



Estimation of Chlorophyll Content in Young and Adult Leaves of Some Selected Plants

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Abstract:

In the present study chlorophyll a (Chl. a) and b (Chl. b) content of ten different plants have been recorded. The qualitative difference of chlorophyll a (Chl.a) and b (Chl.b) content between young and adult leaves were observed. A total of ten plant species were selected namely Mango (*Magnifera indica*), Hibiscus (*Hibiscus rosa-sinensis*), Gavua (*Psidium guajava*), Almond (*Prunus dulcis*), Bryophyllum (*Bryophyllum pinnatum*), Sapodilla (*Manikara zapota*), Neem (*Azadiracta indica*), Ashoka (*Polyalthia longifolia*), Ficus (*Ficus benjamina*) and Datura (*Datura metal*) and were used for analysis of chlorophyll content. In all the cases the adult leaves showed higher chlorophyll content in comparison to young leaves. This has been proved that the age of leaves was an important factor for chlorophyll content.

Keywords: Young and Adult leaves, Plant material, Chlorophyll a and b

1.0 Introduction:

Leaf chlorophyll concentration is an important parameter that is regularly measured as an indicator of chloroplast content, photosynthetic mechanism and of plant metabolism. Chlorophyll is an antioxidant compounds which are present and stored in the chloroplast of green leaf plants and mainly it is present in the green area of leaves, stems, flowers and roots (Mirza et al., 2013; Srichaikul et al., 2011). However the chlorophyll production is mainly depended on penetration of sun light and it is the main source of energy for plant (Srichaikul et al., 2011). In the laboratory it is commonly determined by using pestle and mortar to extract the pigments using an organic solvent such as acetone or dimethyl formamide (Arnon 1949; Porra et al., 1989; Ling et al., 2011). Chlorophyll a and Chlorophyll b are essential pigments of the plant photosystems (Richardson et al., 2002). Moreover the chlorophyll A is the primary photosynthetic pigment in plants which helps to produce energy in plant (Srichaikul et al., 2011). However the chlorophyll A concentration is 2-3 times higher than that of secondary chlorophyll b in plants (Srichaikul

et al., 2011). To obtain ratio of chlorophyll a and b, the readings should be taken at the wavelength of 650 nm, which was in between the absorption maxima of both (Arnon 1949; Porra et al., 1989; Devmalkar et al., 2014).

Green plants have different characters because of the presence of various pigments like chlorophyll, carotenoid, other pigments and water content which together constitute the spectral characters of a plant body (Philip and Shirly, 1978; Jan-Chang Chen and Chaur-Tzuhn Chen, 2006). However the chlorophyll content has medicinal qualities. The chlorophyll is also plays important role in plant physiology and it can be act as nutrition in decline blood sugar conditions, detoxification, digestion, excretion and decreasing allergens (Srichaikul et al., 2011, Singh et al., 2011). However in using modern technique like satellite remote sensing technology being used for analysis of leaf chlorophyll concentration can also be measured. Variation in leaf chlorophyll content can provide information about the physiological condition of a leaf or plant. Destructive methods of leaf chlorophyll content quantification include

traditional method using extraction and spectrophotometric or HPLC measurement, but they are considered time consuming and expensive. Biochemical components (green pigment and nutrient) of forest canopies are among essential parameters that control physiological processes (Gamonand Quio, 1999; Chen *et al.*, 2006). Present paper records the chlorophyll content of different plant leaves. Difference between chlorophyll content in young and adult leaves of same plant species were studied. It is essential to do this kind of study to know the photosynthetic activity of physiological changes of young and adult leaves of plants. This work was an experimental study and objective of study was to analyze chlorophyll a and b content in young and adult leaf of selected plants. However the chlorophyll is very important macromolecule which indicates performance of photosynthesis and energy utilization rate. Also it gives us energy in the form of food or plant material. Chlorophyll bears antioxidant properties which can be used in a medicinal drug discovery. In the article it has explicitly been explained the chlorophyll content in young and adult leaves and its interaction with other macromolecules.

