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Absorbance Characteristics of Chlorophyll Extracts of Spinach (Amaranthus Hybridus & Amaranthus tricolor) and Water Spinach (Ipomea spp.) as Sensitizers

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The development of dyes has moved from natural sources to synthetic substitutes since the discovery of mauveine in 1856. Environmental concerns have sparked a renewed interest in natural colors. This work investigates chlorophyll extracts from water spinach (Ipomoea spp.) and spinach (Amaranthus tricolor) as photosensitizers in dye-sensitized solar cells (DSSCs). Chlorophyll content analysis showed Amaranthus tricolor has a high total chlorophyll content of 10.367 mg/g, with 9.551 mg/g chlorophyll a and 0.818 mg/g chlorophyll b. Amaranthus hybridus also showed significant levels at 10.462 mg/g total chlorophyll, comprising 9.181 mg/g chlorophyll a and 1.283 mg/g chlorophyll b. Conversely, Ipomoea species had a lower concentration of 5.380 mg/g. Acetone extraction and UV–Vis spectrophotometry revealed significant absorption peaks in the 380–460 nm range. Amaranthus tricolor's performance makes it ideal for solar applications. However, storage-induced degradation from stressors like senescence and light intensity caused a blueshift in absorbance and decreased efficiency. Despite this, Amaranthus tricolor remains a viable, environmentally benign natural chlorophyll alternative for industrial and renewable energy applications.

Keywords: *characteristics, chlorophyll, sensitizer, spinach, water spinach*

1. INTRODUCTION

Dyes have been used since ancient times, with the first dyes being made from natural materials like minerals, plants, and animals. Indigo, derived from Indigofera tinctoria and madder, is a well-known natural dye used to color fabrics, leather, and hair. Tyrian dye, made from sea snails, was considered a symbol of wealth and status in Greek and Roman society (Alegbe & Uthman, 2024). Natural dyes grew in popularity, especially in Europe, and advances in weaving and dyeing techniques resulted in better quality and color diversity. The industrial revolution led to the discovery of mauveine, the first synthetic dye made from aniline, in 1856. This led to the development of the synthetic dye industry, which grew in variety, quality, and diversification in the 20th century (Ciccola et al., 2024). However, the late 20th century saw increased environmental concerns due to the production and use of synthetic dyes, leading to a resurgence of natural dyes in niche industries like organic textiles, cosmetics, and food. Technological advances have led to new inventions such as color-sensitive solar dyes and energy- and water-efficient digital dyeing technologies, as can be seen in Figure 1 (Khan et al., 2024).

Spinach (Amaranthus hybridus L.) is a plant known to contain high chlorophyll, so spinach is a candidate worthy of research as a potential source of natural photosensitizers. Previous studies recommended spinach chlorophyll extract with good stability and effective performance in converting light energy into chemical energy (Sankaranarayanan et al., 2022). It has also been previously revealed that spinach chlorophyll has an ideal chemical structure to interact with metal oxide surfaces, which is

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a critical component for DSSC applications. The high concentration of antioxidants in spinach ensures that phytonutrients are protected from photochemical degradation (Zhao et al., 2024).

Water spinach, the Latin name Ipomoea spp., also shows interesting potential as a source of chlorophyll. Water spinach is a fast-growing, gastropod vegetation plant in various geographical and ecological conditions, which allows its extraction to be carried out easily and sustainably. Studies have found that water spinach chlorophyll exhibits light absorption similar to that of spinach in light, and its elimination is sufficient when applied to various photochemical applications(Asih et al., 2024). What's more, the additional composition of kale extract in the form of carotenoids and flavonoids can provide a higher percentage of photosensitizer efficiency due to synergistic effects.

