

Comparative study of the chlorophyll content of the leaves of some commercially available plants and their extracts

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Abstract

Chlorophyll, Nature's gift pigment, gives green color to plants. There is an elevating demand for natural color in the food and cosmetic industry. As chlorophyll is a green pigment and reported to have a series of health benefits, its importance as natural color is increasing in the market. Even though chlorophyll is available in all the plants; industry is looking for commercially viable source for making chlorophyll products. In this study, some commercially available plants are compared for their chlorophyll content using UV-Visible Spectrophotometry. The yield and chlorophyll content of the extracts made from these plants were also estimated. Chlorophyll content was found to be highest in Bamboo leaves. The yield and chlorophyll content of the extract were also found to be high in the extract made from Bamboo leaves.

Keywords: - Chlorophyll, UV-Visible Spectrophotometry.

1. Introduction

A pigment is a molecule which absorbs light at a particular wavelength and thus gives a specific color to that molecule. There are different types of pigments in nature and the green pigment found in the plants and algae is chlorophyll

There are different chlorophyll molecules existing in plants such as Chlorophyll a, Chlorophyll b, Chlorophyll c and Chlorophyll d and the most predominant molecules in higher plants and algae are Chlorophyll a and b [1]. Chlorophyll a and b are present in the ratio 3:1 in green plants [2].

Structure of Chlorophyll molecule consists of a porphyrin ring with a central magnesium atom. A long phytol chain is attached to the porphyrin ring which makes it hydrophobic. Chlorophyll a and b differ very slightly in their structure particularly at one of the side chains. In Chlorophyll a, the side chain has a methyl group (-CH₃) whereas Chlorophyll b has an aldehyde (-CHO) [4].

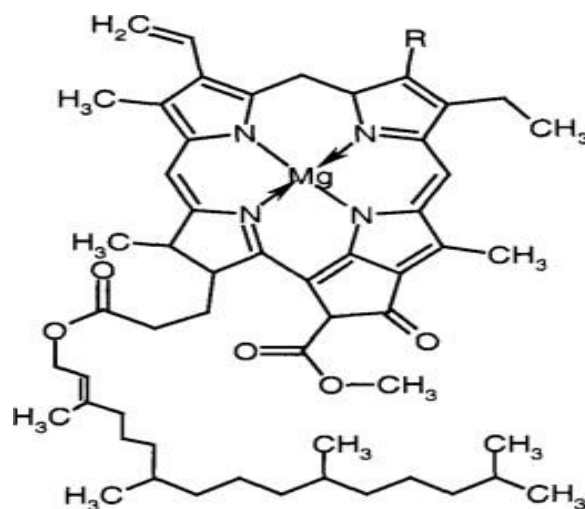


Figure-1 Structure of Chlorophyll

If R = -CH₃, it is Chlorophyll a and R = -CHO it is Chlorophyll b

Chlorophyll absorbs light in the red and blue regions of the visible spectra and reflects the green region and thus makes the plant green. Chlorophyll a is bluish green in color and Chlorophyll b is greenish yellow in color.

The natural colors derived from plants are gaining high importance in food color industry [5]. Chlorophyll is used as natural green pigment in processed foods. As it is a strong green pigment and the consumer's growing preferences for natural foods, Chlorophyll is gaining importance as food additive. Also, several studies reported the health benefits by the consumption of Chlorophyll. It is reported to have antioxidant and antimutagenic activities upon consumption [6,7]. In recent studies Chlorophyll has been used in photodynamic therapy as a sensitizer [8]. All these factors encourage the natural ingredient developers to switch from artificial green pigments to Chlorophyll-based natural coloring solutions.

Even though Chlorophyll is available in almost all the green plants, for making this green color pigment as a food additive, the raw material plant used for processing should be commercially available. The main objective of this study is to compare some of the commercially available green plants for its Chlorophyll content in their leaves and their extracts.

2. Materials and Methods

2.1 Collection of Samples

Samples of buffalo grass (*Bouteloua dactyloides*), banana leaves (*Musa paradisiaca*), mulberry leaves (*Morus alba*) and bamboo leaves (*Bambusa vulgaris*) were collected from different parts of Kerala. CO3 – Napier grass (*Pennisetum purpureum* Schum) samples were collected from Tamil Nādu and Spinach was collected from Karnataka (*Spinacia oleracea*).

2.2 Estimation of Chlorophyll Content

Chlorophyll content of the leaves and the extract was estimated using UV-Visible Spectroscopic method developed by Inskeep & Bloom [9] with suitable sample preparation methods. Moisture content of the fresh leaves were estimated using Toluene by AOAC method [10,11,12].

2.3 Estimation of Chlorophyll Content in Fresh Leaves

A uniform sample of fresh plant parts was taken and grinded using a mixer grinder. About 10g sample was accurately weighed into a conical flask. 50ml of 80% acetone was added into the sample and sonicated for 15 minutes in an ultrasound sonicator. The solution was decanted through a Whatman filter paper 1 into a 250ml standard flask. The procedure was repeated for 3-4 times until the solution became colorless and then it was made up to 250ml using 80% acetone. 5ml of this solution was pipetted out into a 100ml standard flask and made up to the mark using 80% acetone. Absorbance of the solution was measured at 664.5nm and 647nm using 80% acetone as blank.

Total chlorophyll content was calculated using the equation

$$\text{Chlorophyll a (mg/ml)} = \frac{[(A_{664.5} \times 12.63) - (A_{647} \times 2.52)] \times \text{Dilution}}{\text{Weight of the sample} \times 1000}$$

$$\text{Chlorophyll b (mg/ml)} = \frac{[(A_{647} \times 20.47) - (A_{664.5} \times 4.73)] \times \text{Dilution}}{\text{Weight of the sample} \times 1000}$$

$$\text{Total chlorophyll content (mg/ml)} = \frac{[(A_{647} \times 17.95) + (A_{664.5} \times 7.90)] \times \text{Dilution}}{\text{Weight of the sample} \times 1000}$$

A_{664.5} = Absorbance at 664.5 nm, A₆₄₇ = Absorbance at 647 nm

2.4 Preparation of Extract and Estimation of Yield and Chlorophyll Content

100g of grinded sample of fresh leaves was taken in a separating funnel and filled with methanol to soaking level for 1 hour. The solvent was drained after 1 hour. Three more washes were given similarly. The extracts were collected, filtered, and evaporated using rotavapor to 20% of the initial volume. Hexane was added to the solution, mixed well and allowed to separate. Hexane layer in which chlorophyll was extracted was collected in a previously weighed round bottom flask and evaporated to dryness at 65°C under vacuum. Weight of the extract after drying was noted and calculated as the yield of the extract. Chlorophyll content of the extract was measured by diluting in 80% acetone using the same method as mentioned above.

3. Results

Table 1: Estimation of chlorophyll content and moisture content in the plant parts

Plant names	Chlorophyll a (mg/ml)	Chlorophyll b (mg/ml)	Total chlorophyll (mg/ml)	Moisture content (%)	Chlorophyll a (Dry basis) (mg/ml)	Chlorophyll b (Dry basis) (mg/ml)	Total Chlorophyll l (Dry basis) (mg/ml)
Spinach leaves	0.49	0.15	0.64	94.10	8.26	2.55	10.81
Buffalo Grass	1.45	0.47	1.92	85.20	9.82	3.16	12.97
Banana leaves	1.35	0.43	1.78	84.30	8.57	2.77	11.34
C03 grass	1.35	0.43	1.78	86.10	9.69	3.12	12.81
Mulberry leaves	1.03	0.33	1.36	89.10	9.44	3.04	12.48
Bamboo leaves	3.03	0.98	4.01	80.30	15.38	4.97	20.36

Graph-1 Comparison of chlorophyll a, chlorophyll b and total chlorophyll in plants