2.0 Materials and Methods:

2.1 Study Area:

Present study was carried out at Astgaon which is located in the Rahata Tahasil of Ahmednagar district, Maharashtra during the month of March 2013. A total of ten different plant species were selected at random from different parts of the study area. Sampling was done for once only. Both the young and adult leaves from the same sample plant were collected and subjected to standard chemical procedures for determination of chlorophyll content.

2.2 Chlorophyll Analysis:

One gram of leaf sample was finely cut and gently mixed with a clean pestle and mortar. To this homogenized leaf material, 20ml of 80% acetone and 0.5gm $MgCO_3$ powder was added. The materials were further grind gently. The sample was then put into a refrigerator at $4^{\circ}C$ for 4 hours. Thereafter, the sample was centrifuged at 500 rpm for 5 minutes. The supernatant was transferred to 100 l volumetric flask. The final volume was made up to 100 ml with addition of 80% acetone. The color absorbance of the solution was estimated by a spectrophotometer using 645 and 663nm wavelength against the

solvent. Acetone (80%) was used as a blank (APHA, 1989).

Leaf material – crushed using mortar and pestle-added Acetone and $MgCO_3$ –kept for 4 hours in freeze at $4^{\circ}C$ –centrifuge at 500 rpm for 5 minute-then measured absorbance on spectrophotometer

Formula:

$$Chl\ a = 11.75 \times A_{662.6} - 2.35 \times A_{645.6}$$

$$Chl\ b = 18.61 \times A_{645.6} - 3.96 \times A_{662.6}$$

Where, Ca and Cb are the chlorophyll a and chlorophyll b, A is absorbance.

3.0 Result and Discussion:

Results of the present study have been presented in Table-1. In mango young leaves the Chl. a and Chl. b contents were 3.24 mg pigment/m³ and Chl. b =3.09 mg pigment/m³, respectively. The adult leaves contained Chl. a=19.28 mg pigment/m³ and Chl. b=13.72 mg pigment/m³. In mango plants the adult leaves contained higher amount of chlorophyll than the young leaves. Similarly, in Hibiscus the chlorophyll content in young leaves were Chl. a =19.60 mg pigment/m³ and Chl. b=12.87 mg pigment/m³ while in adult leaves the Chl. a and Chl. b content were 5.50 mg pigment/m³ and 2.29 mg pigment/m³, respectively indicating that, the young leaves of Hibiscus contained higher amount of chlorophyll than the adult leaves. In guava plants the chlorophyll content in young leaves was Chl. a =6.27 mg pigment/m³ and Chl. a =5.40 mg pigment/m³ while in adult leaves it was Chl. a =3.91mg pigment/m³ and Chl. b=5.73 mg pigment/m³. The Ca content was higher in young leaves, but Cb content was higher in adult leaves. Chlorophyll content in young leaves of almond trees was Chl. a =5.25mg pigment/m³ and Chl. b=3.04 mg pigment/m³. However, in adult leaves it was Chl. a =14.24 mg per pigment/m³ and Chl. a =12.19 mg pigment/m³ indicating higher chlorophyll content in adult leaves. In the Bryophyllum, the chlorophyll content in young leaves were Chl. a =6.04mg pigment/m³ and Chl. b=7.33 mg pigment/m³ and in adult leaves it was Chl. a =9.09 mg pigment/m³ and Chl. b=9.98 mg pigment/m³ which implies that the adult leaves contained higher amount of chlorophyll than the young leaves (Table no 1). Sapodilla the chlorophyll in young leaves were Chl. a =4.62 mg pigment/m³ and Chl. b=8.87 mg pigment/m³ and in adult leaves were observed Chl. a =3.35 mg pigment/m³ and Chl. b =5.55 mg pigment/m³ so In the Sapodilla plant the young leaves contained

higher chlorophyll than the adult leaves. Similarly, in neem, ficus and datura the adult leaves contained higher chlorophyll (both Chl. a and Chl.b) in adult leaves in comparison to the younger leaves (Table-1). In case of total chlorophyll content, higher chlorophyll content has been observed in Datura

(*Datura metal*) in adult leaves while highest chlorophyll content in young leaves was observed in Hibiscus (*Hibiscus rosa-sinensis*). The lowest chlorophyll content was observed in Ashoka (*Polyalthia longifolia*) for both young and adult leaves (Fig. 1).

