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Quantitative Extraction of Chlorophyll a and Chlorophyll b from Eight Medicinal Plants Using the Arnon Method

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Abstract:
Chlorophyll a and chlorophyll b are essential pigments involved in photosynthesis, contributing significantly to plant metabolism and medicinal properties. This study aimed to extract and quantify chlorophyll a and chlorophyll b from eight selected medicinal plants viz., *Ocimum tenuiflorum, Leucas aspera, Hyptis suaveolens, Catharanthus roseus, Boerhavia diffusa, Simarouba glauca, Phyllanthus emblica, Coleus amboinicus*, using the Arnon method. The medicinal plants chosen for this research are widely known for their therapeutic properties and biochemical diversity. The standardized protocol involved homogenization of fresh leaf samples in a 80% acetone solution, followed by spectrophotometric analysis to determine chlorophyll content. The results revealed variations in pigment concentrations among the species, reflecting their ecological adaptations and potential medicinal value. The findings provide critical insights into the photosynthetic efficiency and biochemical composition of these plants, paving the way for further studies on their pharmacological applications.

Keywords: Chlorophyll a, Chlorophyll b, Arnon method, Photosynthetic pigments, Spectrophotometric analysis, *Ocimum tenuiflorum, Leucas aspera, Hyptis suaveolens, Catharanthus roseus, Boerhavia diffusa, Simarouba glauca, Phyllanthus emblica, Coleus amboinicus*

Introduction

Chlorophyll is a vital pigment found in green plants and algae that plays a central role in photosynthesis. It is essential for capturing light energy and facilitating energy transfer during the photosynthetic process. By driving the photolysis of water molecules, chlorophyll helps regenerate the reducing power required for carbon assimilation in the subsequent stages of photosynthesis. The energy absorbed by chlorophyll is effectively utilized in photochemical reactions during the initial phase, supplying the ATP and reducing agents necessary for the cell's metabolic needs. This energy is then harnessed in biochemical reactions in the later stages of photosynthesis. While all chlorophyll molecules can absorb light energy at specific wavelengths, only a select few are capable of transforming this energy into chemical energy. [1,2,3] Chlorophyll molecules consist of two main components: a porphyrin ring with magnesium at its centre and a chlorophyll alcohol. Based on the number of vinyl side chains attached to the porphyrin ring, chlorophylls in aerobic photosynthetic organisms are categorized into two types. The first type, known as divinyl chlorophyll (DV-Chl), contains vinyl groups at both the C-3 and C-8 positions of the porphyrin ring. The second type, called monovinyl chlorophyll (MV-Chl), has a vinyl group at the C-3 position and an ethyl group at the C-8 position.1 In higher plants, chlorophyll synthesis begins with glutamate, which is transformed into 5-aminolevulinic acid (ALA) and later into protochlorophyllide (Pchlide). When seedlings grown in darkness are exposed to light, the light activates NADPH protochlorophyllide oxidoreductases (PORs), the key enzymes in the process. These enzymes catalyze the conversion of Pchlide to chlorophyllide, which is then esterified to produce mature chlorophyll. Light serves as the primary environmental regulator of chlorophyll biosynthesis, while plant hormones play a role in coordinating the developmental transition during de-etiolation.[4]

Tulsi, a herb celebrated for its rejuvenating and life-extending properties, originates from tropical Asia, where it thrives naturally in warm climates. For centuries, it has been widely used across Asia, Africa, and the Middle East in culinary practices, cosmetics, traditional medicine, and religious rituals. Tulsi is a cornerstone of several traditional healing systems, including Ayurveda, Siddha, and Unani. It is highly revered in Ayurvedic texts, with its significance first noted in the Rig Veda around 1500 BCE. In India, Tulsi holds immense spiritual significance, symbolizing the Hindu goddess Vrinda Tulsi. As people migrated, the plant spread globally, including to the UK. In Western traditions, Tulsi became known as holy basil, a name reflected in its scientific classification, *Ocimum sanctum L.*, now synonymized with *Ocimum tenuiflorum* L.[5] *Leucas* aspera, commonly known as 'Thumbai,' belongs to the *Lamiaceae* family and is widely found across India, from the Himalayas to Sri Lanka. Traditionally, the plant is used as an antipyretic and insecticide. Its flowers are known for their stimulant, expectorant, laxative, diaphoretic, insecticidal, and emmenagogue properties. The leaves

