

Original Research

Investigation of Pigment Transition Patterns in Terminalia Catappa Leaves to Determine Optimal Harvesting Time for Maximum Pigment Yield

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Abstract

This study examined how the amounts of chlorophyll and carotenoids in Terminalia catappa leaves changed over four growth stages that occurred every two weeks. We used three different solvents for extraction: ethanol, dimethyl sulfoxide (DMSO), and methanol. We extracted pigments through solvent-assisted centrifugation and measured them using UV-Visible spectrophotometry. The results showed that the pigment concentrations increased as the leaves matured. Of the solvents tested, methanol had the best extraction efficiency, reaching the highest Chlorophyll-a content of 7.69 mg/g at the fourth growth stage (60 days). Ethanol followed, while DMSO had lower extraction yields and less stable pigments. Chlorophyll-a was always present in higher amounts than Chlorophyll-b and carotenoids in all stages and solvents. The data indicate that methanol is the best solvent for extracting pigments from Terminalia catappa leaves, and harvesting at later maturity stages improves pigment yield. These findings provide useful information for creating natural pigment sources for food, pharmaceuticals, and biotechnology, and they also help in valuing Terminalia catappa biomass. Unlike previous studies that only looked at solvent-based extraction, this research links extraction efficiency to leaf maturity stages, marking the first systematic identification of the best time to harvest Terminalia catappa for maximum pigment yield. This approach adds new insights into strategies for using biomass and optimizing pigment yield.

Keywords: Terminalia catappa; Chlorophyll-a; Chlorophyll-b; Carotenoids; Pigment extraction; Methanol extraction; Ethanol extraction; DMSO; UV-Visible spectrophotometry; Leaf maturation stages; Fortnightly growth variation; Biomass valorization.

Introduction

Terminalia catappa known as Indian almond, tropical almond, or sea almond, is a large deciduous tree found in tropical and subtropical areas of Asia, Africa, and the Pacific. It grows in both coastal and inland regions of Bangladesh [1, 2]. Taxonomically, it belongs to the Combretaceae family, Myrtales order, Magnoliopsida class, and Magnoliophyta division. This species has a broad, spreading canopy with branches that grow horizontally. It has significant ornamental value in urban areas and plays important ecological roles, such as controlling coastal erosion and regulating microclimates [1]. In Bangladesh, people commonly call Terminalia catappa “badam.” It grows naturally along roadsides, in school yards, parks, riverbanks, and other environments. It adapts well to various soil types, especially saline soils, which helps it thrive with little farming intervention. This makes it a strong candidate for afforestation, phytoremediation, and developing resource-based bioproducts [2].

Its most distinctive features include large, leathery leaves shaped from ovate to obovate, measuring around 15 to 25 cm long and 10 to 14 cm wide. As the leaves mature, they change color from bright green to yellow, red, and brown before falling off. This color change shows the breakdown of photosynthetic pigments like chlorophylls and the increased visibility or creation of secondary pigments such as carotenoids and anthocyanins [3]. The pigment changes in Terminalia catappa leaves are mainly driven by the amounts and ratios of chlorophylls (chlorophyll-a and chlorophyll-b) and carotenoids (including β -carotene, lutein, and zeaxanthin) [3, 4]. Chlorophylls are the main pigments used in photosynthesis, with chlorophyll-a mostly absorbing light in red and blue-violet areas, while

chlorophyll-b helps extend light absorption [4]. Carotenoids are accessory pigments that aid in energy transfer and provide protection against photooxidative stress [4, 5].

Chlorophyll molecules consist of a porphyrin ring that coordinates with a central magnesium ion, which is essential for capturing solar energy. On the other hand, carotenoids are tetraterpenoids made of 40-carbon polyene chains and fall into two main groups: carotenes and xanthophylls [5, 6]. Both groups of pigments respond well to environmental conditions like light intensity, temperature changes, and nutrient availability, making them effective physiological and ecological markers [5, 6]. Beyond their roles in plants, chlorophylls and carotenoids are also important for health. Chlorophylls have detoxifying, anti-inflammatory, and wound-healing abilities, while carotenoids are powerful antioxidants and precursors to vitamin A [4, 7]. Many studies show that dietary carotenoids can lower the risk of chronic illnesses like heart disease, age-related macular degeneration, and certain types of cancer [6-8]. This dual ecological and therapeutic aspect drives increasing scientific interest in *Terminalia catappa* as a reliable source of bioactive pigments for food, pharmaceutical, and cosmetic industries.

