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Effect of Operating Condition of Natural Dye Production from Red Spinach (*Amaranthus Dubius*) using Solvent Extraction

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Abstract. Due to synthetic dyes' health and environmental impacts, there is a growing demand for natural dyes as a sustainable alternative. This study investigates the production of natural dye from red spinach (*Amaranthus dubius*) using solvent extraction methods. The dominant pigment, betacyanin, was extracted and analyzed under various operating conditions. The effects of different solvents (water, ethanol, acetone, ethanol-water, and acetone-water), extraction temperatures (20°C, 40°C, 60°C, and 80°C), and extraction times (30, 60, 90, and 120 minutes) on dye yield were evaluated. The highest yield of 34% was achieved using acetone water (50:50) at 60°C for 60 minutes. The extracted dyes were analyzed using a chroma meter. These findings suggest optimal conditions for maximizing dye yield from presenting a viable alternative to synthetic dyes at 60℃ and 60 minutes can get *ca*. 34% of yield. FTIR analysis showed that the extracted natural dye contains antioxidants compared with synthetic dye.

Keywords: Natural Dye, Red Spinach, Solvent Extraction

INTRODUCTION

Nowadays, the developing focus on the harm of using chemical products to the environment, the destruction of plant life, the pollutants of soil and water assets, and herbal merchandise are progressively regaining importance. These are considered renewable assets whose capacity merits being studied to optimize their manufacturing and development (Souissi *et al*., 2018).

Synthetic dyes are widely used in several industries, such as dyeing nylon, silk, leather, and cotton (Shindhal *et al*., 2021; Sivakumar *et al*., 2011; Slama *et al*., 2021). However, the synthetic dyes are highly cytotoxic (Al-Tohamy *et al*., 2022) carcinogenic to mammalian cells, and act as a liver tumor promoter. They can harm health and the human body. Such as growth and fertility rates and liver, kidney, and heart damage. Other than that, synthetic dyes also affect the

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environment. Due to the harmful and toxic synthetic dyes, today, natural dyes are being developed to substitute for the usefulness of synthetic dyes.

Since natural dyes are mainly from plants, animals, and mineral sources (Yadav *et al.*, 2023), they are eco-friendly and produce beautiful shades. They are safe because they are non-toxic, non-allergic, non-carcinogenic, and biodegradable (Che & Yang, 2022). Natural dye does not affect or create any environmental problems at the stage of production or use and maintains ecological balance (Sinha *et al.*, 2012).

Red spinach, which is also known as Chinese spinach. *Amaranthin* is a natural red pigment belonging to the betacyanin group, a subgroup of betalains. It is one of the key pigments found in plants of the *Amaranthus genus*, including red spinach *(Amaranthus dubius*). It is responsible for these plants' vibrant red-to-purple coloration. The natural dye color from red spinach can be extracted using the conventional method. Nowadays, the developing focus is on the harm of using chemical products to the environment, the destruction of plant life, the pollutants of soil and water assets, and herbal merchandise, which are progressively gaining importance. These are considered renewable assets whose capacity is to be studied to optimize manufacturing and development (Souissi *et al.*, 2018).

Natural dyes, derived from renewable plant sources, have gained attention as sustainable alternatives to synthetic dyes, which are associated with environmental pollution and health risks. Red spinach (*Amaranthus dubius*), rich in betacyanin, is a promising candidate for producing natural red dyes. However, the extraction process often needs to improve its efficiency, such as low pigment yield, degradation during extraction, and the co-extraction of impurities. These challenges arise from the need for optimized operating conditions, including solvent selection, extraction time, and temperature, which directly influence the efficiency and quality of the extracted dye.

This research focuses on three key parameters: time, temperature, and type of solvents to address these concerns and enhance the extraction of betacyanin from red spinach. These factors significantly influence the extraction process, determining the natural dye's efficiency, yield, and stability. Solvent types, including water, ethanol, acetone, and mixtures, were explored to assess their polarity and effectiveness in dissolving betacyanin. Extraction times ranging from 30 to 120 minutes were examined to balance pigment yield and quality, while temperatures from 20°C to 100°C were tested to optimize solubility without compromising pigment stability. By systematically studying these parameters, the research aims to establish sustainable practices for maximizing dye yield while preserving the natural pigment's structural integrity.