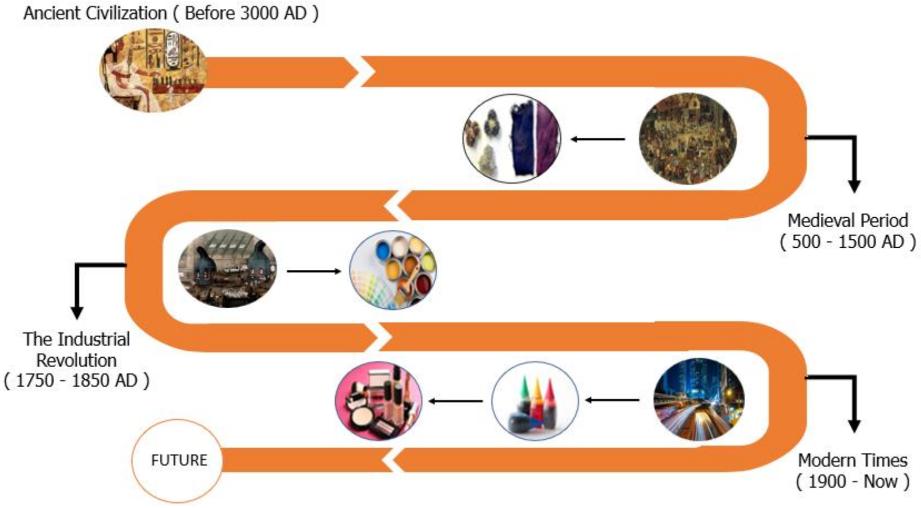


Figure 1. History of dye from the ancient civilization to the modern era, and for future concern to the green technology and environment (Khan et al., 2024)

Despite these advancements, significant exploration gaps remain. There's a need for a comprehensive comparison of chlorophyll from different growth stages of spinach to estimate their effectiveness and environmental impact. There is limited exploration in the world for the performance of chlorophyll extracts from spinach and water spinach in practical applications such as DSSCs and other green technologies. Further disquisition is demanded to compare the effectiveness of chlorophyll from different factory sources and to explore the benefits of fresh composites on photosensitizer performance. Addressing these gaps will optimize the growth environment and enhance the effectiveness of natural photosensitizers for supporting sustainable green technologies. This study aims to address these gaps by assaying the absorbance characteristics of spinach and water spinach chlorophyll extracts and assessing their eventuality as photosensitizers, thereby supporting the development of further sustainable and effective green technologies.

The extraction and purification process of chlorophyll from water spinach and spinach is a very important first step in this research. To ensure that the chlorophyll produced has high purity and is not contaminated by other compounds that can interfere with its sensitizer function, an appropriate extraction method must be chosen. To be adopted on a larger scale, the method should also be environmentally friendly and economical. Several methods, such as extraction with organic solvents and ultrasonic methods(VEREP et al., 2023), have been proposed and tried in previous studies, but the results were different. Thus, this study will focus on the in-depth absorbance characteristics of spinach and water spinach chlorophyll extracts, exploring their potential use as photosensitizers in various technological applications. It provides new insights into the ability of chlorophyll as a natural sensitizer to encourage more sustainable use of natural resources in green technology.

2. METHOD

The research involved selecting and testing fresh leaves from water spinach and spinach plants to ensure they were in good condition and free from contamination. The samples were then placed into a microwave and heated for 2 minutes. The samples were checked every 30 seconds to ensure the leaves did not burn. After that, the samples were cut into small pieces, blended and pulverized (Figure 1), then mixed with 80% acetone and stirred at high speed for 10 minutes. The chlorophyll extract was then heated at various temperatures and tested for temperature stability. The absorbance was measured using a UV-Vis T90 spectrophotometer at 300-700 nm. This study also aims to measure the characteristics of the absorbance spectrum, measure the chlorophyll content, and determine the peak width. For the mental details about the experimental processes in this study, see Figure 2.