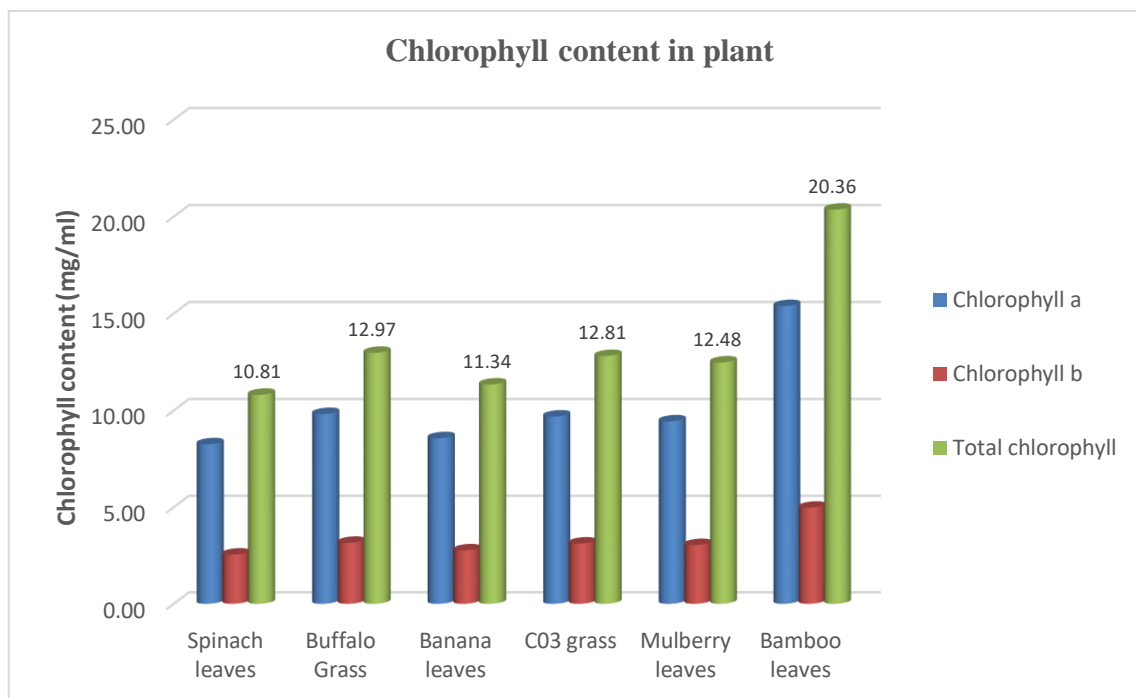
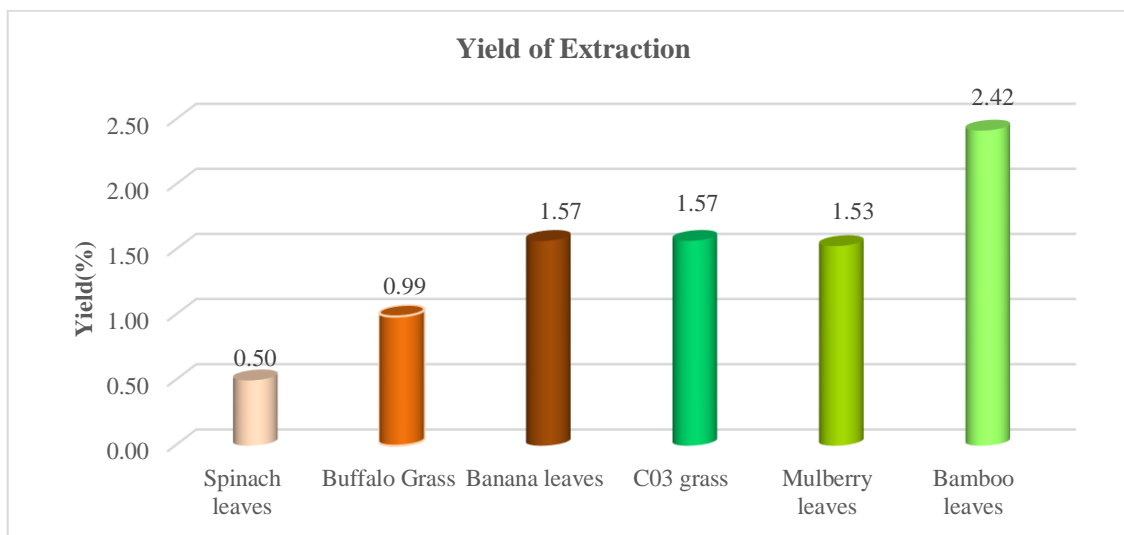