MATERIALS AND METHODS

The materials used in this research were red spinach, a natural dye source; ethanol (95% purity) and acetone (99% purity) were obtained from Merck, Germany, as solvents, and distilled water, which was used to extract natural dye from red spinach.

Sample Preparation

The red spinach (Figure 1) freshly purchased from the local market in Pasar Borong Klang, Selangor. The stems were washed and rinsed with distilled water to eliminate the impurities and ensure reliable determination and reproducibility of solutions extracted from red spinach. After washing, the stems were left to dry at room temperature (*ca.* 27°C) to remove the excessive water from the washing. Once the material was dried, the stems and leaves were cut into smaller pieces (2-4 cm) for extraction.

Fig. 1: Fresh red spinach

Effect of Different Solvents

Five grams of red spinach were placed in a 250 mL Erlenmeyer flask. Five different solvents— water, ethanol, acetone, and a mixture of water at a 1:1 ratio were mixed with ethanol and acetone, respectively. The flasks were placed in an incubator shaker Protech SI-50-D (Malaysia) set at 60°C and 150 rpm for 1 hour. The crude dye was obtained by filtering the solution using a cotton cloth filter. The crude dye solutions were concentrated using a vacuum rotary evaporator (Heidolph Laborota 4000 Efficient, Germany) below the solvent's boiling point to remove the solvents. The purified dye was collected for further analysis, and the yield (%) of extracted natural dye collected was calculated using Eq. (1).

$$Yield(\%) = \left(\frac{\text{mass dye after solvent removal}}{mass\ raw\ material + solvent}\right) x\ 100\% \quad (1)$$

Effect of Extraction Temperature

Five grams of red spinach were weighed and taken into an Erlenmeyer flask of 250 ml, and 50 ml of the selected solvent ratio from the previous section was added into the conical flask. The incubator shaker was prepared at temperatures (20, 40, 60, and 80°C), at a speed of 150 rpm and shaken for 1 hour. Crude dyestuff was obtained by filtering the heated solution using a cotton cloth filter. The highest extraction will be chosen for the next parameter. The crude dye solutions were concentrated using a vacuum rotary evaporator below the solvent's boiling point to remove the solvents. The purified dye was collected for further analysis, and the yield (%) of extracted natural dye collected was calculated.

Effect of Extraction Time

Five grams of sample was weighed and taken into an Erlenmeyer flask of 250 ml. The 50 ml of the best solvent ratio from the first section was added into the conical flask at the best temperature from the second section. Then, the shaker was set at a speed of 150 rpm and heated for (30, 60, 90, and 120) minutes. Crude dyestuff was obtained by filtering the heated solution using a cotton cloth filter. The crude dye solutions were concentrated using a vacuum rotary evaporator solvent's boiling point to remove the solvents. The purified dye was collected for further analysis, and the yield (%) of extracted natural dye collected was calculated.

Analysis

Color Measurement

Konica Minolta Chroma Meter CR-400 was used in color measurement. The red color reading was obtained through reading from the device. The extracted dye must be stored away from light by wrapping the sample bottles with dark paper in the refrigerator. This was because betacyanin pigments were very labile to light and heat degradation. Failure to do so will cause the extract to turn brownish and greenish because of pigment degradation (Aztatzi-Rugerio *et al.*, 2019) (Groeneveld *et al.*, 2023). The color profile was measured as lightness *ca.* 27.42 (L*), redness *ca.* 0.88 (a*), and yellowness *ca.* 2.8 (b*), and there was no significant variation in lightness. The color measurement was based on the chromameter graph, as shown in Figure 2 (Javaid *et al.*, 2018).