Traditionally, people have used *Terminalia catappa* leaves in folk medicine across Asia and Africa. Ethnomedicinal uses include treating skin issues, diarrhea, dysentery, hepatitis, and respiratory problems [2, 9]. Research has uncovered a variety of secondary metabolites, such as flavonoids, hydrolyzable tannins (especially punicalagin and corilagin), saponins, alkaloids, phenolic acids, and triterpenoids, which contribute to its wide-ranging biological effects [2, 10]. Notably, dried *Terminalia catappa* leaves are used in aquaculture, particularly in ornamental fish farming. The leaves release bioactive compounds that lower water pH, inhibit microbial growth, and improve fish health by reducing stress and increasing resistance to infections [10, 11]. Their use as a natural alternative to antibiotics highlights their value in sustainable aquaculture [11].

Given the ecological abundance, medicinal importance, and rich phytochemistry of *Terminalia catappa*, this study aimed to explore how pigment content changes throughout different stages of leaf maturity. We extracted pigments using methanol, ethanol, and dimethyl sulfoxide (DMSO) and analyzed them with UV-Visible spectrophotometry. The main goal is to find the best harvesting stage for maximum pigment yield, promoting *Terminalia catappa* as a natural pigment source for biotechnological, nutritional, and renewable energy applications.

While several studies have looked into general pigment extraction from plant leaves, there have been few comprehensive analyses connecting solvent effectiveness with the developmental stages of *Terminalia catappa* leaves. Past research has mainly focused on qualitative extractions or medicinal uses. In contrast, this study provides a quantitative assessment of chlorophyll and carotenoid variation across specific growth intervals. It establishes a clear link between leaf maturity and pigment yield, creating a unique contribution to existing pigment research by identifying the best harvesting period and comparing the performance of different solvents.

Literature Review

Previous research on *Terminalia catappa* has mainly centered on its medicinal and pharmacological uses, such as its antioxidant, liver-protective, antibacterial, and antidiabetic effects, attributed to its rich variety of flavonoids, tannins, and phenolics. Recently, pigment extracts from *Terminalia catappa* leaves have shown promise as natural dyes in dye-sensitized solar cells and as eco-friendly colorants for the food and cosmetic industries. However, few studies have focused on how chlorophylls and carotenoids change during leaf development or how to determine the ideal harvesting time for maximizing pigment extraction. This information gap motivates the current study. The observed drop in chlorophylls, along with steady carotenoid levels, aligns with previous findings about pigment changes during leaf aging.

Materials and Methods

Collection and Authentication of Plant Material

The fresh leaves of *Terminalia catappa* were collected from several healthy, mature trees growing on the campus and riverbanks in the Savar region of Dhaka District, Bangladesh, between June and November 2024. Leaves were gathered at different developmental stages: young green, mature green, early senescent (yellowing), and late senescent (reddish-brown) to capture pigment variations as the leaves matured. This study took place at Gono Bishwabidyalay, located in Savar, Dhaka, Bangladesh. The area offers a good natural environment for the growth of *Terminalia catappa* trees.

The research occurred over 60 days from March to May 2022, during the active growth phase when *Terminalia catappa* produces new leaves. More than 15 healthy trees were chosen for sampling. Both mature and young leaves were collected from the same trees to ensure consistency. The leaves were processed right after collection without any storage to keep the pigment intact.



Figure 1: *Terminalia catappa* leaf samples collected at different time points such as, (a) 1st Fortnight (15th day) – young green leaves, (b) 2nd Fortnight (30th day) – moderately matured leaves, (c) 3rd Fortnight (45th day) – mature leaves with deeper green color, (d) 4th Fortnight (60th day) – fully matured leaves.

