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Extraction of Chlorophyll and its application as a natural dye for fabrics

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Abstract

As the world faces growing concerns over the harmful effects of synthetic dyes on both the environment and human health, chlorophyll presents itself as a revolutionary, eco-friendly alternative in the dye industry. This naturally occurring pigment, vital in photosynthesis, is renowned for its renewability, bioactivity, and versatility, making it an ideal candidate for applications in textiles, food, and medicine. The study delves into various methods for extracting chlorophyll, including aqueous, solvent-based aiming to maximize yield and quality. In addition, it investigates chlorophyll's structural and functional roles, its nutritional advantages, and its potential as a sustainable substitute for harmful synthetic dyes. Laboratory trials show promising results in using chlorophyll to effectively dye fabrics, reinforcing its viability as a green solution for industries moving towards eco-conscious practices.

Key Words:

Chlorophyll, Extraction, Natural Dye, Green Plants, and Fabrics.

1. Introduction:

In recent decades, plants have become important s ources of phytochemicals, which have been used i n many fields, including the food industry and me dicine. For example, phytochemicals extracted fro m plants are used for the design and development of numerous functional ingredients and/or nutrace uticals (Uğuz *et al.*, 2023).

There has been a resurgence of interest in natural dyes worldwide (Habib *et al.*, 2022). In fact, some synthetic dyes are banned in Western countries due to their toxic, carcinogenic and polluting properties. For centuries, natural dyes were the lifeblood of textile coloring, dominating the industry up until the late 19th century. These vibrant, earth-derived hues not only defined the look of fabrics but also shaped the evolution of textile craftsmanship during that era. The growing demand for eco-friendly products has sparked a notable shift among environmentally conscious consumers, who are now gravitating toward textiles crafted with natural dyes. This shift is fueling the rise of a niche market dedicated to sustainable fabrics, offering a greener alternative for those seeking to align their lifestyles with eco-conscious values (Verma *et al.,* 2016; Ragab *et al.,* 2022). Artificial colorants have long been a cause for concern due to their harmful effects on human health, including their association with cancer risks and genetic mutations. Beyond the health implications, these substances also pose significant environmental challenges, particularly through the toxic wastewater discharged by the paint industry. In response to these pressing issues, the sector has been increasingly motivated to explore and implement safer, more sustainable methods of dye production. Such initiatives are not just a step forward in safeguarding health but also represent a crucial effort in minimizing ecological harm (Adeel *et al.*, 2022, Adeel *et al.*, 2022, Hussaan *et al.*, 2023). Natural dyes provide a sustainable, eco-conscious alternative to synthetic counterparts, frequently delivering additional biological advantages. (Batool *et al.,* 2022, Andriamanantena *et al.,* 2023).

Natural dyes stand out as champions of sustainability, adored for their environmentally friendly nature. Sourced from renewable materials and capable of breaking down naturally, they leave no harmful trace on the planet, making them a perfect choice for eco-conscious living. Soft and soothing on the skin, they not only bring comfort but could also offer potential health advantages to those who wear them. They can be used to dye almost any natural textile (Verma *et al.,* 2016, Ragab *et al.,* 2022, Saxena *et al.,* 2014).

Natural pigments are packed with an impressive blend of vitamins, minerals, bioactive plant compounds, and essential nutrients that contribute to overall well-being. These vibrant compounds aren't just about providing color; they offer a wealth of health benefits. From delivering powerful antioxidants to reducing inflammation, they also play a role in cancer prevention, safeguarding brain health, and promoting heart health. Their multifaceted properties make them indispensable allies in supporting a healthy lifestyle. (Lu *et al.,* 2023, Azmin *et al.,* 2022). Plant pigments hold immense promise across industries such as cosmetics, pharmaceuticals, and food production. Their natural properties make them versatile and valuable, paving the way for innovative applications and sustainable solutions in these sectors. (Azmin *et al.,* 2022). Over the past few years, natural dyes have experienced a surge in global demand, fueled by rising health consciousness and the uncovering of exciting new pharmacological benefits linked to these vibrant, nature-derived pigments. As more people embrace wellness-oriented lifestyles and seek safer, eco-friendly alternatives, the appeal of natural dyes continues to expand, carving out a prominent space in today's market. (Lyu *et al.,* 2022, Yu *et al.,* 2024).

Conventional production techniques place considerable emphasis on meticulously chosen sources, underscoring the importance of extraction in obtaining pure bioactive pigments from specific plants.

The conventional method for extracting natural pigments from plants typically relies on solid-liquid extraction. This process involves grinding plant tissues and soaking them to encourage the release of pigments into the chosen solvent. When tissue undergoes mechanical degradation, it leads to the breakdown of cell walls and the release of various cellular components. This process creates the need for extra cleaning measures to ensure all remnants are properly removed.

Solvent extraction faces a multitude of challenges, making it far from perfect in many applications. For starters, the process can be time-consuming, often requiring extended periods to achieve results. Despite this, it frequently falls short in terms of extraction efficiency, yielding lower-than-expected outputs. Additionally, the method necessitates the use of substantial volumes of solvents, which isn't just impractical but also costly. The situation is further compounded by the naturally low concentrations of target pigment compounds, which hamper overall efficiency even more. Beyond these operational drawbacks, the technique raises red flags for its environmental footprint and potential health hazards, posing serious concerns about its sustainability and long-term impact. (Yu *et al.,* 2024, Rodríguez-Mena *et al.,* 2023, Garcia-Vaquero *et al.,* 2020).

Natural pigments add a splash of vibrant colour to our world while offering impressive health benefits, thanks to their rich bioactive properties. These natural compounds are more than just visually appealing; they carry a host of advantages that make them a fascinating ally for overall well-being. (Di Salvo *et al.,* 2023).