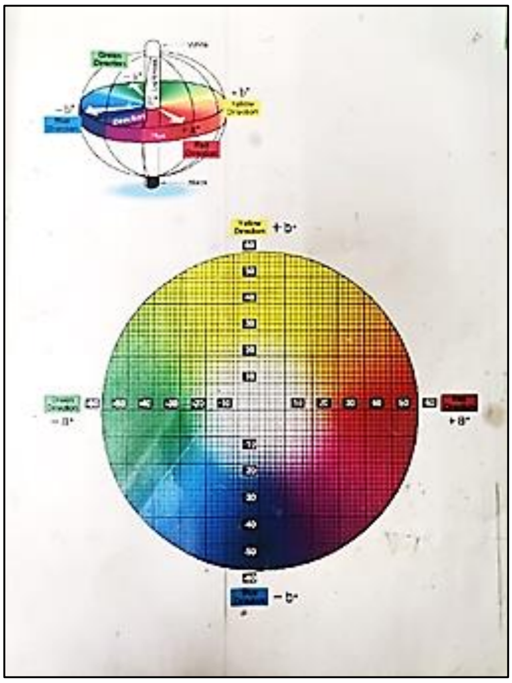

Fig. 2. Colour direction of Chomameter

Fourier-Transform Infrared (FTIR) Spectroscopy

Approximately 5 mg of each sample was used. The FT-IR spectrophotometer (Spectrum One, Perkin Elmer, USA) was operated at a spectral range of 515–4000 cm−1 and a resolution of 8 cm−1. The spectra were generated by Spectrum version 5.0.1 software and interpreted using the guidelines described by (S. Kumar & Brooks, 2018). The natural dye extracted from red spinach and synthetic dye was analyzed to evaluate the difference.

RESULTS AND DISCUSSION

Effect Type of Solvents

Figure 3 shows the effect of different solvents on the product yield percentage (%) of red spinach dyes. Previous studies evaluated natural dye extraction, commonly used in water, ethanol, and acetone (Adedokun *et al.*, 2018) and (Sutrisna *et al.*, 2020). In this study, the type of solvents influenced the production of dyes. The result shows that the mixture of acetone and water reached the highest extracted 31.72 %. Meanwhile, the lowest yield percentage (%) obtained from the experiment was acetone as a solvent, with 8.42 %.

Organic solvents are widely used in extracting phenolic components from plants. Therefore, Dirar *et al.* (2019) found that ethanol and acetone are the best organic solvents for this purpose. The acetone water mixture (50:50) achieves a balanced polarity that aligns well with betacyanin's amphiphilic nature.

Betacyanin is primarily polar but has some hydrophobic (nonpolar) regions due to its aromatic and aliphatic structures. Meanwhile, pure acetone is too nonpolar to interact efficiently with all parts of the pigment molecule, reducing solubility. In addition, pure water is overly polar, limiting its ability to dissolve the less polar components of betacyanineffectively.

While pure ethanol is a viable solvent for many extractions, it is less effective than acetone-water and ethanol-water for extracting betacyanin. This is due to ethanol’s lower polarity, limited hydrogen-bonding ability, slower penetration into the plant matrix, and reduced solubilization of highly polar compounds. Lastly, a mixture of ethanol-water mixtures is less effective than acetone-water because ethanol's lower polarity than acetone reduces its ability to solubilize highly polar components. Therefore, the mixture of acetone and water is more efficient than other solvents in extracting the dyes from red spinach (Nielson & Harley, 1996).

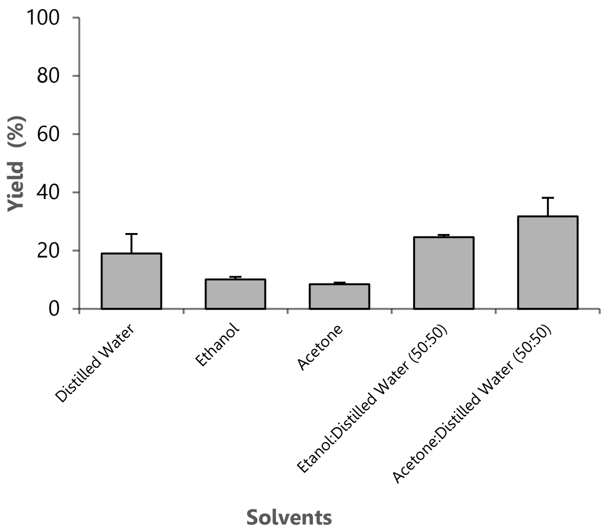

Fig. 3: Yield of natural dye extracted (%) at different types of solvents

The color of dyes extracted from red spinach in different solvents is different. A* value represents the red color detected by the chroma meter device. Figure 4 shows the result of the color analysis, which is represented by a*. The highest value of a* was obtained from acetone: distilled water with a ratio (50:50) of 2.11. Meanwhile, the lowest value of a* is – 0.38, which is green color. This is because the betacyanin compound is soluble in an alkaline solution. The red color obtained from the dyes extracted is referred to as betacyanin (Howard *et al.*, 2022) and Figure 5 shows the samples of dyes extracted from red spinach using different solvents.