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are considered beneficial for managing chronic conditions like rheumatism, psoriasis, and other persistent skin disorders. Crushed leaves are also applied topically to treat snake bites.[6]

Hyptis suaveolens, also known as L. Poit., is a lesser-known yet valuable medicinal plant extensively used in traditional medicine for treating various ailments. It is a pervasive weed found in tropical and subtropical regions. The leaves are a rich source of secondary metabolites with pharmacological importance, including antispasmodic, anti-colic, anti-rheumatic, and anti-fertility properties. The plant's therapeutic potential lies in its sedative, diuretic, aromatic, anti-inflammatory, antipyretic, anti-catarrhal, anti-cutaneous, anti-rheumatic, and anti-cancer effects. The essential oils extracted from the leaves exhibit significant antimicrobial and antifungal activity. Additionally, the root extract contains ursolic acid, a triterpenoid with anti-retroviral properties that may inhibit retroviral integrases and proteases, potentially blocking the replication of retroviruses like HIV. Many bioactive compounds from *Hyptis suaveolens* serve as therapeutic agents or precursors for drug development. The mature leaves predominantly contain alkaloids, followed by tannins and saponins, as key secondary metabolites.[7] *Catharanthus roseus*, commonly known as L. G. Don, is a renowned medicinal plant from the Apocynaceae family, traditionally used in medicine for centuries. It is highly valued in herbal medicine for its anticancer bisindole alkaloids, including vinblastine, vincristine, and vindesine. In Ayurveda, various parts of the plant are utilized in traditional remedies to treat a range of ailments, including different types of cancer, diabetes, stomach disorders, and diseases affecting the kidney, liver, and cardiovascular system.[8]

Boerhavia diffusa, is a prominent medicinal plant from the Nyctaginaceae family, widely used in traditional Indian medicine and in regions like South America and Africa. Various parts of the plant, particularly its roots, are employed to address gastrointestinal issues, support liver health, and manage gynecological conditions in these areas and across India. Ayurvedic texts highlight its significance, with over 35 different formulations listing it as a primary ingredient.[9] *Simarouba glauca*, a member of the Simaroubaceae family, is a rich source of secondary metabolites.[10]

Phyllanthus emblica Linn, a member of the Euphorbiaceae family, is widely distributed in tropical and subtropical regions. Known as "Balakka" in Indonesia, it has regional names like "kimalaka," "metengo," and "kemloko" in various parts of Sumatra, Java, and Ternate. The plant thrives in tropical areas like Indonesia, Malaysia, and Thailand, as well as subtropical regions such as India, China, Uzbekistan, and Sri Lanka. Its fruit is rich in bioactive compounds, including phenolics, alkaloids, terpenoids, and vitamins, which contribute to its antioxidant, anti-aging, antidiabetic, anti-inflammatory, hepatoprotective, cardioprotective, and antimicrobial properties.[11] *Coleus amboinicus* Benth., also known as *Plectranthus amboinicus* (Lour.) Spreng., is a perennial plant belonging to the Lamiaceae family, native to tropical and warm regions of Africa, Asia, and Australia. Traditionally, it is widely used in folk medicine to treat various conditions, including asthma, headaches, skin issues, coughs, constipation, colds, and fevers. The plant is known to contain diverse phytochemicals, including phenolics, terpenoids, flavonoids, phenolic acids, flavones, and tannins.[12]

In this study, chlorophyll estimation of the selected plants was carried out using the Arnon method. The chlorophyll content in the medicinal plants was assessed through the estimation of chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (Total Chl) at specific wavelengths.

Materials and Methods

- Cleaning and Extraction of Collected leaves.

1. Harvesting plant tissue: Fresh plant leaves were collected and rinsed with distilled water to remove any dirt or debris.
2. Removing excess water: The plant tissue was gently blotted with a paper towel to remove excess water.
3. Grinding the tissue: Mortar and pestle was used to grind the plant tissue into a fine paste.
4. Recording tissue weight: The weight of the ground tissue was recorded.