Table 1: Chlorophyll content of different plant species (mg/l)

Sr.No	Plants Name	Young leaves		Total Chl. Content young leaves	Adult leaves		Total Chl. Content adult leaves
		Chl. a	Chl. b		Chl. a	Chl. b	
1.	Mango (<i>Magnifera indica</i>)	3.24	3.09	6.48	19.28	13.72	33
2.	Hibiscus (<i>Hibiscus rosa-sinensis</i>)	19.60	12.87	39.2	5.50	2.29	7.79
3.	Gavua (<i>Psidium guajava</i>)	6.27	5.40	12.54	3.91	5.73	9.64
4.	Almond (<i>Prunus dulcis</i>)	5.25	3.04	10.5	14.24	12.19	26.43
5.	Bryophyllum (<i>Bryophyllum pinnatum</i>)	6.04	7.33	12.08	9.09	9.98	19.07
6.	Sapodilla (<i>Manikara zapota</i>)	4.62	8.87	9.24	3.35	5.55	8.9
7.	Neem (<i>Azadiracta indica</i>)	18.09	9.76	36.18	18.10	12.98	31.08
8.	Ashoka (<i>Polyalthia longifolia</i>)	1.30	1.65	2.6	1.36	1.21	2.57
9.	Ficus (<i>Ficus benjamina</i>)	6.66	4.7	13.32	10.44	28.21	38.65
10.	Datura (<i>Datura metal</i>)	13.96	8.84	27.92	19.1	24.2	43.3

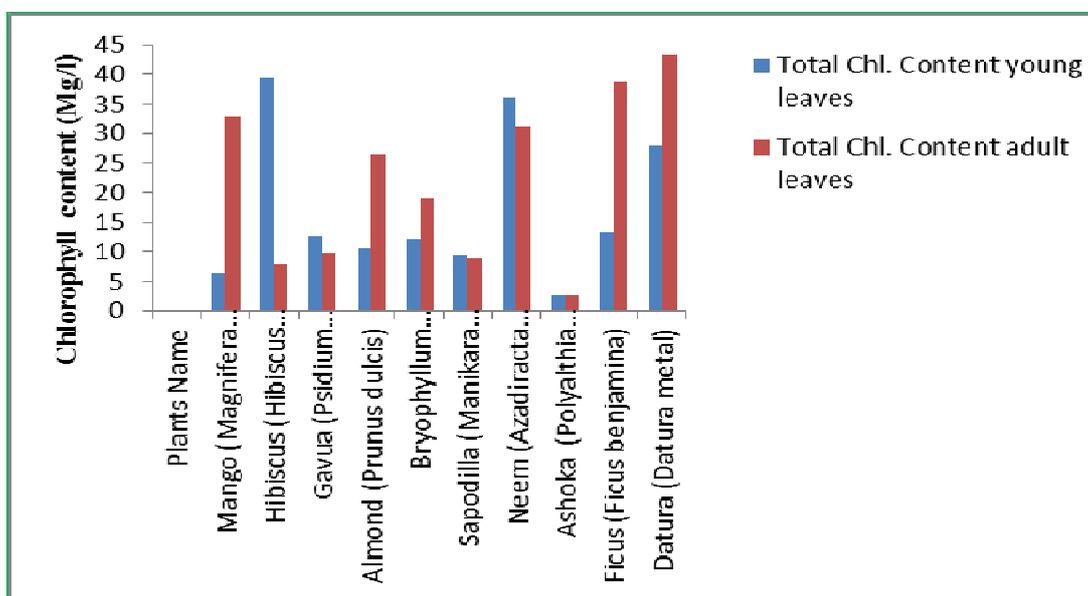


Fig. 1: Total Chlorophyll content in young and adult leaves of different plant species