The mixture of acetone and water creates a solvent system that can solubilize a wide range of compounds, leveraging water's strong polar interactions and acetone's moderate polarity. Water assists in breaking down the hydrogen bonds in the plant matrix, while acetone penetrates deeply into the cell walls and interacts with the pigment molecules, enhancing overall extraction efficiency. Acetone has better permeability through cell walls than ethanol, allowing for a more effective release of intracellular pigments like betacyanin. Combined with water, it facilitates better extraction by softening and breaking down plant cell walls without excessive solvent evaporation.

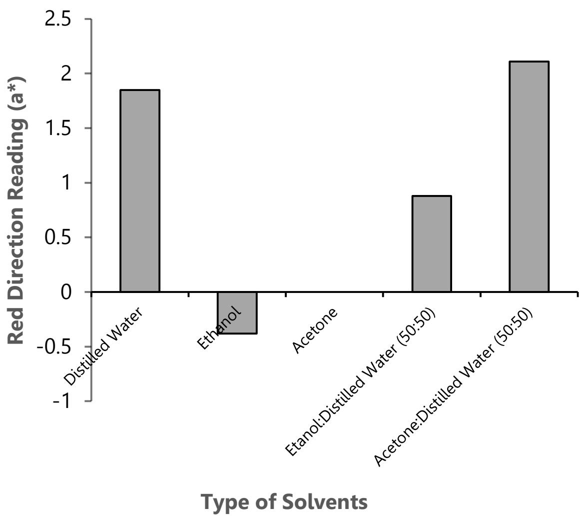

Fig. 4: Amount of Red Direction (a*) on different types of solvents

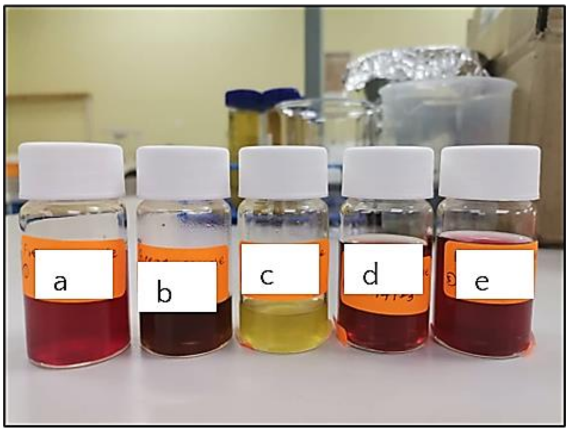

Fig. 5: Natural dyes extracted from different types of solvents where a) water, b) ethanol, c) acetone, d) ethanol:water (50:50), and e) acetone:water (50:50)

This can be observed in the extracted green color using pure acetone (Figure 5c) and red color in a mixture of acetone and water (Figure 5e). So that no red color value is detected using the Choma Meter, as shown in Figure 5. Therefore, weak acid-base conditions are near-neutral environments (pH 5-7) and are more suitable for betacyanin extraction, ensuring better stability and yield (R. Kumar *et al.*, 2023) (Li *et al.*, 2022) and (Stintzing *et al.*, 2004).

Effect of Extraction Temperature

The impact of extraction temperature on natural dye yield was examined using acetone-water (50:50). The results indicate that the dye yield increased with temperature up to 60°C, achieving a yield of 32% (Figure 6). The optimal temperature of 60°C aligns with the thermal sensitivity of betacyanin, which can degrade at higher temperatures due to the breakdown of its chromophore structure. A chromophore structure refers to the part of a molecule responsible for its color. It consists of a group of atoms within a molecule that absorbs specific wavelengths of light in the visible spectrum, resulting in the perception of color. The increases in the result between the range of 20℃ to 60℃ promote the dissolution of red spinach pigment.