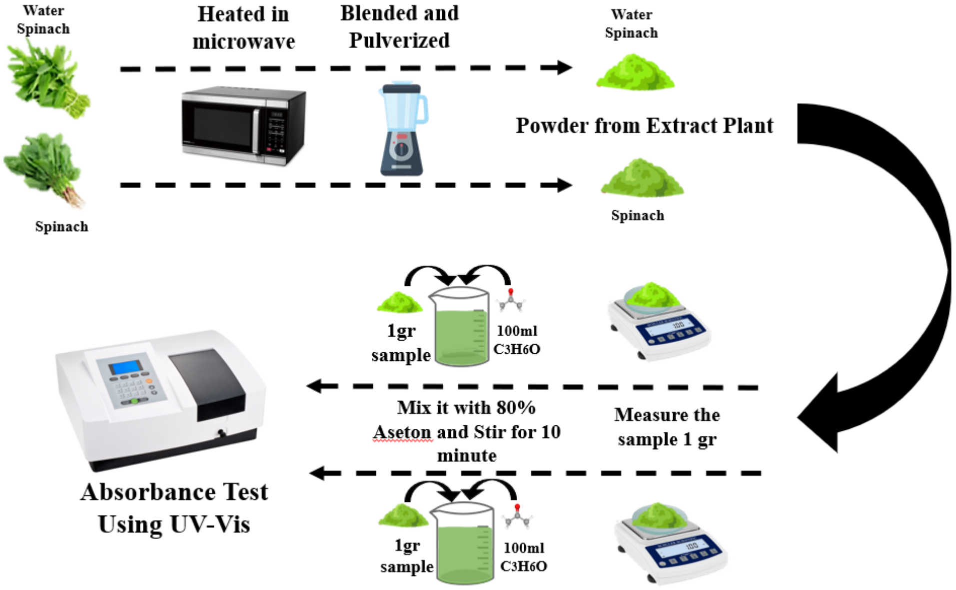


Figure 2. The process of extracting chlorophyll from spinach and water spinach

3. RESULTS AND DISCUSSION

Adopting natural coloring powders from plants such as spinach and water spinach is an environmentally friendly choice and a smart strategy to support the dye production for various applications in industries, as can be seen clearly in Figure 3. These natural dyes have proven to be as effective as a sensitizer for dye-sensitive solar cells (DSSCs)(Ansari et al., 2021). DSSCs can convert light with high efficiency into electrical energy. It is an increasingly important technology in the global campaign to adopt renewable energy. Natural dyes, on the other hand, provide beautiful and long-lasting colors in the textile industry while reducing the environmental impact usually caused by synthetic dyes(Bechtold et al., 2023).

As consumers are increasingly wary of harmful synthetic chemicals in food and cosmetics, natural dyes are becoming a safe choice for consumers, enhancing product appeal and strengthening brand loyalty(Rai et al., 2023;Chopra et al., 2023). These dyes are also used in printing inks that support environmentally friendly business practices(Shahin et al., 2022). Natural dyes can be used in the pharmaceutical industry to coat medicines, ensure product safety and stability, and facilitate identification(Goel et al., 2024). Natural dyes are even used in arts and crafts to provide a rich and authentic color palette, which supports socially responsible production and adds aesthetic value(Alegbe & Uthman, 2024). Natural coloring has many benefits, so it's clear that it's not just a trend. It's a big step towards a more eco-friendly and healthy future.

Figure 4 shows the light absorption spectra of three plants: pulled water spinach (*Ipomea spp.)*, wild spinach (*Amaranthus hybridus)*, and spinach (*Amaranthus tricolor)*, at wavelengths from 300 to 700 nanometers. All three plants show significant absorption peaks in the 380-460 nm range, indicating the presence of photosynthetic pigments such as chlorophyll. However, Spinach (*Amaranthus tricolor)* was the most prominent, showing "higher pigment concentration or better absorption efficiency compared to the others". Spinach (*Amaranthus tricolor)* and Wild Spinach (*Amaranthus hybridus)* seem to have an advantage in the 620-700 nm range, which is usually associated with the absorption of red light. In contrast, water spinach (*Ipomea spp.)* showed lower absorption. This suggests a difference in pigment composition among the three plants, where the spinach appears to excel in absorbing a wide range of the light spectrum. Due to differences in pigment composition, different plant species have different absorption spectra(Ullaha et al., 2024). These differences can affect the efficiency of light utilization, which has an effect on their growth, health, and nutrient content. This may provide important insights for the use of plant pigments in nutrition and health.

