



Beetroot Extract as Fluorescence Probes for Copper Ion Detection in Aqueous Solution

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Ganesan Krishnan*, Nurul Badrina Aiman Rashidi, Zakirah Buhari

Abstract:

Background of Study: Copper contamination in water poses serious health and environmental risks. Excessive exposure can lead to critical human health issues and is toxic to aquatic organisms. Existing detection techniques are often expensive and complex, highlighting the need for more accessible, eco-friendly alternatives.

Aims and Scope of Paper: This study explores betanin from beetroot as a low-cost, eco-friendly fluorescence probe for detecting copper ions, highlighting its sensitivity and potential for environmental and biomedical use.

Methods: Beetroot extract was analyzed using UV-Vis, PL, and FTIR spectroscopy to examine changes in betanin's optical properties in response to different copper ion concentrations.

Result: The fluorescence intensity of betanin decreased as copper ion concentration increased. A linear correlation ($R^2 = 0.792$) was observed between copper levels and absorbance, and the sensitivity was calculated at -6.21828×10^{-4} A.U./ppm, demonstrating betanin's strong potential as a copper ion detector.

Conclusion: The study confirms betanin from beetroot shows strong potential as a low-cost, eco-friendly copper ion detector, supporting sustainable sensing solutions across various fields.

Keywords: Aqueous Solution; Beetroot dye; Fourier Transform Infrared; Photoluminescence; Ultraviolet-visible;

1. INTRODUCTION

The detection of copper ions in aqueous solutions is of critical importance due to their harmful effects on both human health and the environment. Acute copper poisoning can result in symptoms such as nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and seizures, and in extreme cases, may lead to death. Additionally, copper is highly toxic to aquatic organisms including fish, algae, and invertebrates, causing mortality, reproductive issues, and developmental abnormalities. However, existing detection methods are often costly and complex, limiting their practicality for routine applications.

Red beetroot, a naturally occurring root vegetable that is rich in phytochemicals such as flavonoids, polyphenols, and betalains, is becoming more and more popular for a variety of uses in the culinary and pharmaceutical sectors (Borjan et al., 2022). Due to governmental acts and increased public interest in the safety, nutritional value, and aesthetics of food and dietary supplements, natural betalains attract more attention as advertisements than synthetic ones.

For the non-invasive, real-time, high-resolution visualization and detection of biomolecules and biomolecular processes in cells, fluorescence spectroscopy and imaging have become standard methods. Typically, they employ extrinsic fluorophores, which can be selectively bonded to target biomolecules chemically or genetically (Maillard et al., 2021).

Furthermore, the widespread use of synthetic dyes as fluorescence probes has raised environmental and toxicity concerns, highlighting the need for alternative detection strategies that are both eco-friendly and cost-effective (Li et al., 2022).

Previous studies have explored the potential of organic dyes as fluorescence probes for metal ion detection. For instance, fluorescence analyses of natural dyes extracted from *Plumeria rubra* (red and white) flowers were performed using absorption luminescence and fluorescence spectroscopy techniques (Vettumperumal et al., 2018). Similarly, a pH-responsive fluorescent sensor

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based on a novel pyranoxanthylum salt was developed and evaluated using UV-Vis and fluorescence spectroscopy (Gomes et al., 2021). Despite these advancements, limited research has focused on natural dyes such as betanin—extracted from beetroot—as fluorescence probes for copper ion detection. Moreover, the comprehensive characterization of betanin dye using UV-vis, photoluminescence (PL), and Fourier Transform Infrared (FTIR) spectroscopy remains underexplored.

This study aims to fill these gaps by investigating the potential of betanin dye as a fluorescence probe for detecting copper ions in aqueous environments. The structural and chemical characteristics of betanin will be examined through UV-vis, PL, and FTIR analyses. Its performance as a copper ion detector will be assessed in terms of fluorescence intensity, sensitivity, and linearity.

2. MATERIAL AND METHODS

Fresh beetroot was purchased from local market and beetroot powder containing betanin dye was extracted from the beetroot using aqueous extraction (Zia et al., 2021). After that, 0.01 mol and 0.005 mol pure Beetroot solution were prepared by mixing the beetroot extract with distilled water. The copper ion solutions of different concentrations (200 ppm, 100 ppm, 50 ppm, 25 ppm, and 12.5 ppm) for copper ion detection were prepared by mixing Copper (II) sulphate (CuSO_4) with distilled water.

The instruments used for the analysis in this study included a spectrofluorometer (FluoroMax-4, HORIBA), a UV-Vis-NIR spectrometer (SHIMADZU, UV-3600Plus) and a Fourier Transform Infrared (FTIR) spectrophotometer. The experimental procedures involved three primary characterization techniques: photoluminescence (PL) analysis, Fourier Transform Infrared (FTIR) spectroscopy, and Ultraviolet-visible (UV-vis) spectroscopy.

3. RESULT AND DISCUSSION

Result :

The photoluminescence (PL) spectrum displayed in Figure 1 shows a distinct emission range between 350 nm and 700 nm. When the beetroot dye was excited with a light source at 525 nm, it emitted a strong fluorescence signal, indicating significant photoluminescent activity. Notably, the highest emission peaks were observed at 580 nm and 653 nm. The PL analysis of the pure beetroot dye highlights its strong fluorescence behavior, reinforcing its potential as a viable fluorescence probe for the sensitive and accurate detection of copper ions in aqueous solutions.

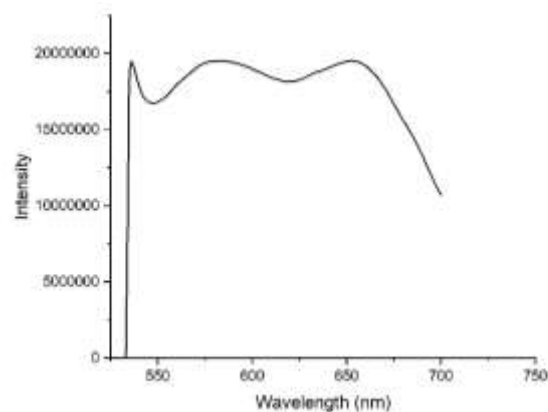


Figure 1. PL graph of Pure Beetroot Powder

In the FTIR analysis in Figure 2, several characteristic peaks were observed in the beetroot powder. The broad peak at around 3380 cm^{-1} indicates the presence of hydroxyl groups (O-H bond), while the peak at 1642 cm^{-1} suggests the presence of carbonyl groups ($\text{C}=\text{O}$ bond). The peak at 1414 cm^{-1} corresponds to the presence of NH_2 wagging and twisting indicating the presence of amines. Additionally, the peaks at 1027 cm^{-1} indicate the presence of aliphatic compounds (C-H and O-H bonds). These findings provide evidence for the presence of compounds such as anthocyanins and betanin in the beetroot powder, which contribute to its red color.

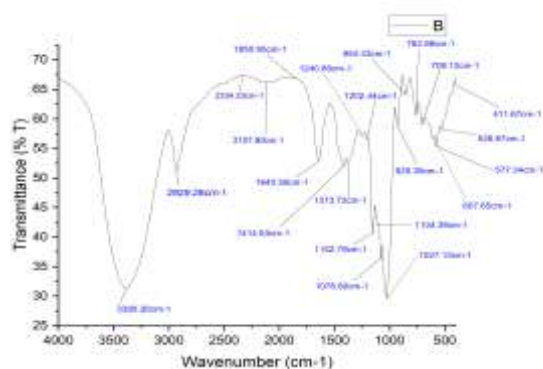


Figure 2. FTIR graph of Pure Beetroot Powder

A comparison was made between 0.01 mol and 0.005 mol pure beetroot dye solutions based on their Ultraviolet-visible characterization is shown in Figure 3. Both concentrations exhibited two distinct absorption peaks at approximately 484 nm and 534 nm. For the 0.01 mol solution, the peak intensities were recorded at 0.799 A.U. (484 nm) and 0.939 A.U. (534 nm). In contrast, the 0.005 mol solution showed lower absorbance values, with 0.461 A.U. at 484 nm and 0.397 A.U. at 534 nm. The reduced intensity in the 0.005 mol solution reflects its lower concentration of betanin and other associated compounds.