This phenomenon helps improve the solubility of color components at higher temperatures. Furthermore, the heat is provided in a selectivity permeable membrane that decreases the solvent's viscosity and makes it easy to pass through the solid substrate mass (Elksibi *et al.*, 2014). However, a further increase to 80°C slightly decreased to 16%. This suggests the potential degradation of betacyanin at higher temperatures, as thermal stability is a known issue for many natural pigments. When the extraction time exceeds 80℃, the increases in temperature cause damage to the pigment.

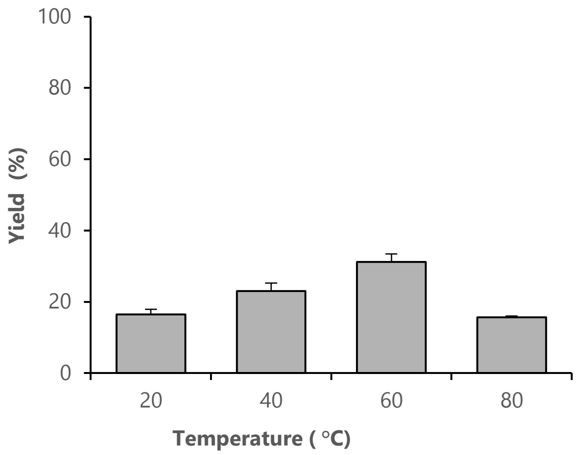

Fig. 6: Yield of natural dye extracted (%) at different temperatures

This is due to the loss of protection from the membrane. It will evaporate easily, and self-oxidation will occur easily. This is due to the process that happens during heat processing. The betanin pigment may be degraded by isomerization, decarboxylation, or cleavage, resulting in a gradually reduced red color (Karthiga *et al.*, 2012). As mentioned by (Kuhn *et al.*, 2022), a betacyanins were significantly degraded, with retention dropping to 50-64% during natural dye extracted from Bougainvillea glabra bracts. This suggests that temperatures above 50°C can lead to substantial degradation of betacyanin.

Figure 7 shows the result of the color analysis, which represents*. The values of a* are slightly different, but all are red since the value of a* is positive. The a* value decreases with increasing temperature. The highest a* value is 20℃, with 8.72 representing dark red. Meanwhile, the lowest is 4.67 at 80℃, which is light red. It may also be the other pigment extracted from red spinach instead of betacyanin. Figure 8 presents the color of dyes extracted from red spinach at different extraction temperatures. It showed that the red pigment (betacyanin) appears in all the natural dyes extracted. They are similar in red color for 60°C and 80°C from physical observations.

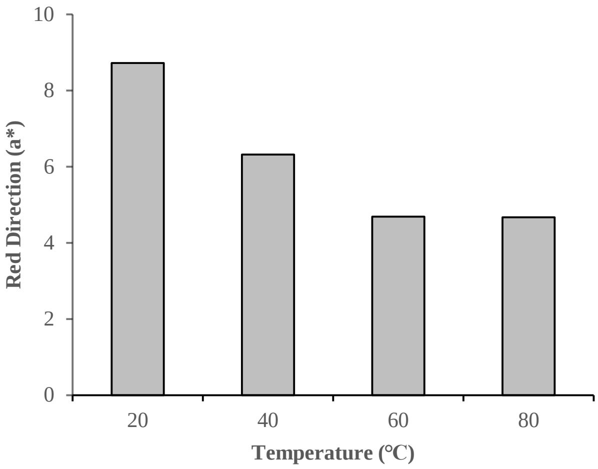

Fig. 7: Amount of Red Direction (a*) at different temperatures

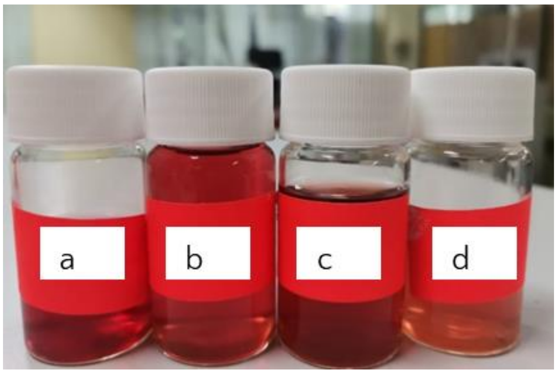

Fig. 8: Natural dyes extracted at different temperatures where a) 20°C, b) 40°C, c) 60°C, and d) 80°C.