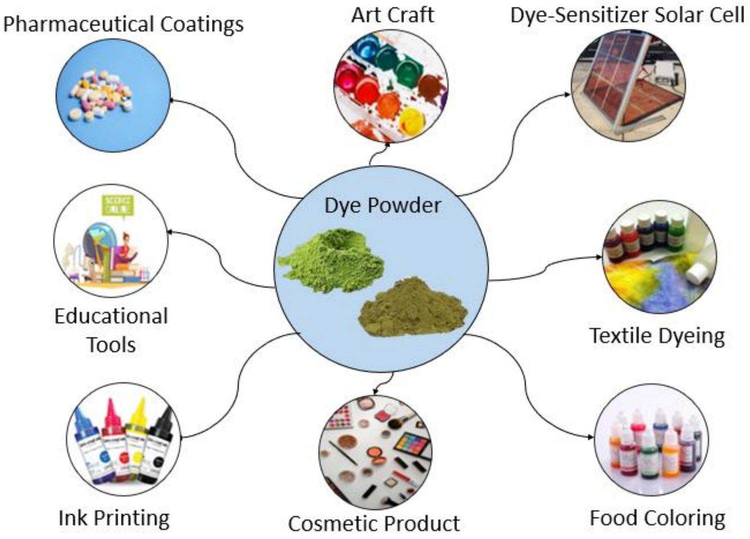
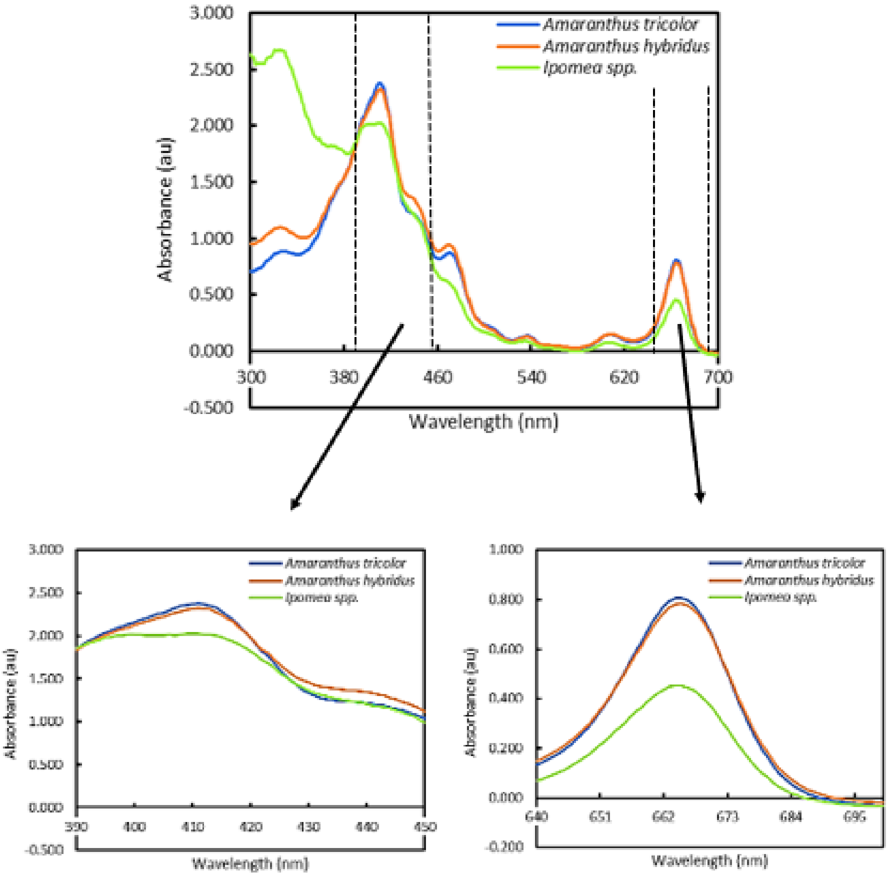


Figure 3. Dye from various types of plant and their application in the cosmetics, food, textile, and pharmacy industries, etc.