Reagents and Equipment

The study used 95% ethanol (analytical grade), dimethyl sulfoxide (DMSO), methanol (analytical grade), and distilled water as solvents. The equipment included a UV-Visible spectrophotometer, a refrigerated centrifuge, a mortar and pestle for sample homogenization, an analytical balance for precise weighing, pipettes (1 mL and 10 mL), and a variable volume micropipette. All chemicals were obtained from trusted suppliers such as Sigma-Aldrich and Merck and were used as received.

Preparation of Plant Material

The healthy, disease-free young leaves of *Terminalia catappa* were collected and prepared for analysis right away. First, the leaves were rinsed under running tap water. Then, they were pre-washed with distilled water to remove any surface contaminants. The wet leaves were gently blotted dry using sterile tissue paper. We carried out extraction procedures directly with fresh leaves to keep as much pigment as possible. To reduce pigment degradation, we processed the leaf samples within 30 minutes of collection. All extraction procedures took place in low light at room temperature (25 ± 2 °C). This helped minimize photooxidation or enzymatic loss of pigments before analysis.

Extraction of Pigments

We extracted pigments using solvent-assisted centrifugation with DMSO, methanol, and ethanol as solvents. We accurately weighed about 0.25 grams of fresh leaf tissue using an analytical balance. Each extraction used 0.25 ± 0.01 grams of homogenized fresh leaf tissue to keep things consistent across all solvent systems and sampling times. We chose this standard mass based on early optimization tests aimed at maximizing pigment recovery. The sample was gently homogenized with a clean mortar and pestle. We transferred the homogenate into clean test tubes with 5 mL of solvent (95% ethanol, DMSO, or methanol) using a pipette. We let the mixture sit for 5 minutes to help dissolve the pigments. Samples were centrifuged at 10,000 rpm for 15 minutes at 4 °C.



Figure 2: Comparative analysis of pigment extraction efficiency using three different solvents: 95% ethanol, dimethyl sulfoxide (DMSO), and methanol.

After centrifugation, we carefully pipetted the clear greenish supernatant, which contained the dissolved pigments, into clean tubes for further dilution and analysis. For the final analysis, we mixed 0.25 mL of the supernatant with 2.25 mL of the respective solvent using a micropipette. This ensured the right concentration range for spectrophotometric measurement.

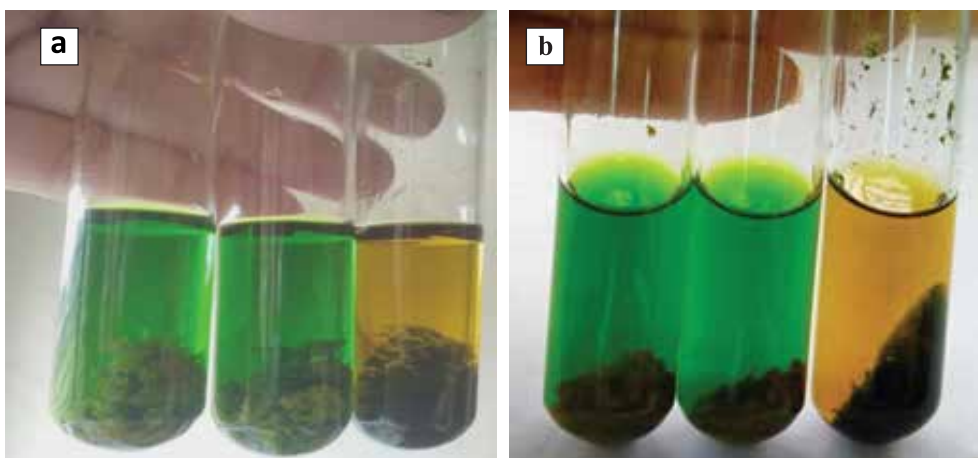


Figure 3: Visual representation of (a) homogenized *Terminalia catappa* leaf samples before centrifugation and (b) clear supernatant obtained after centrifugation.