Effect of Extraction Time

The effect of extraction time on dye yield was assessed at the optimal temperature (60°C) and acetone-water (50:50), as shown in Figure 9.

The yield increased on time, peaking at 60 minutes with 34%. Prolonging the extraction to 90 and 120 minutes resulted in decreased yields of 33.25% and 32.75%, respectively. This decrease is likely due to oxidative and thermal degradation of betacyanin over extended periods. This observation underscores the importance of balancing extraction duration to maximize yield while minimizing pigment degradation. This is because the effect of the extraction time is related to the higher contact time with the solvent and the sample, which grasped more coloring components into the solution. The prolonged extraction time would expose more oxygen and increase the chances of self-oxidation and evaporation (Elksibi *et al.*, 2014).

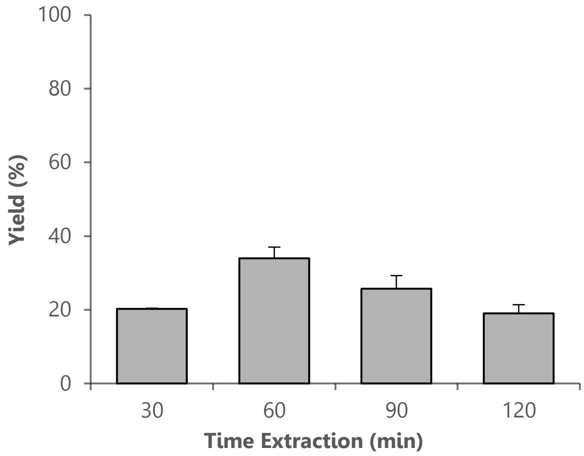

Fig.9: Yield of natural dye extracted (%) at different extraction times

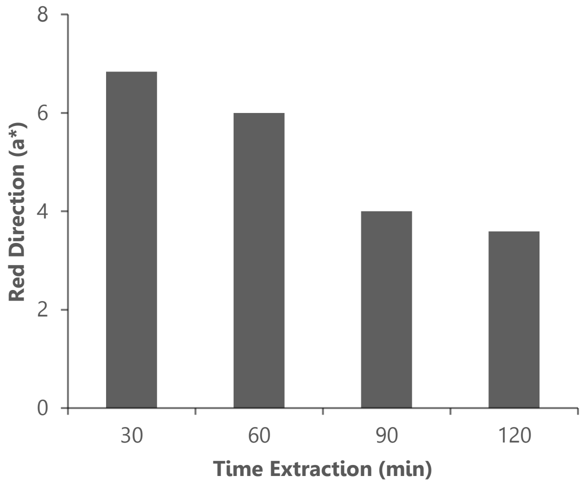

Fig. 10: Amount of Red Direction (a*) at different extraction times

Figure 10 shows the result of the color analysis, which is represented by a*. The values of a* are slightly different, but all are red since the value of a* is positive. The a* value decreases with increasing extraction time. The highest a* value is 30 minutes, with 6.84 representing dark red. Meanwhile, the lowest is 3.59 at 120 minutes, which is light red.

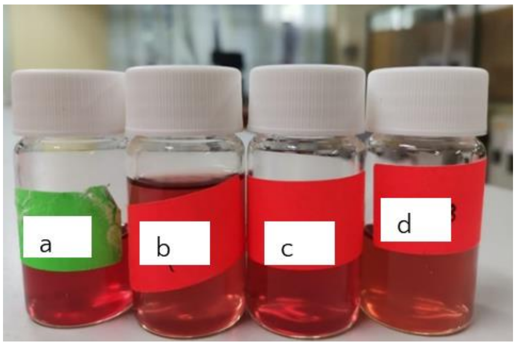

Fig. 11: Natural dye extracted at different extraction times where a) 30, b) 60, c) 90, and d) 120 minutes

Some studies reported that increasing the extraction time increases the betacyanin and betalain decomposition rates (R. Kumar *et al.*, 2023) (Li *et al.*, 2022). This is due to prolonged contact time. The betanin pigment may be degraded by isomerization, decarboxylation, or cleavage, which gradually reduces the red color (Karthiga *et al.*, 2012). It may also be the other pigment extracted from red spinach instead of betacyanin. Figure 11 shows samples of dyes extracted from red spinach at different extraction times. It showed that in physical observations, all the extracted natural dyes showed the red color was produced using a mixture of acetone-water and almost similar pattern.