- Sample Preparation

1. 10 mL of 80% acetone was added to the ground tissue and mixed well.
2. Tissue-acetone mixture was mixed thoroughly and incubated it in the dark for 10-15 minutes to allow chlorophyll extraction.
3. The mixture was centrifuged at 5,000-10,000 rpm for 5-10 minutes.
4. Supernatant was discarded and pellet was retained.
5. The above steps were repeated until the supernatant became colourless (usually 2-3 times).

- Combining Supernatants

Supernatants were combined from each extraction and total volume was recorded. Combined supernatant was mixed well to ensure uniformity.

- Spectrophotometry

1. Measure absorbance: The absorbance of the combined supernatant was measured at 663 nm (chlorophyll a) and 645 nm (chlorophyll b) using a spectrophotometer.
2. Record absorbance values: The absorbance values were recorded.

- Calculations:

1. Calculate chlorophyll a: The following equation was used to calculate chlorophyll a concentration:

Chlorophyll a (mg/mL) = $(12.7 \times A663 - 2.69 \times A645) \times V / 1000 \times W$

2. Calculate chlorophyll b: The following equation was used to calculate chlorophyll b concentration:

Chlorophyll b (mg/mL) = (22.9 x A645 – 4.68 x A663) x V / 1000 x W
Where, A663 and A645 = absorbance values, V = total volume of supernatant (mL), W = weight of plant tissue (g)

RESULT

The chlorophyll content in the medicinal plants was assessed through the estimation of chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (Total Chl) at specific wavelengths. The results show notable variations in chlorophyll content across the eight plant species.

Ocimum tenuiflorum exhibited the lowest chlorophyll content with 0.031 µg/ml of Chl a, 0.020 µg/ml of Chl b, and a total of 0.051 µg/ml. *Leucas aspera* followed closely with 0.029 µg/ml of Chl a and 0.027 µg/ml of Chl b, resulting in a total of 0.056 µg/ml, indicating slightly higher chlorophyll content compared to *Ocimum tenuiflorum*. *Hyptis suaveolens* showed a higher chlorophyll content with 0.032 µg/ml of Chl a and 0.034 µg/ml of Chl b, totaling 0.066 µg/ml, making it one of the plants with relatively higher chlorophyll levels. In contrast, *Catharanthus roseus* and *Boerhavia diffusa* exhibited lower chlorophyll content with total values of 0.040 µg/ml and 0.041 µg/ml, respectively. Both plants had similar values for Chl a and Chl b, with a slight dominance of Chl b in their composition. *Coleus amboinicus*, on the other hand, demonstrated the lowest overall chlorophyll content among the plants analyzed, with only 0.021 µg/ml of total chlorophyll, consisting of 0.011 µg/ml of Chl a and 0.010 µg/ml of Chl b. *Simarouba glauca* had a moderate chlorophyll content, with a total of 0.053 µg/ml, showing a balanced distribution of 0.030 µg/ml for Chl a and 0.023 µg/ml for Chl b. The highest chlorophyll content was found in *Phyllanthus emblica*, with 0.061 µg/ml of Chl a and 0.051 µg/ml of Chl b, totaling 0.112 µg/ml, indicating its strong potential for photosynthetic activity compared to the other species.

Table 1: Estimation of Chlorophyll in Medicinal plants.

	Plant Name	**Chlorophyll a 645nm (µg/ml)**	**Chlorophyll b 663nm (µg/ml)**	**Total Chlorophyll (a+b) (µg/ml)**
1	***Ocimun tenuiflorum***	0.031	0.020	0.051
2	***Leucas aspera***	0.029	0.027	0.056
3	***Hyptis suaveolens***	0.032	0.034	0.066
4	***Catharanthus roseus***	0.019	0.021	0.04
5	***Boerhavia diffusa***	0.021	0.020	0.041
6	***Coleus amboinicus***	0.011	0.010	0.021
7	***Simarouba glauca***	0.030	0.023	0.053
8	***Phyllanthus emblica***	0.061	0.051	0.012