Chlorophyll has been described to exhibit antimut agenic and anticarcinogenic properties due to its a bility to accumulate in tumor tissues and to induce a photodynamic effect upon laser irradiation, prod ucing reactive oxygen species and leading to the de ath of cancer cells (Hojnik *et al.,* 2007).In addition, some data have reported that chl orophylls are able to accelerate wound healing (Hos ikian *et al.,* 2010). Aid in cell repair, and increase hemoglobin levels i n the blood (Kong *et al.,* 2014).

Recently, it has been shown that chlorophyll deriv atives extracted from spinach are able to prevent o xidative damage to DNA. human lymphocytes (Da merum *et al.,* 2015).

In addition to their recent medicinal applications, chlorophylls are widely used as natural colorants in the food and cosmetic industries (Hosikian *et al.,* 2010, Ferreira *et al.,* 2021).

Chlorophyll, the vibrant green pigment that gives plants their signature hue, is a natural wonder widely found in the plant kingdom. Its chemical structure is as fascinating as its role in life on Earth. At its core, chlorophyll boasts a tetrapyrrole framework that forms a porphyrin ring, intricately bonded to a magnesium atom. This unique arrangement is not just a structural detail—it's the key to its remarkable ability to capture sunlight and fuel the life-sustaining process of photosynthesis. Chlorophyll plays a remarkable role in the life of green plants by harnessing the power of sunlight. It enables plants to convert solar energy into chemical energy, kick-starting the process of synthesizing organic compounds from carbon dioxide and water in the air. In doing so, it not only fuels the plant's growth and reproduction but also releases oxygen into the atmosphere, supporting life on Earth. Chlorophyll isn't just the green pigment that gives plants their vibrant hue; it also boasts an impressive array of biological benefits. This natural powerhouse offers potent antioxidant properties, helps combat inflammation, and even exhibits antibacterial effects, making it a multifaceted asset for health and wellness. (Pucci *et al.,* 2021, Hub *et al.,* 2024).

Chlorophylls are the main photoreceptors in phot osynthesis and are common in nature; They are fo und in plants, algae, and cyanobacteria (Hosikian *et al.,* 2010).

In plants, there are two chemical forms, namely ch lorophyll a and b, usually present in a 3:1 ratio. Th e difference between the two forms of chlorophyll lies in the presence of a methyl group at carbon C3 for chlorophyll a, while in chlorophyll b a formyl group has been found at the same position (Hosikian *et al.,* 2010, Ferreira *et al.,* 2021, Makarska-Bialokoz *et al.,* 2014).

In conclusion, recent research highlights those natural dyes, such as chlorophyll, offer promising alternatives to synthetic dyes, both in terms of health benefits and reduced environmental impact. With growing consumer awareness of environmental and health issues, the demand for these dyes is expected to continue rising across various industries. This underscores the importance of ongoing research into their extraction methods and applications. Using natural dyes not only promotes sustainability but also enhances the health benefits for individuals, making them an ideal choice for the future.

2. The Theoretical Framework

2.1. Natural Dyes

Natural dyes are celebrated for their eco-friendly qualities, stemming from their roots in renewable resources and their natural ability to break down, making them fully biodegradable and kinder to the environment. (Saxena et al., 2014). Natural colors are harmonizing and soothing to th e human eye. The ingredients have been thoughtfully designed to minimize allergy risks, care for sensitive skin, and put human health and safety first. (Samanta *et al.,* 2011, Khan *et al.,* 2018). Synthetic dyes are derived from petroleum and coal tar, whereas natural dyes come from the rich bounty of nature itself. Synthetic dyes are highly preferred for their vibrant colors, durability, user-friendly application, and affordability. Growing awareness of the potential risks and adverse effects associated with synthetic dyes is garnering more attention than ever before. In today's world, there's a growing spotlight on the use of natural raw materials in textile production, alongside a rising awareness of the ecological impact of dyeing processes. As sustainability takes center stage, many are turning their attention to the environmental benefits of using natural dyes, sparking conversations about their role in creating a greener and more responsible future for the textile industry. (Saxena *et al.,* 2014, Salauddin *et al.,* 2021).

2.1.1. The benefits of using natural dyes

In the past few years, the timeless art of natural dyeing has made an extraordinary comeback, drawing admiration from artisans and eco-conscious individuals around the globe. This renewed interest stems from the numerous advantages natural dyes offer, often surpassing their synthetic counterparts in both sustainability and charm.: -

1. Natural dyes stand out as a sustainable alternative to their synthetic counterparts due to their remarkable properties. Unlike synthetic dyes, which are derived from non-renewable raw materials, natural dyes are typically sourced from renewable and agro-renewable resources. Not only are they biodegradable, but they are also far more environmentally friendly, minimizing the ecological footprint of their usage. What's even more fascinating is their potential to offer medicinal benefits, adding an unexpected layer of value to their application. This makes natural dyes a compelling option, not just for sustainability advocates but for industries and individuals seeking

eco-conscious and health-enhancing solutions. (Ahmed *et al.,* 2018, Helmy *et al.,* 2020, Mohan *et al.,* 2020).

2. Natural plants like harda and indigo, along with the byproducts generated during their extraction and dyeing processes, often serve as exceptional fertilizers in agriculture. As a result, these organic residues are efficiently reused, completely eliminating the necessity for waste disposal. (Ragab *et al.,* 2022, Kasiri *et al.,* 2013).

3. Natural dyes, sourced from renewable materials, stand out as an environmentally friendly choice that harmonizes beautifully with nature, leaving no harmful impact on the planet. (Affat *et al.,* 2021).

4. Natural tones possess an undeniable allure, effortlessly infusing any space with a sense of tranquility and enduring sophistication. From soft, understated hues that whisper serenity to soothing shades that envelop the senses, these versatile colors hold the key to crafting a haven of peace and refinement. Embrace the subtle allure of a neutral palette and watch as it weaves a harmony that feels both luxuriously elegant and comfortably grounded. (Affat *et al.*, 2021).