=**Figure 4.** Sample Absorbance Spectrum of pulled water spinach (*Ipomea spp.)*, wild spinach (*Amaranthus hybridus)*, and spinach (*Amaranthus tricolor)*

Figure 4 also shows the absorbance peaks of three plant samples (water spinach, wild spinach, and spinach) at different wavelengths of light. These three plants absorb the most blue-violet light, as each shows the highest absorbance peak at around 380 – 460 nm. This corresponds to the nature of chlorophyll, the main plant pigment that absorbs light at this wavelength to trigger photosynthesis. After the peak at a wavelength of 460 – 540 nm, the absorbance decreases drastically, indicating that green light is reflected rather than absorbed, giving the plants their green color. Furthermore, around 620 – 700 nm, there is a small rise in absorbance again, indicating the absorption of red light, which is an important part of the photosynthesis process. This fact is supported by research that states that “chlorophyll a has absorption peaks at around 430 nm and 662 nm, while chlorophyll b absorbs strongly at 453 nm and 642 nm(Mehmood & Zeb, 2023). Both types of chlorophyll are very important in maximizing photosynthetic efficiency in plants”. Therefore, the absorption peaks in this graph are clearly related to the main role of chlorophyll in capturing light energy for photosynthesis.

The three plant species (*Amaranthus tricolor, Amaranthus hybridus,* and *Ipomoea spp.)* have different chlorophyll absorbance spectra, as shown in Figure 5. The absorption patterns of all species are similar in the wavelength range of 390-450 nm, associated with chlorophyll b, although there are slight differences in peak intensity. However, in the 640-695 nm range, associated with chlorophyll a, Amaranthus tricolor has a higher absorbance peak than the other species. This shift is no coincidence; more likely, it is the result of a complex biological adjustment to specific environmental conditions. Environmental changes that impact the molecular structure of chlorophyll are the main cause of this shift. For example, environmental stress factors such as changes in light intensity, temperature extremes, or drought can alter the way chlorophyll interacts with proteins within the thylakoid membrane. These interactions can change the absorbance wavelength, as chlorophyll often shows a slightly altered peak compared to in vitro measurements. In plant biochemistry, this is a long-recognized phenomenon. Chlorophyll embedded in a protein environment turns red to about 10 nm, in contrast to chlorophyll dissolved in organic solvents(Cherepanov et al., 2024). Moreover, these adaptations demonstrate the intelligent evolutionary response of plants to light conditions and their environment. In addition to changes in the spectrum of light that plants receive in various habitats, natural selection pressure drives plants to adjust their absorption spectra more efficiently. As such, these shifts indicate not just changes in molecular structure but also the adaptation of plants to environmental stresses, which makes them more efficient at photosynthesis in certain environments. This suggests that changes in plant chlorophyll absorbance are critical for plant survival and productivity under various environmental conditions(Gao et al., 2024).