Characterization

The pigment contents, which include chlorophyll-a, chlorophyll-b, and carotenoids, were identified and measured with a UV-Visible spectrophotometer. The absorbance of the prepared extracts was recorded at specific wavelengths based on the extraction solvent: for 95% ethanol at 664 nm, 649 nm, and 470 nm; for DMSO at 665.1 nm, 649.1 nm, and 480 nm; and for methanol at 665.2 nm, 652.4 nm, and 470 nm. Only the clear supernatant was carefully transferred into clean quartz cuvettes to prevent any disturbance from sediment or debris. We performed baseline correction using the respective pure solvent as the blank for each measurement. Pigment concentrations ($\mu\text{g/mL}$) were calculated with the following solvent-specific equations (Table 1). All experiments were done in triplicate ($n = 3$) for each solvent and developmental stage. The data are shown as mean $\pm$ standard deviation (SD).

We carried out statistical analysis using Microsoft Excel 2021. Differences among means were evaluated using one-way ANOVA, with $p < 0.05$ considered statistically significant.

Table 1: Equations for determination of Chlorophyll-a (Chl-a), Chlorophyll-b (Chl-b), and total Carotenoids (Cx+c) by different extractant solvents in spectrophotometer.

Solvent	Equation for Chl-a	Equation for Chl-b	Equation for Cx+c
95% Ethanol	$\text{Chl-a} = 13.36 \times A_{664} - 5.19 \times A_{649}$	$\text{Chl-b} = 27.43 \times A_{649} - 8.12 \times A_{664}$	$\text{Cx+c} = (1000 \times A_{470} - 2.13 \times \text{Ca} - 97.63 \times \text{Cb}) / 209$
DMSO	$\text{Chl-a} = 12.47 \times A_{665.1} - 3.62 \times A_{649.1}$	$\text{Chl-b} = 25.06 \times A_{649.1} - 6.5 \times A_{665.1}$	$\text{Cx+c} = (1000 \times A_{480} - 1.29 \times \text{Ca} - 53.78 \times \text{Cb}) / 220$
Methanol	$\text{Chl-a} = 16.72 \times A_{665.2} - 9.16 \times A_{652.4}$	$\text{Chl-b} = 34.09 \times A_{652.4} - 15.28 \times A_{665.2}$	$\text{Cx+c} = (1000 \times A_{470} - 1.63 \times \text{Ca} - 104.96 \times \text{Cb}) / 221$

Note: A = Absorbance at the given wavelength, Ca = Chlorophyll-a concentration, and Cb = Chlorophyll-b concentration.

Result and Discussions

The present study concerns the variation in the pigment content of the leaves of *Terminalia catappa* during a 60-day growth period, sampled at 15-day intervals. Fifteen trees from a uniform site were selected to reduce variability in environmental conditions. The pigments were extracted using ethanol, DMSO, and methanol and quantified by UV-Visible spectrophotometry. The results show dynamic changes in pigment accumulation (chlorophyll-a, chlorophyll-b, and carotenoids) over the maturation period and marked variations in the efficiency of extraction among the studied solvents.

Pigment Extraction Using Ethanol

The ethanol-extracted samples showed different pigment accumulation patterns across the four Fortnightly stages. As shown in Figure 4, Chlorophyll-a concentration increased sharply from 2.98 mg/g at the 1st Fortnightly to a peak of 7.08 mg/g at the 2nd Fortnightly (30 days). This suggests an active phase of chlorophyll production during early leaf maturation. A slight decline to 6.72 mg/g and 6.69 mg/g was observed during the 3rd and 4th Fortnightly stages, likely due to the start of leaf aging and pigment breakdown. Chlorophyll-b followed a similar pattern, rising from 1.19 mg/g to 3.19 mg/g between the 1st and 2nd Fortnightly and stabilizing around 2.86–2.95 mg/g thereafter. Carotenoids showed a modest but steady increase from 1.19 mg/g to 1.75 mg/g.