5. Certain natural colorants, such as carmine, which is a popular ingredient in many lipsticks, are widely regarded as safe and non-toxic, even if accidentally ingested. (Affat *et al.*, 2021).

2.1.2. **Drawbacks of natural dyes**

Although natural dyes offer several benefits, they also come with significant drawbacks. (Victoria *et al.,* 2015, Sarfarazi *et al.,* 2015). Natural dyes hold immense historical and cultural value, yet their role in the modern textile industry remains surprisingly small—barely 1% of the sector. This modest figure can largely be attributed to the significant technical hurdles and sustainability concerns associated with their production and application. While these challenges stifle broader adoption, they also highlight an urgent need for innovation and adaptation to harness the true potential of natural dyes within a more sustainable fashion ecosystem. (Verma *et al.,* 2016, Ragab *et al.,* 2022). The constraints and obstacles can be summarized in the following points: -

1. Achieving consistent hues with natural dyes is notably challenging due to the inherent unpredictability of these agricultural resources. The final color outcomes are shaped by a range of variables, including seasonal fluctuations, geographical origins, plant varieties, and the stages of ripeness. (Habib *et al.,* 2022, Ragab *et al.,* 2022, Affat *et al.,* 2021).

2. Designing a recipe for natural dyeing can often feel like unraveling a mystery. Achieving bold, enduring colors goes far beyond the dye material itself; it hinges heavily on the selection of your base materials. The type of fabric, the nature of the fibers, and even the surrounding environmental conditions are all critical factors that influence the resulting shades. This intricate balance transforms natural dyeing into a captivating blend of experimentation, instinct, scientific principles, and time-honored practices (Ragab *et al.,* 2022).

3. Colored textiles often face challenges in maintaining their original hues when exposed to the forces of nature and daily wear. Sunlight, sweat, and exposure to air can cause noticeable shifts in their coloration over time. However, a handful of exceptional fabrics stand out—designed to endure these conditions, they boast impressive fade resistance and continue to shine with vibrant colors, even after repeated washes and extended exposure to light (Ragab et al., 2022).

4. Natural dyes often struggle to compete with synthetic alternatives in terms of cost efficiency. Reaching comparable levels of vibrancy and

saturation on fabrics generally requires a significantly larger amount of natural dye. For instance, coloring one gram of cotton might need as few as five grams of synthetic dye, whereas the same process could demand an astonishing 230 grams of natural dye. This stark difference underscores why natural dyes tend to increase costs and pose challenges for large-scale production, even though they are more environmentally friendly (Affat *et al.,* 2021).

5. Solubility: The solution has low water solubility and a tedious extraction procedure may be required (Affat *et al.*, 2021).

6. The downside of natural dyes is that they're not entirely free from harm. While often hailed as eco-friendly alternatives, they, too, can have their own set of drawbacks. Logwood, a natural resource known for producing the compounds hematein and hematoxylin, carries certain health risks that shouldn't be overlooked. When these substances are inhaled, ingested, or come into direct contact with the skin, they can potentially lead to significant harm. It's essential to handle them with care and take appropriate precautions to minimize exposure. Bloodroot, often celebrated for its role as a natural dye, carries an important warning. While its vivid pigmentation has long been cherished, the fine particles it releases can pose a risk if inhaled, potentially causing irritation or even inflammation. The application of natural dyes frequently relies on the use of mordants, which play a vital role in ensuring the dyes adhere effectively to the material. These compounds play a crucial role in helping the paint bond securely to fabrics; however, they often come with a major downside—they can be dangerous and potentially harmful. Mordants play a crucial role in the art of natural dyeing, acting as agents that help fix colors to fabrics and intensify their vibrancy. Some commonly used mordants include aluminum, known for brightening hues; copper, which can deepen shades; iron, often used to create darker, muted tones; and chromium, a traditional choice for enhancing color durability. Each of these substances not only locks in natural dyes but also transforms their results, offering an array of beautiful, lasting finishes (Affat *et al.,* 2021).

2.2. Methods of Extraction of Natural Dyes

The process of extraction entails the isolation of specific color components by breaking down plant cell walls through the application of either physical or chemical methods. This operation is conducted within a solvent medium under precisely controlled environmental parameters (Habib *et al.,* 2022, Ragab *et al.,* 2022).

Extracting dyes from natural sources plays a pivotal role in textile production, acting as an essential step to achieve the desired dyeing characteristics while ensuring high-quality outcomes. Developing a standardized extraction process and optimizing parameters specific to a particular natural resource are crucial for achieving economic efficiency. Such measures directly contribute to lowering production costs for the end products (Ragab *et al.*, 2022).

colors were extracted using water and organic solv ents. Water-based dyes were prepared by bleaching, grinding, a nd filtering the plant material using distilled water (Saxena *et al.,* 2014, Samanta *et al.,* 2011, Khan *et al.,* 2018). They were more concentrated by freezing (Khan et al., 2018).

Spinach and mint were extracted using acetone as a solvent (Khan et al., 2018).

2.2.1. The method of aqueous extraction

Water extraction involves a time-honored technique where the dyestuff is typically broken down into fine pieces or powdered form. It is then submerged in water to break down the cellular structure, thereby enhancing the process's overall effectiveness. The dye solution is prepared by bringing the mixture to a vigorous boil and then carefully filtering it to achieve a clear and refined result (Pizzicato *et al.,* 2023, Merdan *et al.,* 2017). Water extraction is a dependable and efficient method, ensuring the extracted material seamlessly integrates into textile applications. The drawbacks include the extended extraction time, the substantial amount of water needed, and the low dyeing efficiency, as only the water-soluble components of the dye are extracted. During the extraction process, not only is the dye obtained, but sugars and other water-soluble compounds are also drawn out. That said, the efficiency of producing thermosensitive dyes takes a significant hit when exposed to elevated temperatures, making temperature control a critical factor in the process. (Saxena *et al.,* 2014, Pervaiz *et al.,* 2017). An easy and effective approach was used to create a natural dye by repurposing leftover calendula flower petals. The process began with soaking the petals in water for several hours to extract their essence. This was followed by boiling the solution to intensify the process. Once boiled, the mixture was left to cool naturally before being carefully filtered through paper to separate the liquid dye from any solid residues. This straightforward method highlights the potential of calendula petals as a valuable resource for sustainable color extraction. Various extraction conditions were analyzed, revealing that optimal results were achieved when the process was conducted at 40°C for a duration of 40 minutes. This setup delivered the highest extraction yield, underscoring its effectiveness in maximizing outcomes. (Pizzicato et al., 2023).