In almost all the selected plants, the chlorophyll content was high in adult leaves in comparison to young leaves. The possible explanation could be that the young leaves were not mature and on the other hand adult leaves were fully mature (Siwach and Gill, 2014; James *et al.*, 1999). However the structure of mesophyll plays a very important role in the photosynthetic process of leaves through functioning of the internal light (Siwach and Gill, 2014; James *et al.*, 1999). Moreover James *et al.* (1999) have studied and observed that the young leaves had maximum mesophyll content; Due to that they appear blue-gray in color while the adult leaves were shown to have low concentration of mesophyll because of that they appear green in color (Johnson, 1926; Jacobs, 1955; Penfold and Willis, 1956; Pryor, 1976; FAO, 1979; James *et al.*, 1999; Siwach and Gill, 2014). In the context of both young and adult leaves the chlorophyll a/b ratio was higher in adult leaves than that of young leaves (Smith and Nobel, 1978). However in case of Hibiscus, Guava, Ashoka, Sapodilla, the chlorophyll content was high in young leaves than that of adult leaves. The possible explanation could be that the micronutrient deficiency in soil e.g. Fe deficiency or less penetration of sunlight can be the cause of low chlorophyll content (Morikawa *et al.*, 2006; Dantas *et al.*, 2007). However the almost in all cases the chlorophyll a concentration was higher than that of chlorophyll b. The possible justification could be that Chlorophyll a is the primary pigment while others pigments including Chlorophyll b are accessory pigments (Srichaikul *et al.*, 2011).

4.0 Conclusion:

From the findings of the present study we conclude that the most of the plant showed higher chlorophyll content in adult leaves as compared to young leaves. Some plants had shown low chlorophyll content in adult leaves (Hibiscus, Guava Ashoka, and Sapodilla). Chlorophyll content can be used as measurement of healthiness of plants canopy and the rate of photosynthesis as well. This study will be helpful to do research in chlorophyll content analysis of various plants species and study the vegetation cover area. Also the effect of pollution on chlorophyll content is an important point of study. It needs to keep continuous monitoring on chlorophyll content to maintain and to check the healthiness of plants. The chlorophyll pigments are an indicator of O₂ production and carbon sequestration.

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References:

- 1) Arnon, D. I. (1949): Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* 24,1-15.
- 2) Dantas, B., F., Pereira M., S., Ribeiro L., De S., Maia J., L., T., Silva D., J., Duenhas L., H., Lima M., A., C., De, Bassoi L., H., (2007): Metabolic Responses Of Guava Trees Irrigated With Different N And K Levels In São Francisco Valley. *Rev. Bras. Frutic., Jaboticabal*, 29, 323-328.
- 3) Devmalkar, V. S., Murumkar C. V., Salunkhe S. M. and Chavan S. J. (2014): Studies on pigment chlorophyll isolation and estimation of different bryophytes for their biochemical properties. *J. Nat. Prod. Plant Resour.*, 4 (2), 56-61.
- 4) FAO., (1979): Eucalypts for planting. Food and Agriculture Organization of the United Nations, Rome, Italy,
- 5) Jacobs, M., R., (1955): Growth habits of the eucalypts. Forestry and Timber Bureau, Canberra, Australia.,
- 6) James S., A., Smith W., K., and Vogelmann T., C., (1999): Ontogenetic Differences in Mesophyll Structure and Chlorophyll Distribution in *Eucalyptus Globulus* sp. *Globulus* (Myrtaceae) *American Journal of Botany*, 86, 198-207.
- 7) