stage. They are subsequently coated with betalain colour, curcumin, and silver nanoparticles during the coating phase. Lastly, during the application process, the coated filter strips are used to quickly identify food decomposition. A discernible colour shift on the strip indicates the presence of bacteria that cause spoiling.

III. RESULTS AND DISCUSSIONS

➢ *Synthesis of Silver Nanoparticles by using A. Niger*

After being effectively separated from the soil and grown on MGPY and PDB medium, *Aspergillus Niger* produced detectable fungal biomass after 72 hours. The culture broth was filtered to produce the fungal filtrate, which was then utilized to synthesize silver nanoparticles (AgNP). When 1 mM $AgNO_3$ was added to the filtrate and it was incubated in a dark environment, a noticeable color shift from yellow to brown was seen, signifying the creation of AgNP. The control configuration did not alter in this way. By using Surface Plasmon Resonance (SPR) to establish the existence of silver nanoparticles, UV-Vis spectroscopy showed a clear absorption peak between 300 and 500 nm.

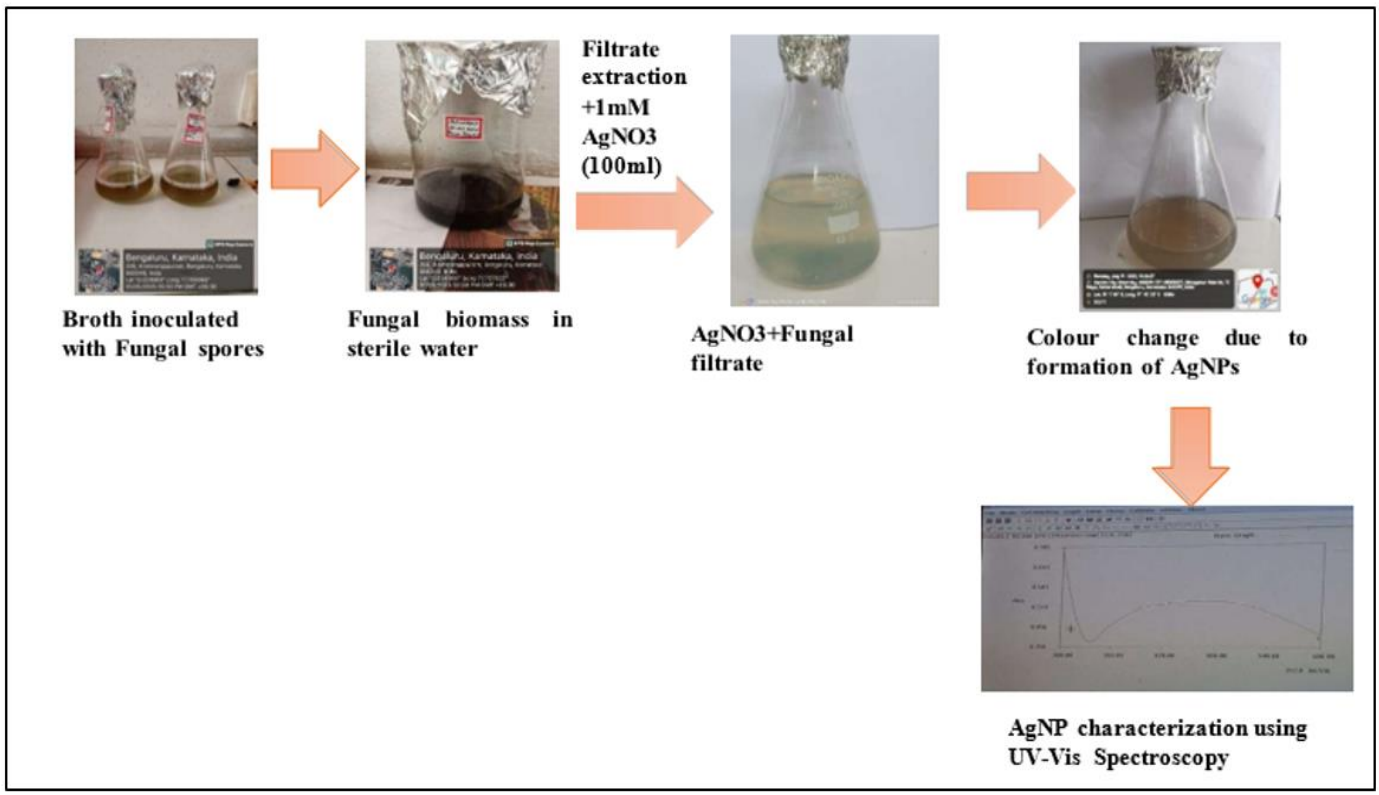

Fig. 2: Extraction and Characterization of AgNPs using A. Niger

➢ *Extraction of Curcumin Pigment from Turmeric (Curcuma Longa)*

Curcumin pigment was successfully extracted from *Curcuma longa* using 95% ethanol. After 24 hours of incubation and centrifugation, a clear yellow supernatant containing curcumin was obtained and stored at 4°C for further use.

Discussion: Curcumin was extracted from *Curcuma longa* using ethanol, following [12]. The extract, containing eight key phenolic compounds, demonstrated strong antimicrobial activity against *E. coli* and other bacteria at 10 mg TE/mL. The curcumin-coated filter papers in this study exhibited visible color changes in response to bacterial presence, supporting its antimicrobial and pH-responsive behavior. These observations align with findings by [13], who used curcumin as a halochromic pH indicator in a DNA-based LAMP assay. Both studies rely on curcumin's keto-enol tautomerism, although the latter focuses on molecular diagnostic cs, while this study emphasizes VOC- triggered colorimetric shifts.

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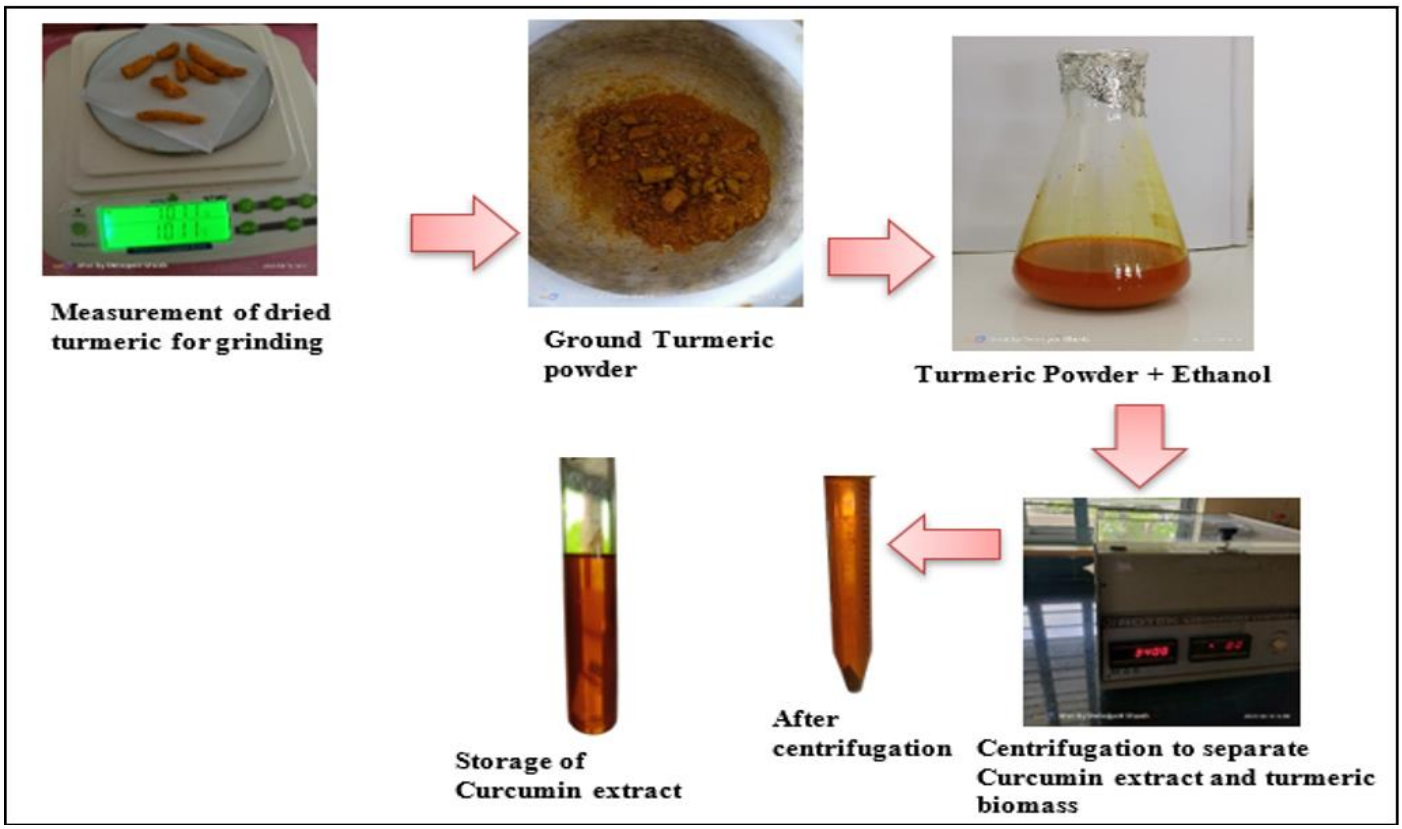

Fig 3 Extraction and Curcumin Pigment from Turmeric (*Curcuma longa*)

➢ *Extraction of Betalain Pigment from Beet Root*

Betalain pigment was successfully extracted from *Beta vulgaris* using acidified ethanol (2% citric acid). After 24 hours of incubation, the extract was filtered and concentrated using a water bath at 40°C. Spectrophotometric analysis at 535 nm confirmed strong absorbance, and the total betalain content was calculated to be 1695.83 mg per 100 g of beetroot, indicating a high pigment yield.

Discussion: Betalains were extracted from *Beta vulgaris* using acidified ethanol following [11], yielding a concentrated red pigment with a strong absorbance at 535 nm. These results align with [7], who emphasized betalains' pH sensitivity and potential as colorimetric biosensors for food quality monitoring. Although curcumin was the primary focus of this study, the observed pigment stability suggests that betalains could serve as a complementary dual-indicator in biosensors, enhancing color contrast and detection sensitivity. Both studies reinforce betalains' utility in VOC-responsive and antioxidant-based sensing platforms.

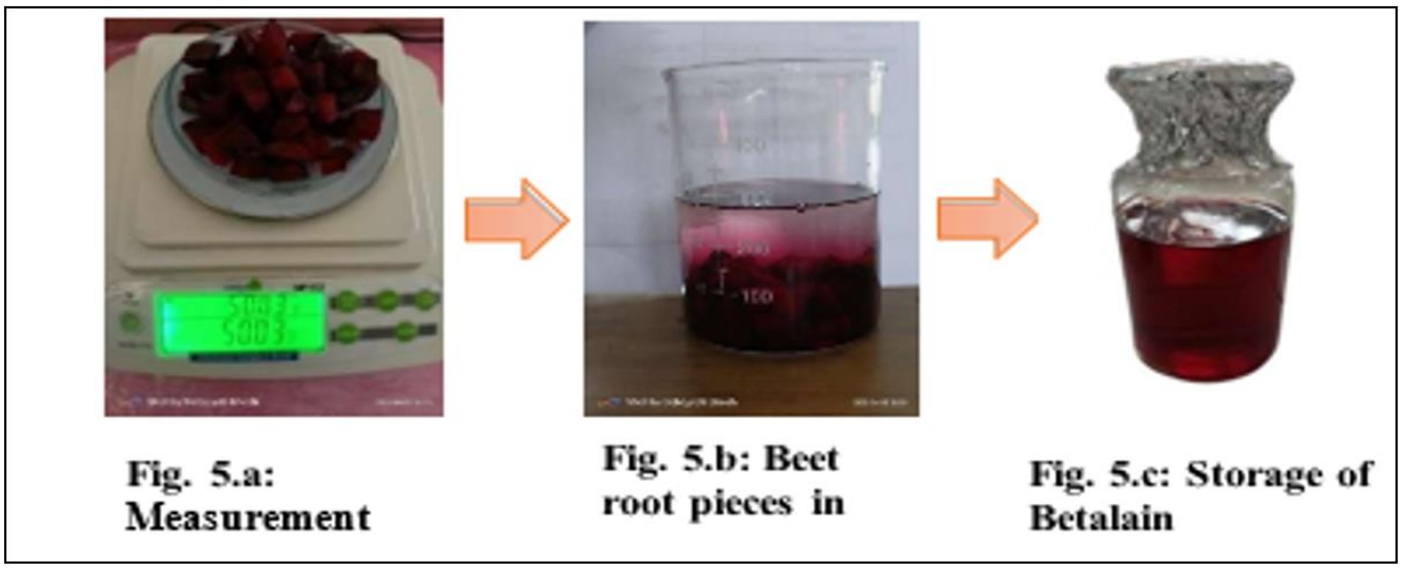

Fig 4 Extraction of Betalain Pigment from Beet Root (*Beta vulgaris)*

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➢ *Extraction and Preparation of Nanocrystalline Cellulose from Water Hyacinth*

Eichhornia crassipes was effectively treated using a multi-step procedure that included alkalization, bleaching, acid hydrolysis, and sonication to extract nanocrystalline cellulose (NCC). Sequential acid hydrolysis refined the cellulose, whereas alkaline and bleaching procedures effectively eliminated non-cellulosic elements. Under UV-Vis spectroscopy, the stable NCC solution created by the last sonication stage had distinctive absorbance in the 300–450 nm region, indicating the existence of nanocellulose. The NCC was kept in storage for further use.

Discussion: Nanocellulose was successfully extracted from water hyacinth following the sequential steps outlined by [18], including alkalization, bleaching, acid hydrolysis, and sonication. Although advanced characterization tools like FTIR and SEM were unavailable, validation through UV-Vis spectroscopy and visual inspection showed stability and clarity comparable to reported findings. This confirms the efficiency of the method and supports the project's goal of creating eco-friendly, biodegradable biosensor materials after bleaching with H2O2 + Acetic Acid (4:1) from invasive biomass.