2.2.2. Solvent Extraction Method

The process of extraction using organic solvents like ethanol, methanol, or various solvent mixtures operates in much the same way as water-based extraction, but it typically achieves higher yields. Using lower temperatures helps minimize the risk of degradation. The water-alcohol extraction method effectively captures both water-soluble and water-insoluble compounds. Furthermore, the solvents can be effortlessly recovered through distillation, allowing them to be reused. One drawback is the presence of leftover toxic solvents in the process. Additionally, the extracted material tends to be poorly water-soluble, and there is often the unintended co-extraction of other compounds like chlorophylls and waxy substances. (Saxena *et al.,* 2014, Pizzicato *et al.,* 2023). A comparative analysis was conducted to assess the effectiveness of nine solvents—n-hexane, ethanol, acetonitrile, chloroform, ethyl ether, ethyl acetate, petroleum ether, n-butyl alcohol, and methanol—in extracting natural dyes from sources including cordyline, pandan, and dragon fruit (Cordyline fruticosusifolia and Hylocereus polyhizus, respectively). The study aimed to determine the optimal conditions for dye extraction. The findings indicated that methanol, ethanol, and water emerged as the most effective solvents for extracting dyes from the analyzed plants (Pizzicato *et al.,* 2023, Al-Alwani *et al.,* 2014).

2.2.3. Alkali or Acid extraction method

The majority of natural colors are glycosides, which can be extracted effectively in both acidic and alkaline environments. Tesu natural dye is obtained from tesu flowers through a process of acidic hydrolysis. Alkaline solutions are ideal for dyes with phenolic groups in their molecular structure (Ragab *et al.,* 2022, Helmy *et al.,* 2020).

2.2.4. Supercritical Fluid Extraction Method

Supercritical Fluid Extraction (SFE) is an advanced technique for isolating specific compounds from plants while operating at temperatures close to ambient levels, effectively minimizing the risk of thermal degradation. SFE stands out as a highly effective analytical technique, rivaling the reliability and precision of conventional chemical analysis methods. It is an excellent choice for both qualitative and quantitative analysis of the components found in natural products (Ragab *et al.*, 2022, Heydari *et al.*, 2014).

2.2.5. Enzyme-Assisted Extraction Method

The enzymatic extraction method offers a powerful approach to boost the efficiency of extracting key compounds from plants. By breaking down the structural barriers of plant cell walls and membranes, this technique enables greater access to and release of the plant's active components, making it a highly effective solution for optimized extractions (Ragab *et al.*, 2022, Helmy *et al.*, 2020). This approach is highly effective for extracting dyes from tough plant materials like bark, roots, and similar

substances.This is an effective approach to isolate d yes from the material under consideration, as it sig nificantly reduces the extraction time compared to the traditional system and is environmentally frien dly (Ragab *et al.*, 2022, Helmy *et al.*, 2020). This approach not only helps minimize solvent usage but also significantly cuts down on energy consumption.Consequently, enzyme-

assisted extraction has been proposed as another w ay to extract the natural product (Ragab *et al.*, 2022).

2.3. Structure, Types, Functional Role and location in plants, and Biosynthesis of Chlorophyll

2.3.1. Structure of Chlorophyll

Chlorophyll molecules are built upon a porphyrin framework, composed of four interconnected pyrrole rings surrounding a central magnesium ion. Additionally, they feature an extended hydrophobic alkyl chain as part of their structure (Ferreira *et al.*, 2021, Ebrahimi *et al.*, 2023). Chlorophylls are a type of porphyrin ring structure characterized by a reduced double bond, known as chlorin, (as shown in Figure 1). Magnesium lies at the core of the chlorine structure, bonded to the tetrapyrrole ring. Chlorophyll are uniquely structured with a hydrophilic head known as the porphyrin group and a lipophilic hydrocarbon tail referred to as the phytol group. These distinct components play a crucial role in their function, creating a perfect balance between their affinity for water and lipids. This dual nature enables chlorophyll to interact efficiently within the cellular environment, making it essential for processes like photosynthesis. Due to the presence of their lipophilic hydrocarbon chains, like phytol tails, these compounds are typically regarded as insoluble in polar solvents. Their structure inherently favors interactions with nonpolar environments, making them less compatible with the polarity of solvents such as water. (Ebrahimi *et al.*, 2023, Sharma *et al.*, 2021). Chlorophylls, the vibrant green pigments that define much of the natural world, are oil-soluble and amphiphilic in nature. These compounds are found abundantly across plants, algae, and cyanobacteria, playing a crucial role in the process of photosynthesis and giving life its characteristic shades of green. Chlorophyll in plants exists in two distinct structural forms (Ebrahimi *et al.*, 2023).