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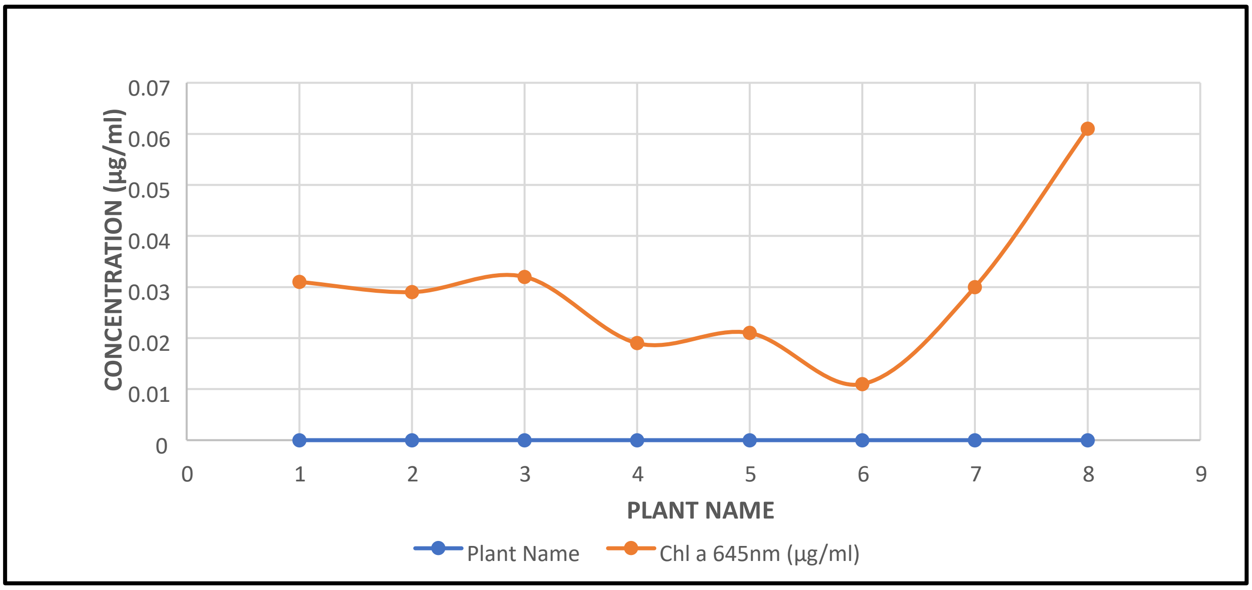
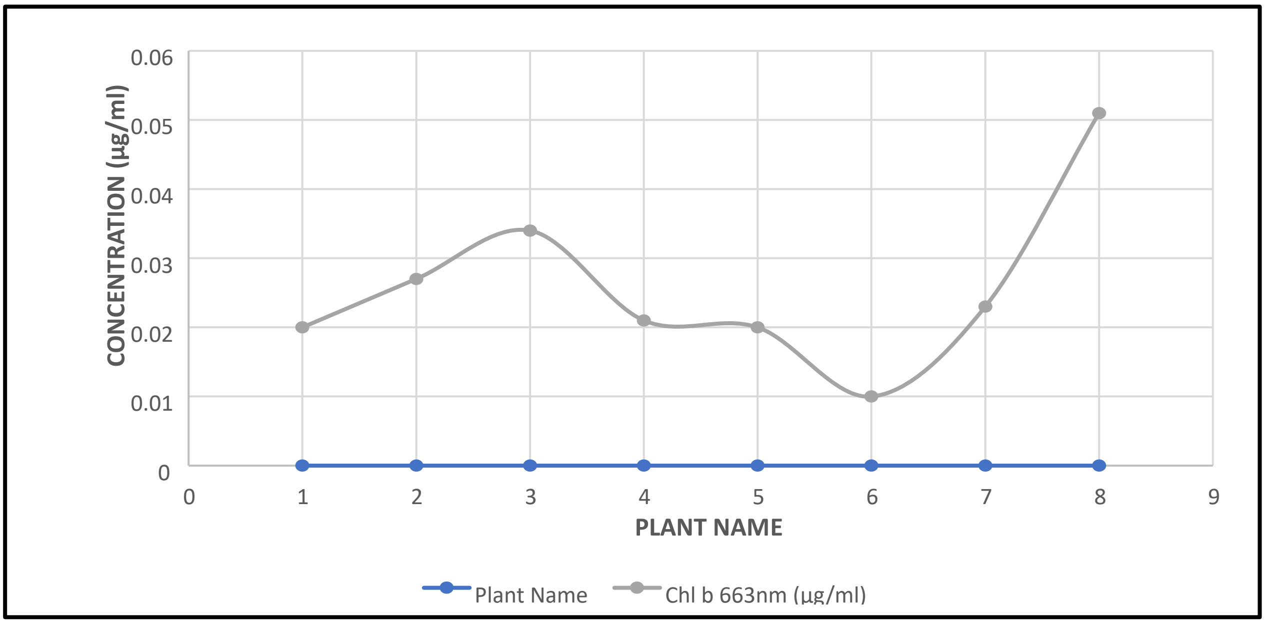