FTIR Analysis

This section discusses the spectral characteristics of dyes prepared from red amaranth spinach and synthetic red dye, as shown in Figure 12. When comparing the transmission FTIR spectra of the red amaranth spinach dye to the synthetic red dye, differences in peak positions were noted within the range of 4000 - 515 cm^{-1} (Kabir *et al.*, 2022). The presence of phenolic compounds was indicated by transmittance peaks in the 3250 - 3550 cm^{-1} wavenumber range. Additionally, two distinct peaks between 2850 - 3300 cm^{-1} were attributed to C-H stretching, which is associated with polysaccharides, lipids, and carbohydrates (Kabir *et al.*, 2019).

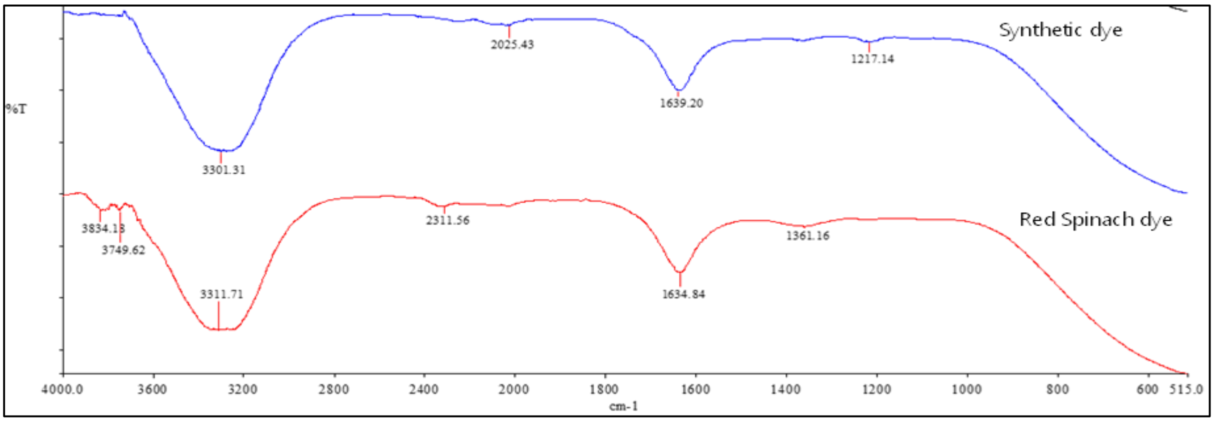

Fig. 12: FTIR analysis

A single peak in this range was specifically identified. Another notable peak was observed at 1680 - 1750 cm^{-1}, corresponding to C=O stretching. This analysis proved that natural dye extraction from red spinach contains antioxidants compared to synthetic dye.

CONCLUSIONS

This study successfully optimized the extraction of betacyanin, a natural red pigment, from *Amaranthus dubius* (red spinach) using solvent extraction methods. The findings highlight the critical role of operating parameters—solvent type, extraction temperature, and extraction time—in determining the yield and stability of the extracted dye. Using acetone-water (50:50) as a solvent mixture at 60°C for 60 minutes was identified as the optimal condition, producing the highest yield of 34%. The extracted pigment was characterized using FTIR, which confirmed the presence of key functional groups associated with betacyanin. These results validate the potential of red spinach as a viable and sustainable source of natural dyes, offering an eco-friendly alternative to synthetic dyes with significant industrial and environmental benefits. Future research should focus on scaling up the extraction process, evaluating the pigment's performance in various applications (e.g., textiles, food, cosmetics), and investigating its stability under real-world conditions. These findings contribute to the growing knowledge on natural dye production and support the development of sustainable and environmentally friendly alternatives to synthetic dyes.

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