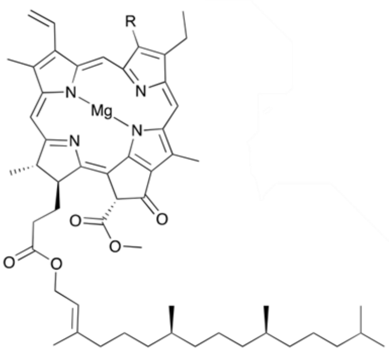

Figure 1. Chemical structure of chlorophyll

2.3.2. Types of Chlorophyll

Chlorophyll exists in various forms, such as chlorophyll a, b, c, d, and e. (as shown in Figure 2) (Martins *et al.,* 2023, Bjorn *et al.,* 2009). Chlorophyll a is the predominant type of chlorophyll present in plants. The chemical structure features a porphyrin ring housing a central magnesium ion, accompanied by a hydrocarbon tail referred to as phytol. The porphyrin ring is made up of four nitrogen-containing structures known as pyrroles, while the phytol tail is composed of repeating isoprenoid units (Durrett *et al.,* 2021). Chlorophyll a captures light most effectively within the red and blue segment of the spectrum, showing peak absorption around 430 nanometers for blue light and 662 nanometers for red light (Martins *et al.,* 2023). Chlorophyll b represents a distinct variant of chlorophyll present in plants, algae, and certain bacterial species. Its chemical composition closely resembles that of chlorophyll a, though it features a modified porphyrin ring structure. The modification enables chlorophyll b to capture light within the blue-green segment of the spectrum, reaching its absorption maximum near 453 nanometers. Chlorophyll b contributes to photosynthesis as well, serving primarily to shield chlorophyll a from damage caused by excessive light exposure. Beyond chlorophyll a and b, several other types of chlorophyll exist, including chlorophyll c, d, and e. They are present in various organisms, including algae, and exhibit distinct absorption spectra while serving diverse functions. Chlorophyll c primarily captures light in the blue-green segment of the spectrum, while chlorophyll d focuses on absorbing light from the red region. Similarly, chlorophyll a also specializes in absorbing light within the red wavelength range (Martins *et al.,* 2023, Durrett *et al.,* 2021).

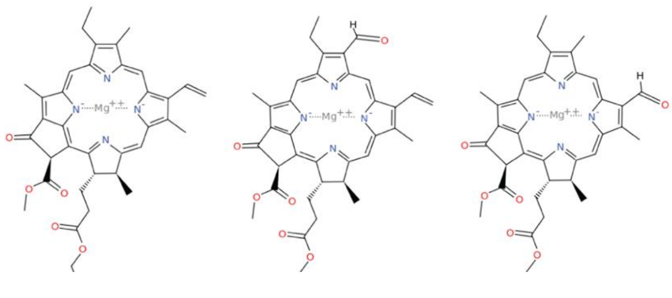

Figure 2. Types of Chemical structure of chlorophyll

Chlorophyll a ($C_{55}H_{72}MgO_5N_4$) features a methyl group ($-CH_3$) attached to the carbon-7, while chlorophyll b ($C_{55}H_{70}MgN_4O_6$) is distinguished by having an aldehyde group (-CHO) at the same carbon (Ebrahimi *et al.,* 2023, Carrillo *et al.,* 2022). The chemical distinction between chlorophylls a and b lies in their structural composition, (as shown in Figure 3). The unique structural characteristics of chlorophyll molecules account for the variation in their coloration, wherein chlorophyll a exhibits a blue-green hue, while chlorophyll b is associated with a blue-yellow tint. In plants, chlorophyll a and b are typically present in a ratio of 3 to 1(Ebrahimi *et al.,* 2023, Carrillo *et al.,* 2022). Research indicates that plants cultivated under shaded conditions exhibit a higher proportion of chlorophyll b relative to chlorophyll a. This variation has been noted in the sunbeam exposure of numerous leaf species and has also been evident when the same species were cultivated under varying levels of light intensity (Ebrahimi *et al.,* 2023, Nguyen *et al.,* 2021).

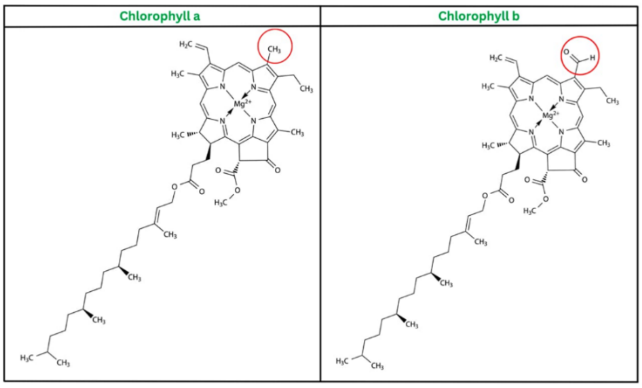

Figure3. Chemical structure of chlorophyll a and b

2.3.3. The functional roles and locations of different components in plants

Secondary metabolites in plants include color pigments, which play a crucial role in various biological processes Play a pivotal role in plant photosynthesis, contributing to essential processes such as capturing sunlight, managing metabolic activities, reducing photooxidative damage, and facilitating a range of other critical functions (Ebrahimi *et al.,* 2023, Das *et al.,* 2022). Chlorophyll gets its name from the Greek words "chloros," which means "green," and "phylon," which translates to "leaf" (Ebrahimi *et al.,* 2023). Chlorophylls are predominantly located within the chloroplasts, which serve as the principal organelles housing substantial concentrations of these pigments. These chloroplasts are widely distributed across nearly all green regions of a plant, particularly in its leaves and stems. Chloroplasts are predominantly located within the mesophyll layer, which constitutes the central region of plant leaves. These organelles are characterized by the presence of thylakoid membranes, within which resides chlorophyll, a green pigment integral to the process of photosynthesis (Ebrahimi *et al.,* 2023). Figure 4 illustrates a diagram representing chlorophyll within plant cells. Adverse environmental factors, including high temperatures and drought stress, can compromise the integrity of chloroplasts and lead to a decrease in the plant's chlorophyll levels. (Ebrahimi *et al.,* 2023, Das *et al.,* 2022).