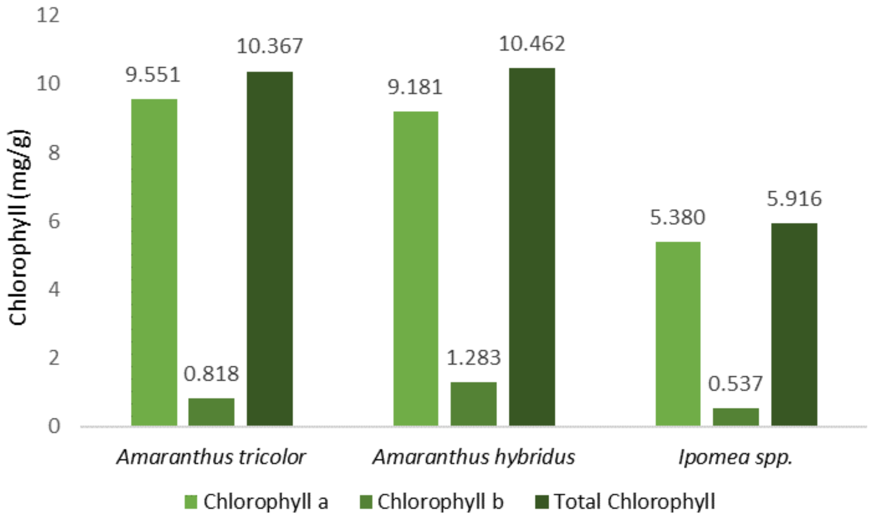


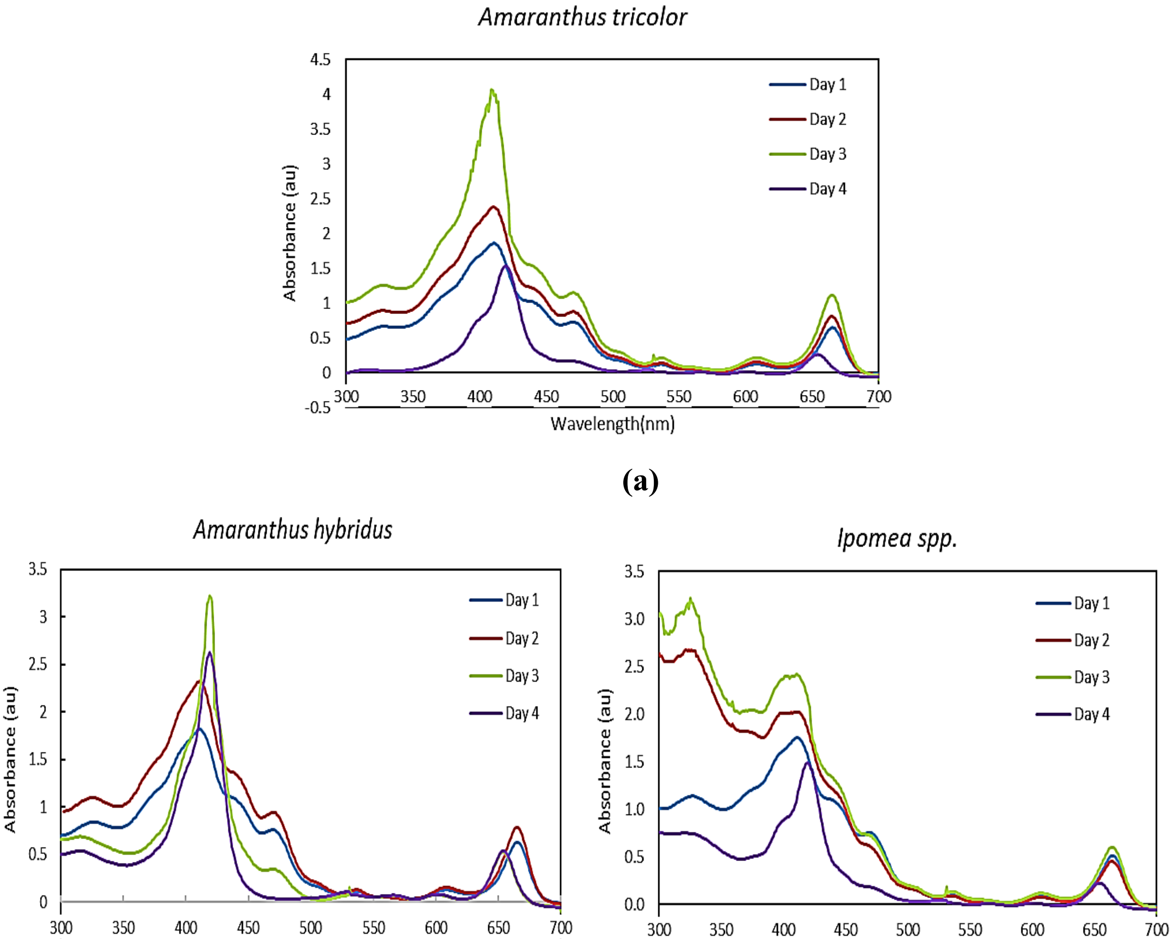
Figure 5. Chlorophyll content in the unit mg/g for water spinach (*Ipomea spp.)*, wild spinach (*Amaranthus hybridus)*, and spinach (*Amaranthus tricolor)*