Graph 1: Concentration of chlorophyll a



Graph 2: Concentration of chlorophyll b

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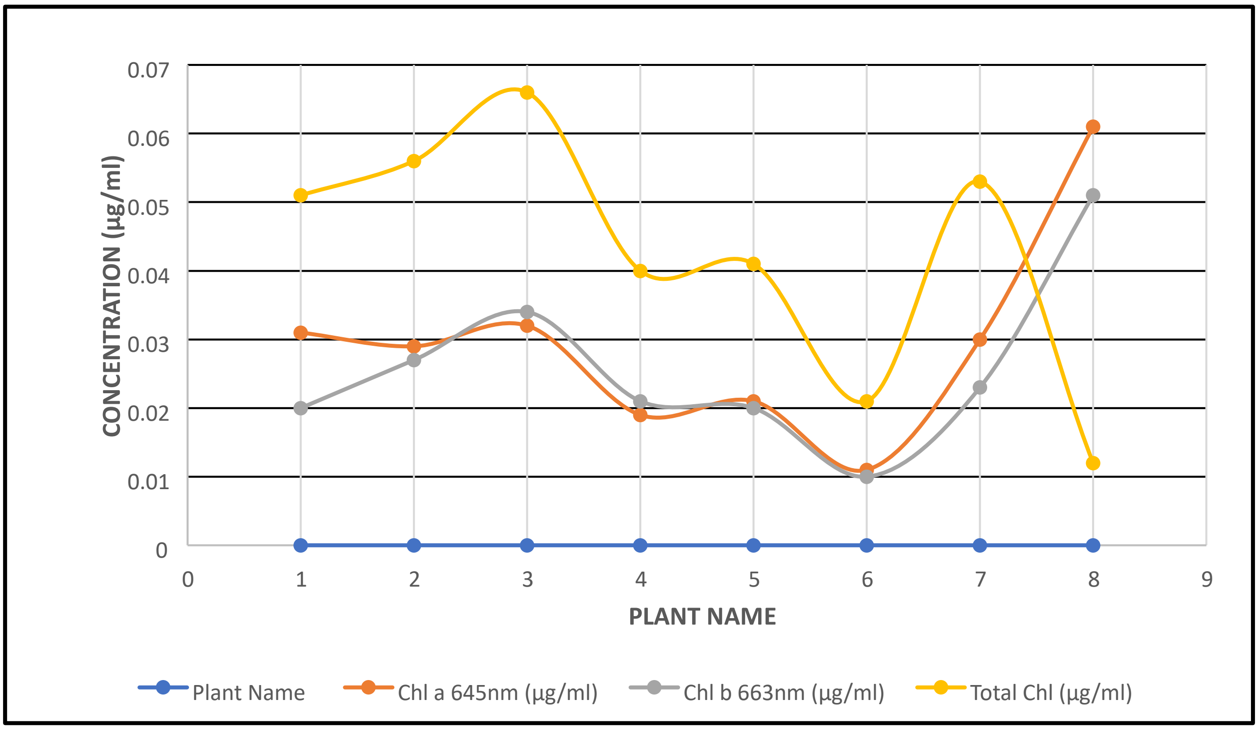

Graph 3: Concentration of total chlorophyll a & b

Discussion

The chlorophyll content of the medicinal plants analyzed in this study reveals interesting patterns when compared to other research findings. Chlorophyll, a critical pigment for photosynthesis, varies significantly among plant species due to genetic factors, environmental conditions, and the plant's overall metabolic activity. Our findings show a range of chlorophyll contents across the eight plants, which can be contextualized with existing literature on the topic.