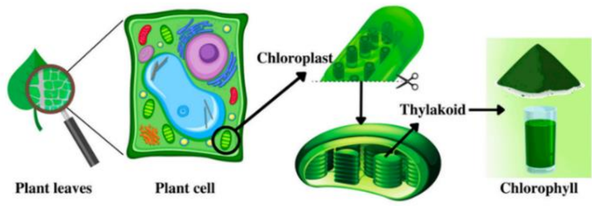

Figure 4. Diagram illustrating the spatial distribution of chlorophyll molecules within plant cells.

The chloroplast is often regarded as the "Food production facility" of the plant cell due to its pivotal role in synthesizing energy-rich compounds, particularly glucose, through the process of photosynthesis. This biochemical conversion is facilitated by the interplay of carbon dioxide, water, and solar energy, which together enable the plant to sustain its metabolic and growth functions. While numerous Light-absorbing pigment, including carotenoids and phycobilins, play a role in shielding against solar radiation, Plant pigment remain the most vital (Ebrahimi *et al.,* 2023). Chlorophyll molecules play a pivotal role in the photosynthetic process by capturing solar energy and transforming it into chemical energy, which is subsequently utilized in the synthesis of vital carbohydrate compounds, such as glucose. These carbohydrates serve as the primary nutritional resource for sustaining the metabolic activities of the plant (as shown in Figure 5). As shown in equation (1), In the process of photosynthesis, chlorophyll utilizes light energy to transform CO_2 and H_2O into carbohydrates.

$$CO_2 + H_2O \rightarrow (CH_2O) + O_2 \qquad (1)$$

CH_2O signifies sugars, carbohydrates, and all cellulose produced by plants (Ebrahimi *et al.,* 2023, Scheepers *et al.,* 2011).

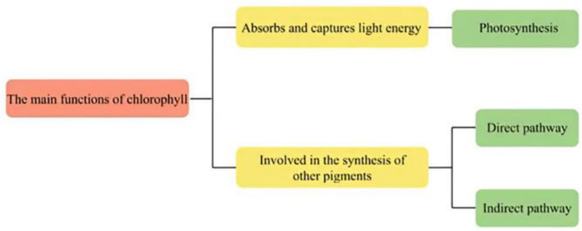

Figure 5. The main functions of chlorophyll.

2.4. Nutritional Properties and Health Benefits of Chlorophylls

Nutritional Benefits and Health Advantages of Chlorophyll:

Green vegetables are widely recognized for their impressive array of health-enhancing properties. These include powerful antioxidants, effective antimutagenic capabilities, and natural detoxifying agents, all of which contribute to overall well-being (Ebrahimi *et al.,* 2023). Consuming green vegetables regularly, including options like spinach and cruciferous varieties, can significantly lower the likelihood of developing chronic illnesses (Al Mijan *et al.,* 2021). Phytonutrients, particularly plant-derived pigments like chlorophyll, carotenoids, and betalains, have historically been explored primarily for their application as natural colorants within technological processes. They are currently under investigation for their remarkable nutritional benefits. Chlorophylls and their related compounds They have shown significant health benefits, including properties that counteract mutations, prevent cancer, and reduce inflammation. Research indicates that chlorophyll is packed with a wealth of nutrients, including vitamins E, A, C, and K, along with β-carotene. It also contains essential minerals like magnesium, potassium, iron, and calcium, as well as vital fatty acids crucial for overall health (Ebrahimi *et al.,* 2023). It has been reported that Including chlorophyll in early-life dietary choices can play a crucial role in minimizing weight gain, enhancing glucose tolerance, and lowering inflammation, potentially serving as a preventive measure against obesity (Li *et al.,* 2019). Furthermore, research examining the impact of chlorophyll on mice with type 1 diabetes has substantiated its efficacy in mitigating the risk associated with this condition (Wunderlich *et al.,* 2020). Plant pigment extracts from mint, broccoli, thyme, and pepper can help control blood sugar levels (Alsuhaibani *et al.,* 2017). Phytol, a derivative formed during the decomposition of chlorophyll, has been observed to alleviate inflammation and joint discomfort by suppressing the activity of Mediators of inflammation (Carvalho *et al.,* 2020).In addition, the chlorophyll pigment in dark green varieties may have a protective effect against certain types of cancer, such as colon and liver cancer. The intricate mechanism of chlorophyll involves its ability to tightly bind with hydrocarbons, aflatoxins, and various other hydrophobic compounds that are often associated with cancer development. Instead of actively eliminating these harmful compounds from the body, chlorophyll works by forming strong components interactions, effectively capturing and sequestering them within its structure.

2.5. Applications of chlorophyll

2.5.1. In medicine

The molecular composition of chlorophyll serves as a critical factor in shaping its bioactive properties, which directly impact its prospective health-promoting effects (Queiroz Zepka *et al.,* 2019). Exploring the connection between molecular composition and biological activity. plays a pivotal role in uncovering the therapeutic potential of chlorophylls and their derivatives. By delving into this intricate relationship, researchers can unlock new insights into how these natural compounds contribute to human health, paving the way for innovative treatments and applications. (Fasakin *et al.*, 2011). Chlorophyll's chemical composition features a porphyrin ring as its central framework, complemented by an extended Nonpolar side group.

This unique structure gives chlorophylls special ph ysicochemical and biological properties. Advancin g research in this field offers significant opportunities to unlock the full capabilities of chlorophylls as bioactive compounds, paving the way for innovative therapeutic strategies. (Figure 7) (Martins *et al.*, 2023).

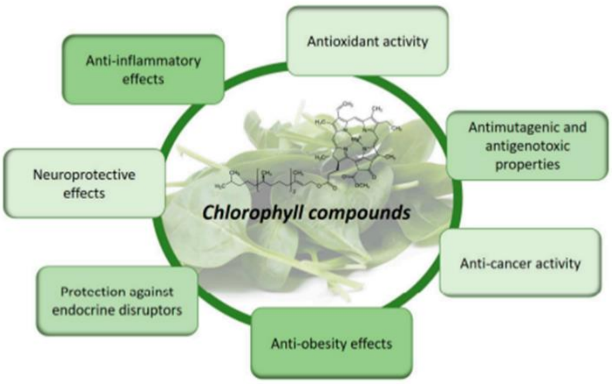

Figure 7. Therapeutic Potential and Bioactive Characteristics of Chlorophyll Derivatives.