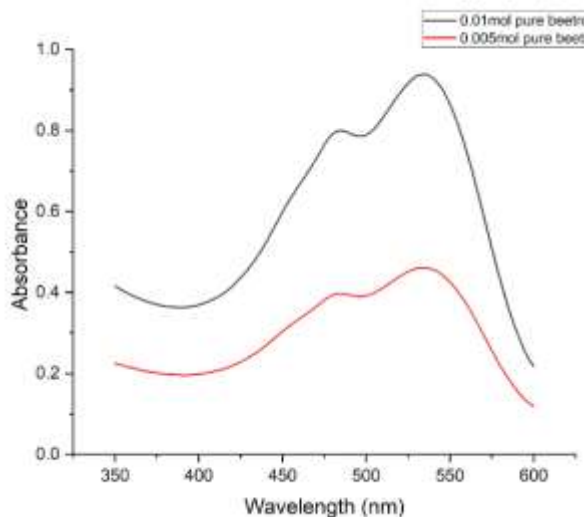


Figure 3. Beetroot solution absorbance for 0.01 mol and 0.005 mol

Figure 4 show the mixture of 80% of 0.01 mol pure beetroot and 20% distilled water. On the other hand, Figure 5 show the photograph of mixture solutions between 80% 0.01 mol of Beetroot solution with 20 % copper solutions with various concentrations. The UV-vis spectra in Figure 6 were analyzed for mixtures: one containing 80% of the 0.01 mol pure beetroot solution with 20% distilled water, and others with 80% of the same beetroot solution combined with 20% copper solution at varying concentrations (200 ppm, 100 ppm, 50 ppm, 25 ppm, and 12.5 ppm). The absorption spectra showed consistent peak positions across all samples; however, a gradual decrease in absorbance intensity was observed as the copper ion concentration increased. Additionally, a visible fading of the solution's color was noted with increasing copper concentrations, suggesting interaction between the dye and copper ions that affects the dye's optical properties.



Figure 4. 80% of 0.01 mol pure beetroot and 20% distilled water



Figure 5. Mixture solution for 0.01 mol of Beetroot solution with copper solution (from right : 200 ppm, 100 ppm, 50 ppm, 25 ppm, and 12.5 ppm)

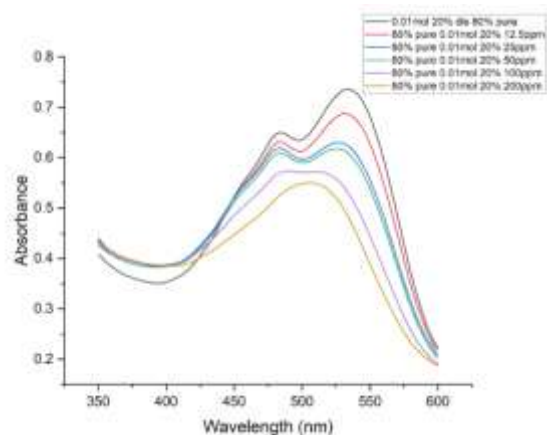


Figure 6. UV-vis graph of 80% 0.01 mol pure beetroot solution with 20% distilled water, and 80% 0.01 mol pure beetroot solution with 20% copper solution.

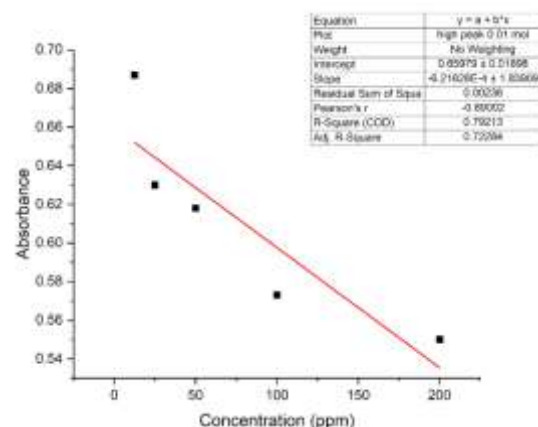


Figure 7. Linear graph of highest peaks 0.01 mol pure beetroot solution with different concentration

The peak value absorbance value for each copper ion concentration extracted from the Figure 6. The absorbance values corresponding to different copper ion concentrations—12.5 ppm (0.687 A.U.), 25 ppm (0.630 A.U.), 50 ppm (0.618 A.U.), 100 ppm (0.573 A.U.), and 200 ppm (0.550 A.U.)—were plotted to assess the dye's responsiveness in the figure 7. The resulting linear fit

yielded an R^2 value of 0.792, indicating a moderately strong correlation between copper ion concentration and absorbance. The calculated sensitivity for the 0.01 mol beetroot solution was -6.21828×10^{-4} A.U./ppm, suggesting that even small changes in copper concentration led to noticeable variations in absorbance. The relatively steep slope of the graph further supports the dye's suitability for sensitive copper ion detection.

Discussion :

Access to reliable electricity remains a significant challenge in remote, hilly, and island areas lacking conventional infrastructure, which negatively impacts essential services and economic development. To address this, the study developed a hybrid renewable energy system integrating solar and wind power with battery storage to provide sustainable electricity for off-grid communities. This system is designed to deliver a clean, consistent, and cost-effective power supply.

The system utilizes solar panels (20V, 2A) and a compact wind turbine (3 feet in diameter, 4V, 4A) as primary energy sources. Due to the fluctuating output from these sources, a DC-DC boost converter is employed to increase the wind turbine's voltage to 13.1–13.5V DC, while a buck converter regulates the solar panel's voltage to a stable 13.5V DC. This voltage regulation is crucial for stabilizing the output and protecting system components. Energy is stored in two 12V, 14Ah batteries connected in parallel, or two 12V, 7Ah batteries connected in parallel, ensuring an uninterrupted supply during periods of low generation. An inverter converts the stored DC power into 220–240V AC, making it compatible with household appliances and commercial equipment.

To optimize energy harvesting, the Maximum Power Point Tracking (MPPT) algorithm is implemented for both solar and wind sources. An ESP32 NodeMCU microcontroller continuously monitors voltage, current, battery level, and power usage. This real-time data is displayed on an LCD and transmitted to the Blynk App via Wi-Fi, allowing remote monitoring and control. This IoT-enabled setup makes the system ideal for remote and off-grid applications.

The proposed system offers several advantages, including providing a reliable power supply even in remote locations, hybrid efficiency by combining solar and wind energy for diverse weather conditions, and smart monitoring capabilities. It is scalable and modular, allowing for easy expansion with more panels, turbines, or batteries. Furthermore, it is cost-effective by replacing expensive controllers with affordable microcontrollers like ESP32, eco-friendly by reducing carbon emissions,

and has educational and practical value for research and rural development projects.

The study's results confirm that this system can provide a stable power supply by optimizing energy harvesting through the MPPT algorithm. The implementation has been proven to improve the energy efficiency and reliability of renewable electricity resources. This developed hybrid microgrid system provides an efficient and environmentally friendly solution for communities with limited access to electricity. With an expandable scale, these technologies have the potential to support energy sustainability and reduce reliance on fossil fuels. The system includes overvoltage and undervoltage protection, enhancing safety and reliability, and its scalability offers a sustainable energy solution with continuous power and proactive maintenance through IoT integration.

4. CONCLUSION

The study aimed to evaluate the potential of betanin dye, extracted from beetroot, as a fluorescence probe for the detection of copper ions in aqueous solutions. The observed interaction between the dye and copper ions led to a reduction in the dye's absorption capacity as copper concentration increased. Notably, the 0.01 mol dye solution exhibited a strong linear correlation between copper ion concentration and absorbance ($R^2 = 0.792$). The calculated sensitivity of the 0.01 mol solution was -6.21828×10^{-4} A.U./ppm. These results affirm that betanin dye is a promising, eco-friendly candidate for the sensitive and cost-effective detection of copper ions in water.

5. ACKNOWLEDGEMENT

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6. AUTHOR CONTRIBUTION STATEMENT

All the authors involved in this study, Nurul Badrina Aiman Rashidi, Zakirah Buhari, and Ganesan Krishnan contributed significantly to the development of the concept, experimental design, data analysis, and writing and editing of this article. Author worked together to ensure this study provides new insights into the use of beet extract as an effective fluorescence probe in detecting copper ions in solutions. In addition, author also play a role in spectroscopic characterization using UV-vis, photoluminescence (PL), and FTIR techniques, which provide in-depth results regarding the optical and chemical properties of betanin. This research is expected to contribute to the development of metal ion detection methods that are more environmentally friendly and sustainable.

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