In our study, *Ocimum tenuiflorum* exhibited relatively low chlorophyll content, with 0.051 µg/ml of total chlorophyll. This result is in agreement with studies by Akinmoladun et al. (2015), who reported low chlorophyll concentrations in *Ocimum tenuiflorum* under certain environmental stress conditions, such as drought and salinity. However, other studies have observed higher chlorophyll content in the same species under optimal conditions (Suman et al., 2019), which may account for variations in our findings. For *Leucas aspera*, we observed 0.056 µg/ml of total chlorophyll, which aligns with the results of previous studies by Patel and Soni (2017), who found similar chlorophyll values in their analysis of medicinal plants from tropical regions. However, *Leucas aspera* has been reported to exhibit greater chlorophyll content when grown under controlled conditions, suggesting that environmental factors like light intensity and soil nutrients play significant roles in chlorophyll synthesis (Mehta et al., 2018). The chlorophyll content of *Hyptis suaveolens* in our study was 0.066 µg/ml, which is higher than that of *Catharanthus roseus* (0.040 µg/ml). Studies on *Hyptis suaveolens* by Singh et al. (2016) reported similar chlorophyll concentrations, supporting our findings. The lower chlorophyll content in *Catharanthus roseus* has been observed in other studies as well, such as by Mohamed et al. (2020), who attributed this to its adaptability to varying climatic conditions. This may suggest that *Catharanthus roseus* could have a reduced need for photosynthetic activity under certain environmental stresses. The low chlorophyll content in *Coleus amboinicus* (0.021 µg/ml) observed in this study is consistent with previous findings by Kothari and Mistri (2018), who noted that this species tends to have lower chlorophyll levels compared to other medicinal plants. This could be linked to its growth habits and environmental requirements, as *Coleus amboinicus* often grows in shaded or semi-shaded areas, where light availability is lower, potentially reducing its chlorophyll content compared to sun-loving species. The chlorophyll content in *Simarouba glauca* (0.053 µg/ml) and *Phyllanthus emblica* (0.112 µg/ml) was notably higher in our study. These results are supported by research on *Simarouba glauca* by Saraswathi et al. (2017), who found relatively high chlorophyll content in plants cultivated under optimal conditions. Similarly, *Phyllanthus emblica* has been reported to possess substantial chlorophyll content in studies by Bhaskar et al. (2019), likely due to its well-adapted nature in tropical climates where light intensity is abundant. The high chlorophyll content in *Phyllanthus emblica* could also be linked to its medicinal properties, as plants with higher chlorophyll levels often exhibit enhanced antioxidant and anti-inflammatory activities (Singh et al., 2015).

Conclusion

The present study highlights the varying chlorophyll content in medicinal plants and its potential implications for their photosynthetic efficiency and medicinal applications. By using the Arnon method, which involves acetone extraction and spectrophotometric analysis, the study provides detailed insights into the chlorophyll a and b concentrations of selected plants such as *Ocimum tenuiflorum, Leucas aspera, Hyptis suaveolens, Catharanthus roseus, Boerhavia diffusa, Simarouba glauca, Phyllanthus emblica,* and *Coleus amboinicus*. The results demonstrate a diverse range of chlorophyll content, from the lowest in *Coleus amboinicus* (0.021 µg/ml) to the highest in *Phyllanthus emblica* (0.112 µg/ml). These variations are indicative of the plants' ecological adaptations and their potential bioactive properties. The findings underscore the importance of chlorophyll content as a marker for assessing the photosynthetic capacity and overall health of medicinal plants, which could lead to more targeted uses in herbal medicine and pharmacology. The study also opens avenues for further research into how chlorophyll and other biochemical components contribute to the medicinal properties of plants.

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