2.5.1.1. Antioxidant activity

Free radical damage

is a significant factor in the progression of numerous illnesses. Natural chlorophylls, known for their antioxidative capabilities, hold great potential in avoiding or reducing the generation of reactive species (Fasakin *et al.*, 2011). In vitro studies have shown that the standard deriv ative of chlorophyll a exhibits superior antioxidant capacity compared to chlorophyll b derivatives. N otwithstanding the possible antioxidant properties of natural plants, studies in this area have been relatively scarce in recent times. This could be linked to the view that chlorophyll is less significant compared to other phytoconstituents present in fruits and vegetables, along with the assumption that the human digestive tract has limited capacity to absorb chlorophyll effectively. Nonetheless, it is important to recognize that the exploration of the antioxidative properties of natural chlorophylls is a burgeoning field, and further research is necessary to thoroughly clarify their capabilities and assess their significance in relation to other phytoconstituents (Martins *et al.*, 2023).

2.5.1.2. Cancer chemoprevention

Cancer, which ranks as the second leading cause of death globally, necessitates the creation of new therapeutic agents that have minimal side effects. Research has shown that chlorophyll may possess anticancer properties effective against various forms of cancer (Martins *et al.*, 2023). The protective benefits of chlorophylls are most pronounced when they are consumed alongside carcinogens, suggesting their ability to form complexes that shield the body. This underscores the vital role of including green leafy vegetables and fruits in your diet, as they act as natural allies against harmful nutritional carcinogens and DNA-mutating compounds. Moreover, diets high in fiber have been linked to improved outcomes in oncology, underscoring the necessity for targeted policies that encourage the consumption of healthier foods instead of processed options, ultimately enhancing public health (McRae *et al.*, 2018).

2.5.2. In industry

Chlorophylls are used in soups, dairy products, and fruit juices. They are widely used as natural food colorings. It is estimated that chlorophyll is produced at a rate of about 1.2 billion tons per year on this planet. The use of dyes is prevalent across multiple sectors, such as medications, beauty products, and food, where they serve as antioxidants and coloring agents. Additionally, it has the potential to function as a biomordant, improving dyeing methods in the textile industry and facilitating the creation of dyes with antimicrobial characteristics. (Figure 8 illustrates the application of chlorophyll derived from alfalfa leaf extract in gelatin and candy products). The foliage of Pandanus amaryllifolius serves as a source of natural pigments, attributed to its elevated chlorophyll levels. artificial dyes should be substituted with green pigment due to the adverse side consequences associated with their use.It is also used as a dye for candles, leather, wax, resin, fabric dye, and other cosmetic preparations such as petroleum jelly (Ghulam Nabi *et al.,* 2023).

Figure 8. Incorporation of chlorophyll into food products

3.Methods of Research and the tools used

3.1. Materials:

- Green plant leaves (e.g., spinach)
- Pure Acetone (as the primary extraction solvent).
- Glass beaker or flask.
- Water bath with a temperature regulator.
- Glass stirring rod or magnetic stirrer.
- Filter funnel and filter paper.

3.2. Steps for Chlorophyll Extraction from Leaves:

3.2.1. Leaf Preparation:

- Washing the leaves: Wash thoroughly with distilled water to remove dust and contaminants.
- Drying the leaves: Allow the leaves to dry completely before use.
- Cutting the leaves: Cut the leaves into medium-sized pieces (2-3 cm) while avoiding crushing or grinding to preserve chemical integrity.

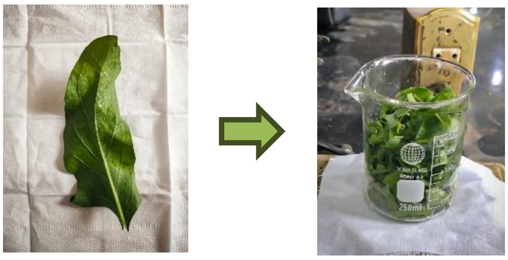

Figure9. Leaf Preparation

3.2.2. Placing the Leaves in the Solvent:

- Place the cut leaves in a clean glass beaker.
- Add the solvent until the leaves are fully submerged to ensure effective chlorophyll extraction.
- Maintain a solvent-to-leaf ratio of 10:1 (i.e., 100 mL of solvent per 10 grams of leaves).
- Ensure the leaves are completely immersed in the solvent for optimal extraction efficiency.

3.2.3. **Extraction in a Water Bath**:

– Set the water bath temperature between 50 – 60°C, preferably 55°C.

– Ensure the temperature remains stable throughout the extraction process.

– The extraction process should last 30 – 60 minutes, depending on the type of leaves:

– If the leaves are fresh and rich in chlorophyll, 30 minutes should be sufficient.

– If the leaves are dry or have lower chlorophyll content, the duration can be extended up to 60 minutes.

– Stir the mixture every 10 minutes to ensure even distribution of the solvent around the leaves.

– The solvent will gradually turn dark green, indicating chlorophyll extraction from the leaves.