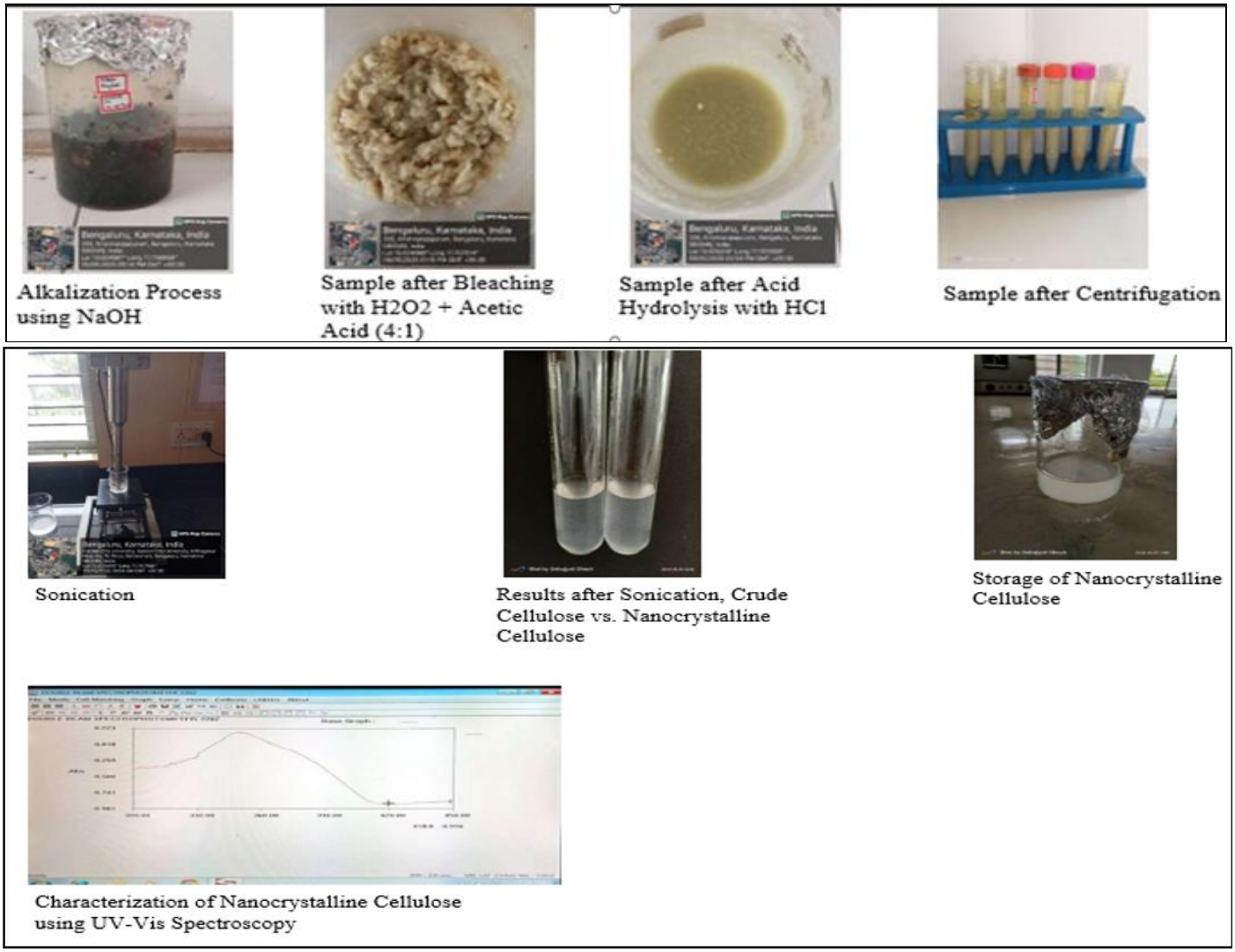

Fig 5 Extraction and Preparation of Nanocrystalline Cellulose from Water Hyacinth

➢ *Evaluation of Microbial Load Via Colour Shift Using Curcumin-Coated Filter Paper Squares*

Squares of filter paper coated with curcumin were subjected to several dilutions of E. coli in order to assess microbial activity by observing a change in colour. Samples with greater bacterial counts showed a noticeable change from brilliant yellow to orange after 10 minutes of incubation. Lower dilutions resulted in a less pronounced colour shift, suggesting a relationship between curcumin degradation and bacterial burden. The control sheets showed no change, indicating that the colour response to microbial presence is unique.

Discussion: The curcumin-coated filter paper, which turned orange upon exposure to *E. coli*, validated the concept of VOC-based colorimetric detection of microbial spoilage. This aligns with [3], who developed a curcumin-based biosensor for *Salmonella typhimurium* using NaOH-induced color change, and [25], who used curcumin films as ammonia sensors in prawn packaging. In all cases, curcumin served as a visual indicator for VOCs, reinforcing its utility in spoilage detection through pH-responsive color shifts.

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Fig 6: Experiment Setup and Result

IV. CONCLUSION

This study successfully developed an eco-friendly, cost-effective biosensing approach for detecting foodborne pathogens using VOC-responsive systems, integrating microbiology, nanotechnology, and environmental sustainability. Key achievements included the green synthesis of silver nanoparticles using *Aspergillus niger*, confirming their potential as visual and antimicrobial agents; the successful extraction of natural pigments (curcumin and betalains) that exhibited measurable color changes in response to microbial presence; and the sensitive detection of *E. coli* concentrations as low as 10^{-6} CFU/mL through curcumin-coated filter papers. Additionally, nanocellulose was extracted from invasive *Eichhornia crassipes*, laying groundwork for biodegradable biosensor substrates. Although fabrication of nanocellulose-based filter strips is ongoing, the project met its core objectives and demonstrated the feasibility of a sustainable, locally sourced biosensor for food safety monitoring in resource-limited environments.

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