Figure 5 shows the large difference in chlorophyll content between three different types of greens: water spinach, spinach, and wild spinach. Spinach (*Amaranthus tricolor)* has the highest chlorophyll content, with chlorophyll a being the main photosynthetic pigment. High chlorophyll content also indicates the potential for more efficient photosynthesis, which supports better plant growth and health. Meanwhile, water spinach (*Ipomea spp.)* has a lower chlorophyll content than both spinach (*Amaranthus tricolor)* and wild spinach (*Amaranthus hybridus)*. This also shows that chlorophyll a is essential for maximizing light absorption, especially in the red and blue spectrum. It is a very important component to produce the energy necessary for optimal plant growth(Li et al., 2024).

Table 1 Absorbance of chlorophyll A and B from various green plants. We have included the absorbance wavelength and its corresponding applications from various references for comparison.

Plant Name	Chlorophyll A Absorbance	Chlorophyll B Absorbance	Applications	Reference
Arabidopsis thaliana	430 nm, 662 nm	455 nm, 640 nm	Chlorophyll a and b absorb effectively in blue and red light.	(Lin & Charng, 2021)
Chlorella vulgaris	430 nm, 665 nm	455 nm, 642 nm	Absorption efficiency of both pigments indicates effective light harvesting.	(Malapascua et al., 2019)
Hordeum vulgare	433 nm, 665 nm	460 nm, 640 nm	The slight shift in absorbance for chlorophyll b suggests that environmental or genetic factors influence pigment interaction.	(Pollard & Nelson, 1971)
Lactuca sativa	432 nm, 662 nm	460 nm, 641 nm	Slight variations in chlorophyll b absorption, possibly brought on by the surroundings.	(Davies & Asker, 1983)
Nicotiana tabacum	430 nm, 665 nm	445 nm, 640 nm	Absorption efficiency of both pigments indicates effective light harvesting.	(Thorpe & Meier, 1974)
Oryza Sativa	431 nm, 664 nm	453 nm, 642 nm	Chlorophyll b shows a consistent absorption pattern, aiding efficient light capture.	(Nguyen et al., 2021)
Pisum savitum	430 nm, 662 nm	455 nm, 642 nm	The absorption pattern is standard, showing effective light harvesting for photosynthesis.	(Ślaski, 1990)
Solanum lycopersium	431 nm, 664 nm	453 nm, 641 nm	Absorption efficiency of both pigments indicates effective light harvesting.	(Zribi et al., 2009)
Spinacia Oleracea	430 nm, 662 nm	455 nm, 642 nm	Chlorophyll a and b absorb effectively in blue and red light.	(Zmuda & Niehaus, 2023)
Zea Mays	431 nm, 663 nm	455 nm, 642 nm	Similar absorbance ranges indicate continuous photosynthetic behavior.	(Adhikari et al., 2016)

Table 1 shows that Spinacia oleracea (spinach) has an excellent light absorption pattern, emphasizing its importance in photosynthesis. The chlorophyll components of spinach, chlorophyll a and chlorophyll b, have exceptional absorption efficiency in wavelength ranges critical to the photosynthetic process. Chlorophyll a absorbs efficiently in the blue wavelength range, precisely around 430 nm, and in the red wavelength region, approximately 665 nm. Chlorophyll b adds to this by absorbing light in the blue-green region at roughly 455 nm and the red-orange region at around 642 nm. Spinach's strong absorption, particularly in the red wavelength region, is noteworthy. Red light is an important part of the light spectrum for photosynthesis because it is most efficiently absorbed by chlorophyll a. This effective absorption enables spinach to capture a greater range of light energy, resulting in increased photosynthetic activity.