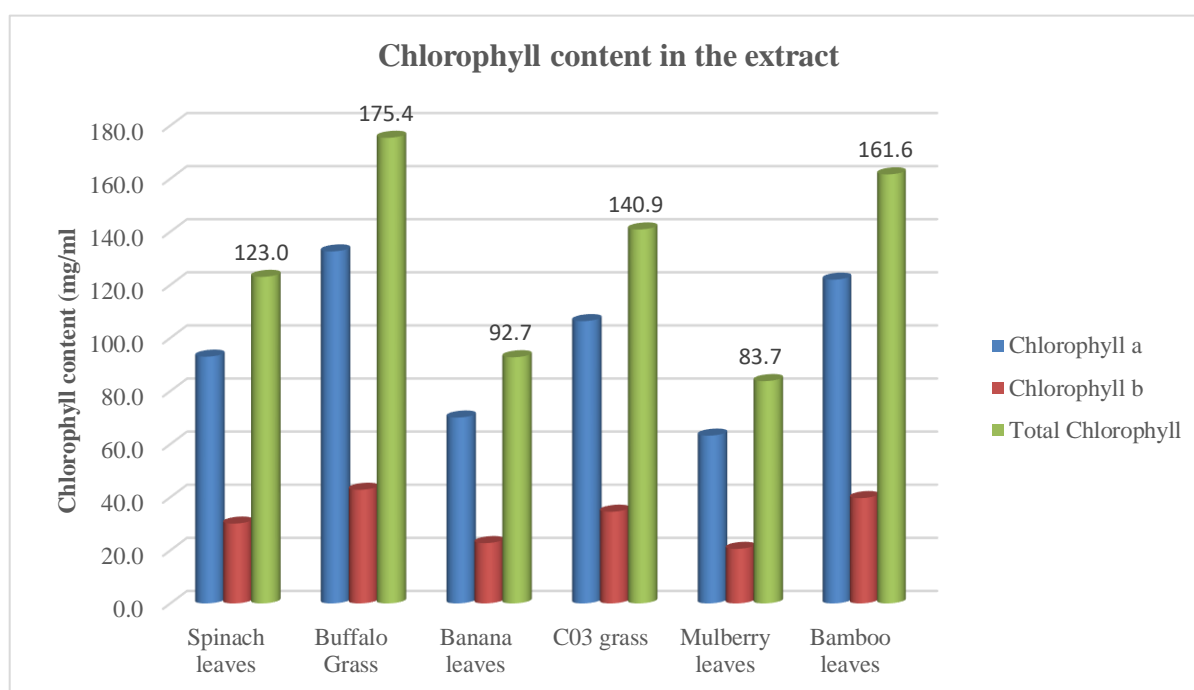
Table 2 Estimation of yield and Chlorophyll content in the extract

Plant names	Yield (%)	Chlorophyll a (mg/ml)	Chlorophyll b (mg/ml)	Total Chlorophyll (mg/ml)
Spinach leaves	0.50	92.9	30.1	123.0
Buffalo Grass	0.99	132.6	42.8	175.4
Banana leaves	1.57	70.0	22.7	92.7
C03 grass	1.57	106.3	34.5	140.9
Mulberry leaves	1.53	63.2	20.5	83.7
Bamboo leaves	2.42	121.9	39.7	161.6

Graph -2 Comparison of yield of the extract



Graph-3 Comparison of the chlorophyll content in extracts of plants



4. Discussion

This study compares some of the commercially available green plants by estimating the Chlorophyll content using UV-Visible spectrophotometric method using 80% Acetone as the solvent [9]. Moisture content of the samples were estimated to find out the chlorophyll content of the leaves on dry basis. A suitable extraction method was developed to prepare the extract using Methanol as the extraction solvent. Hexane was used to back extract the chlorophyll as there was moisture accumulation in the extract during the evaporation which will degrade the Chlorophyll.

Table 1 gives the values of Chlorophyll a, Chlorophyll b and total Chlorophyll of fresh leaves, moisture content of the leaves and the Chlorophyll values on dry basis. The values of chlorophyll content on dry basis were expressed based on the moisture content in the leaves. From graph 1 it clear that Chlorophyll a (3.03mg/ml) and Chlorophyll b (0.98mg/ml) were found to be high in Bamboo leaves. Moisture content was found to be less (80.30%) in Bamboo leaves compared to other leaves. On dry basis also Bamboo leaves contain the highest chlorophyll content. Chlorophyll content was found to be less in Spinach leaves (Chlorophyll a – 0.49mg/ml and chlorophyll b 0.15mg/ml) and moisture is high compared to other samples.

Table 2 represents the values of the yield and the chlorophyll content of the extract made from the plants. From graph 3 it is evident that Chlorophyll content of the extract was highest in the extract prepared from buffalo grass (175.4mg/ml). Chlorophyll content of the extract from bamboo leaves was found to be slightly less than that of Buffalo grass (161.6mg/ml). Graph 2 gives the indication that the yield of the extract was high in bamboo leaves (2.42%) compared to buffalo grass (0.99%). For raw material to be commercially viable both yield and quality plays important roles. Considering

these two factors Bamboo leaves were found to be best for making Chlorophyll commercially when compared with all other plants taken in this study.

5. Conclusion

Spinach leaves (*Spinacia oleracea*), Buffalo grass (*Bouteloua dactyloides*), Banana leaves (*Musa paradisiaca*), CO3-Napier Grass (*Pennisetum purpureum* Schum), Mulberry leaves (*Morus alba*) and Bamboo leaves (*Bambusa vulgaris*) were compared with their Chlorophyll content for evaluating its viability for preparing Chlorophyll extract commercially. Bamboo leaves were found to be the best in terms of Chlorophyll content in the fresh material as well as in terms of yield and quality of the extract.

6. Acknowledgement

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7. Conflicts of Interest

The authors declare that there is no conflict of interest

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