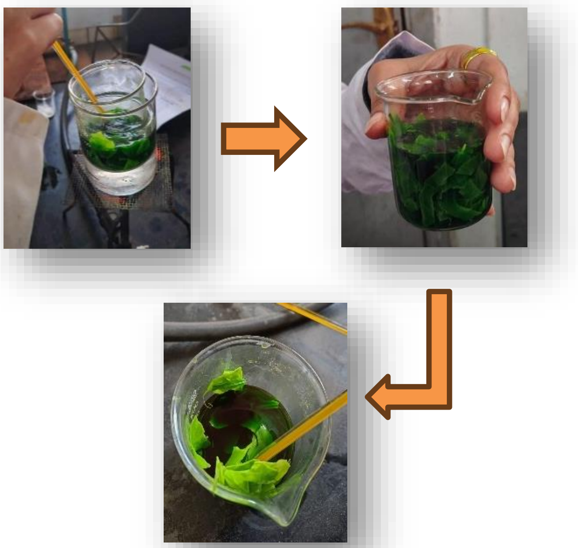

Figure10. Placing the Leaves in the Solvent and Extraction in a Water Bath

3.2.4. **Filtration and Separation of the Extract**:

– After the extraction process is complete, the beaker is removed from the water bath.

– It is then cooled quickly to ensure the stability of the extracted components.

– The extract is filtered to separate any solid impurities, such as:

– Plant fibers.

– Small leaf residues.

– The filtration process must be carried out carefully to ensure a completely pure extract.

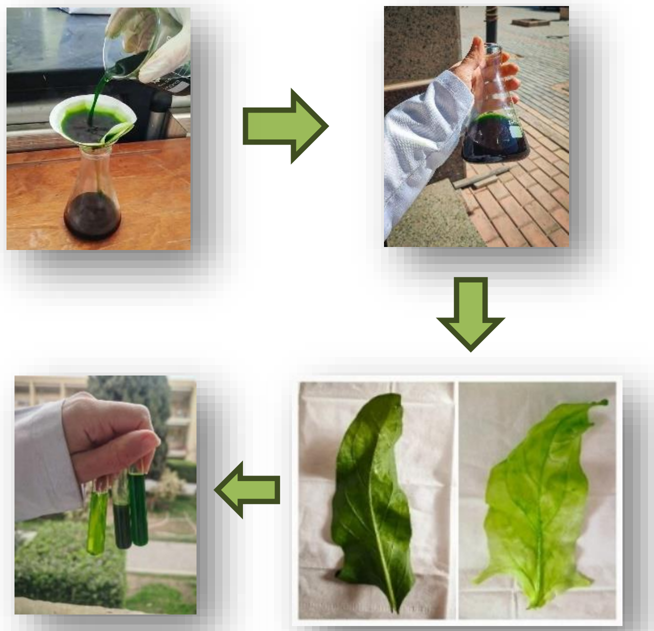

Figure11. Filtration and Separation of the Extract

4. Results of Research

4.1. Steps for Dyeing Fabric with Chlorophyll Extract

4.1.1. Soaking the Fabric in Ferric Chloride

– solution of ferric chloride ($FeCl_3$) its concentration 0.5% to 1%.

– Soak the fabric in this solution for 5 minutes. Ferric chloride helps fix the dye to the fibers.

– After soaking, remove the fabric from the solution and rinse it with cold water.

Figure12. Soaking the Fabric in Ferric Chloride

4.1.2. Soaking in Chlorophyll Extract

- After preparing the fabric with ferric chloride, Soak it in chlorophyll extract as previously explained.
- Soak the fabric in the solution for 30–60 minutes, depending on the desired color intensity.
- At this stage, ferric chloride will enhance chlorophyll absorption into the fibers and improve color fastness.

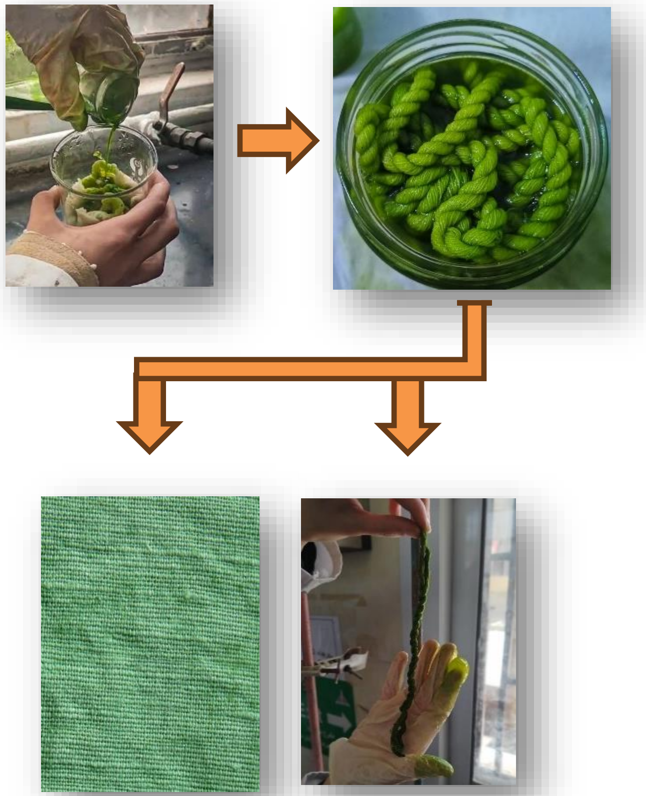

Figure13. Soaking in Chlorophyll Extract

5. Conclusion

This study highlights the potential of chlorophyll as a sustainable and eco-friendly fabric dye, demonstrating its effectiveness as a natural alternative to synthetic dyes. It reveals that natural dyes like chlorophyll offer numerous benefits, including biodegradability, non-toxicity, and health advantages, making them the ideal solution for addressing environmental challenges. The various extraction methods explored provide valuable insights into optimizing processes for greater yield and efficiency. With rising environmental awareness and consumer demand for green products, chlorophyll-based dyes present a promising alternative for industries striving for sustainability. Further exploration of its applications across different sectors could unlock innovative, eco-friendly advancements, paving the way for a greener future in various industries. Future exploration should focus on refining extraction techniques and expanding chlorophyll's applications, particularly in areas such as food and medicine, to maximize its potential impact. Future exploration should focus on refining extraction techniques and expanding chlorophyll's applications, particularly in areas such as food and medicine, to maximize its potential impact.

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