(a)

(b) (c)

Figure 6. Sample storage time spectrum (a) spinach (*Amaranthus tricolor),* (b) wild spinach (*Amaranthus hybridus)*, and (c) *water* spinach (*Ipomea spp.)*

Spinach's propensity to absorb large volumes of light, particularly in the red zone, connects directly with its increased photosynthetic efficiency. This means that spinach can transform light energy into chemical energy more efficiently, promoting overall growth and production. This finding is consistent with previous research that emphasizes the relevance of chlorophyll's absorption properties in photosynthesis. The plant's high absorbance at red wavelengths demonstrates not just efficient light absorption, but also its ability to use available light more effectively, resulting in stronger photosynthetic performance. This property of spinach makes it a great contender for a variety of applications, including those requiring effective light-to-energy conversion, such as dye-sensitized solar cells (DSSCs). Thus, the data support the hypothesis that spinach chlorophyll is extremely adept at absorbing light energy, highlighting its importance in both fundamental biological processes and practical industrial applications.

Amaranthus tricolor, Amaranthus hybridus, and Ipomoea spp. Absorbance spectra from day one to day four are displayed in Figure 6, with the main peaks occurring around 400–450 nm. Environmental stresses like high light intensity, leaf senescence, or food insufficiency can cause or contribute to the loss of chlorophyll, the principal pigment that absorbs light in the blue and red wavelengths. A blue shift, or the absorbance peak shifting to shorter wavelengths, is caused by this deterioration process. Chlorophyll molecules' structural alterations and shifts in the molecular environment cause this shift.

A shift in the wavelengths at which chlorophyll absorbs light results from the breakdown of the compound's conjugated system and electronic structure. Chlorophyll b reductase and pheophorbide an oxygenase (PAO), two enzymes that aid in the removal of chlorophyll from photosynthetic structures, are commonly involved in the breakdown process of chlorophyll. Further affecting the absorbance spectra, these enzymes aid in the breakdown of chlorophyll. The loss of chlorophyll's capacity to absorb light at particular wavelengths, such as 430–450 nm (chlorophyll a) and 650–680 nm (chlorophyll b), is reflected in the decrease in the absorbance peak seen in Figure 6 for the spectra of Amaranthus tricolor, Amaranthus hybridus, and Ipomoea spp. from day one to day four. This spectral shift is unlikely to be greatly impacted by the addition of anthocyanins, which absorb at different spectra and do not shift wavelengths. The observed blue shift for Figure 6 and decreased absorbance in the spectra demonstrate that overall, chlorophyll degradation leads to a diminished capability for light absorption (Biswal et al., 2024).

4. CONCLUSION

By lowering environmental impact and improving product appeal, the use of natural plant-based dyes, such as those from spinach, wild spinach, and water spinach, offers considerable advantages across industries like solar energy, textiles, food, and pharmaceuticals. These dyes, which are high in chlorophyll, show significant absorption in the red (620–700 nm) and blue-violet (380–460 nm) wavelengths, which are vital for photosynthesis. The plant spinach (Amaranthus tricolor) had the highest concentration of chlorophyll; absorption maxima for chlorophyll a were seen at 430 and 662 nm, while those for chlorophyll b were seen at 453 and 642 nm. The total chlorophyll content of spinach is 10.367 mg/g, whereas that of Amaranthus hybridus is 10.462 mg/g and that of Ipomoea spp. is 5.916 mg/g. This composition's high chlorophyll content facilitates effective light-to-energy conversion, supporting uses such as dye-sensitized solar cells (DSSCs). As seen in Amaranthus tricolor, Amaranthus hybridus, and Ipomoea spp. from day one to day four, however, chlorophyll degradation brought on by environmental stressors like light intensity and leaf senescence causes shifts in absorbance peaks to shorter wavelengths, emphasizing the vital role that chlorophyll plays in optimizing light absorption and plant productivity.

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