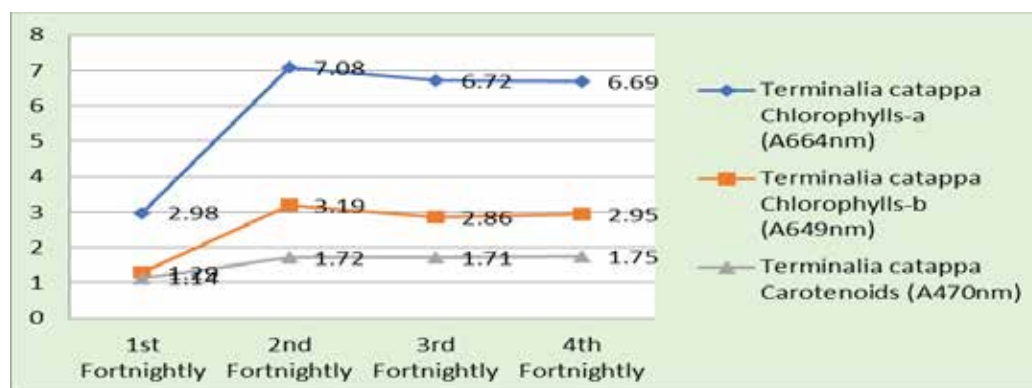


Figure 4: Variation in Chlorophyll-a, Chlorophyll-b, and Carotenoid's content (mean $\pm$ SD, $n = 3$) in *Terminalia catappa* leaves over four fortnightly stages using ethanol as extraction solvent, used pigment concentration (mg/g $\pm$ SD).

Their relatively stable levels indicate a continued need for protection from light and for gathering light throughout the growth cycle. These findings demonstrate ethanol's effectiveness in extracting chlorophylls and carotenoids during the active growth phase of *Terminalia catappa* leaves, especially in the early-to-mid maturation stages.

Pigment Extraction Using DMSO

In contrast to ethanol, DMSO extraction showed a different pigment profile. As shown in Figure 5, pigment concentrations stayed relatively low during the early stages but rose significantly by the 3rd Fortnightly (45 days).

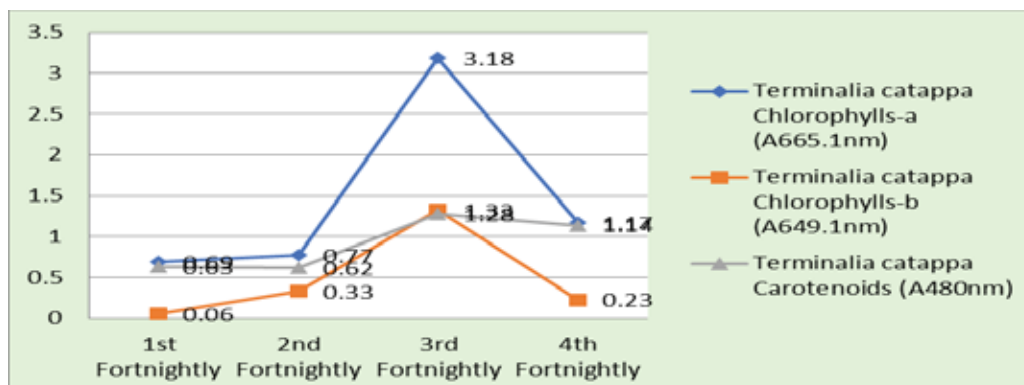


Figure 5: Variation in Chlorophyll-a, Chlorophyll-b, and Carotenoids content (mean $\pm$ SD, n = 3) in *Terminalia catappa* leaves over four Fortnightly stages using Dimethyl Sulfoxide (DMSO) as extraction solvent, used pigment concentration (mg/g $\pm$ SD).

Chlorophyll-a content increased from 0.69 mg/g at the 1st Fortnightly to 3.18 mg/g at the 3rd Fortnightly, then dropped sharply to 1.14 mg/g by the 4th Fortnightly. Similarly, Chlorophyll-b and Carotenoid contents peaked at 1.38 mg/g and 1.14 mg/g, respectively, during the 3rd Fortnightly stage. The rapid rise and subsequent decline suggest that DMSO is more sensitive to changes in the leaves and may not preserve pigment integrity well over longer periods. Potential oxidation of pigments during extraction could explain the observed patterns. Therefore, DMSO seems suitable for short-term pigment analyses, especially during mid-growth stages, but less ideal for extensive, long-term pigment studies.

Pigment Extraction Using Methanol

Methanol consistently produced the highest pigment concentrations out of all the solvents tested. Figure 6 shows that Chlorophyll-a levels increased from 4.29 mg/g in the 1st Fortnightly to a peak of 7.69 mg/g in the 4th Fortnightly. This was the highest pigment yield recorded across all treatments and stages. Chlorophyll-b concentrations also rose from 1.37 mg/g to 2.04 mg/g.

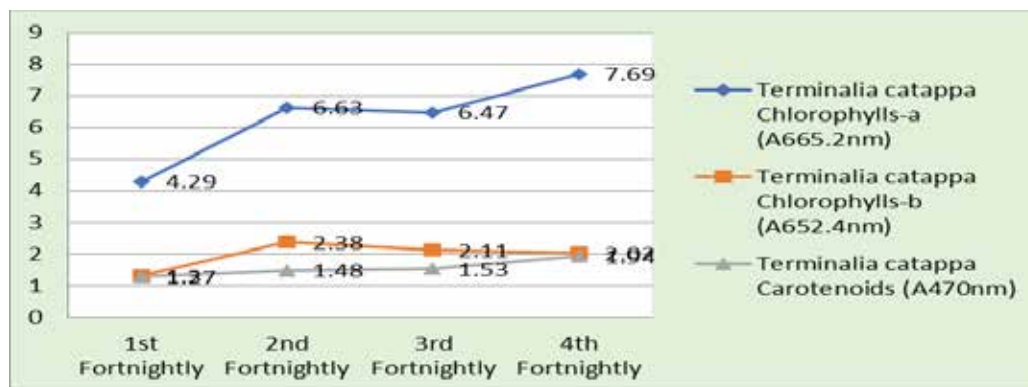


Figure 6: Variation in Chlorophyll-a, Chlorophyll-b, and Carotenoids content (mean $\pm$ SD, n = 3) in *Terminalia catappa* leaves over four Fortnightly stages using Methanol as extraction solvent, used pigment concentration (mg/g $\pm$ SD).

Carotenoid levels increased from 1.27 mg/g to 1.94 mg/g. The similar upward trends for all pigments indicate that the processes to produce chlorophylls and carotenoids remained active as the leaves matured. Methanol effectively

helped in their extraction and stabilization. Methanol's strong performance comes from its high polarity, strong hydrogen-bonding ability, and excellent tissue permeability, which improve pigment solubilization. Its relatively low boiling point also reduces thermal degradation during sample preparation.

Comparative Analysis Across Solvents

A comparison of the solvents showed clear differences in how well they extracted pigments. Methanol consistently produced the highest concentrations of Chlorophyll-a, Chlorophyll-b, and Carotenoids. Ethanol followed next, with DMSO coming in last. Throughout the study, the amount of Chlorophyll-a remained higher than both Chlorophyll-b and Carotenoids, which supports its key role in capturing light for photosynthesis. Ethanol was particularly effective during the early and mid-stages, providing a good option when methanol use may be limited due to safety or regulatory issues. On the other hand, DMSO showed limited efficiency and caused pigment breakdown over time, making it a poor choice for long-term pigment stability studies. These findings agree with previous research, which shows that methanol and ethanol are dependable solvents for extracting plant pigments. The consistent rise in pigment levels when using methanol supports the idea of focusing on late maturation stages, especially the 4th Fortnightly, for the best pigment harvest.

Summary of Extraction Efficiencies Across Solvents

To compare pigment extraction performance quantitatively, the average amounts of Chlorophyll-a, Chlorophyll-b, and Carotenoids collected at the peak (4th fortnightly) stage are summarized in Table 2. The results clearly show that methanol produced the highest pigment concentrations in all three categories, followed by ethanol and DMSO.

Table 2. Summary of extraction efficiencies (mean $\pm$ SD, n = 3) of Chlorophyll-a, Chlorophyll-b, and Carotenoids in *Terminalia catappa* leaves at the 4th fortnightly (60-day) stage using different solvents.

Solvent	Chlorophyll-a (mg/g $\pm$ SD)	Chlorophyll-b (mg/g $\pm$ SD)	Carotenoids (mg/g $\pm$ SD)	Relative Efficiency Trend
Methanol	7.69 $\pm$ 0.21	2.04 $\pm$ 0.10	1.94 $\pm$ 0.08	Highest
Ethanol	6.69 $\pm$ 0.18	2.95 $\pm$ 0.12	1.75 $\pm$ 0.09	Moderate
DMSO	1.14 $\pm$ 0.05	1.38 $\pm$ 0.07	1.14 $\pm$ 0.06	Lowest

The differences in efficiency relate to the polarity of the solvents and how well they can move through tissue. The specific measurement of chlorophyll levels using UV-Vis spectrophotometry follows the methods outlined by Porra (2002).

3.6 Influence of Growth Stage on Pigmentation

The developmental stage of the leaves greatly affected pigment accumulation. Younger leaves, sampled during the 1st Fortnightly, showed much lower pigment levels. This likely happened because chloroplast structures were not fully developed in the early stages. As the leaves matured, pigment concentrations gradually rose, peaking during the 3rd and 4th Fortnightly stages, depending on the solvent. This trend reflects how the leaves adapt physiologically as they change. Chlorophyll production increases to meet the growing demands of photosynthesis. Higher levels of carotenoids in the later stages indicate a greater need for protection from light as the photosynthetic system becomes fully functional. These findings are important for improving leaf harvesting strategies to maximize pigment yield. This has significant implications for areas like phytochemistry, natural coloring, nutraceuticals, and biotechnology. The changes in chlorophyll and carotenoid levels observed during leaf maturation and aging show important physiological adaptations related to photosynthesis and stress management. As leaves age, chlorophyll breaks down faster through a pathway that involves chlorophyllase and pheophytinase. This process converts chlorophyll into non-fluorescent products. This degradation reduces photosynthesis but allows carotenoids to become more prominent. Carotenoids, especially xanthophylls like lutein and zeaxanthin, either build up or remain stable during this time to protect against excess light energy and prevent damage from oxidation. The decline of chlorophyll alongside the persistence of carotenoids indicates a protective shift in metabolism. This ensures controlled aging and nutrient redistribution. These insights explain the pigment transition pattern seen in *Terminalia catappa* leaves, highlighting the late maturation stage as the best time for harvesting pigments.

Conclusion and Future Directions

This study shows that the extraction solvent and leaf maturity have a significant impact on the pigment content of *Terminalia catappa* leaves. Methanol was the most effective solvent for extracting chlorophylls and carotenoids, with pigment levels peaking at the 4th fortnightly (60-day) stage, representing late leaf maturation. Beyond laboratory findings, these results matter for industries that use natural pigments. Identifying the best harvesting time provides a scientific foundation for large-scale pigment extraction from *Terminalia catappa* biomass for eco-friendly dye production, food and beverage color, cosmetic formulations, and pharmaceutical uses where natural antioxidants are important. Using *Terminalia catappa* leaves, which are readily available and often underused, promotes sustainable bioproduct development and waste valorization in tropical areas. While this work focused on chlorophylls and carotenoids, future studies could include anthocyanin pigments that become noticeable during late aging, offering additional insight into color changes in *Terminalia catappa* leaves. Additionally, research examining seasonal variation, environmental effects, and pigment stability during storage or processing could enhance the practical understanding of these natural colorants. Such studies could strengthen the use of *Terminalia catappa* pigments in sustainable dyeing, food, and cosmetic applications.

Authors Contributions

Md. Fuad Hossain performed the fieldwork, laboratory experiments and initial data organization, and contributed to the first draft. Suparna Rahman Tuchy assisted with experimental design, data interpretation and manuscript revision. Nilay Kumar Dey conceived and supervised the study, carried out the main analyses and prepared the final manuscript. All authors read and approved the final version. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that they do not have any conflict of interest.

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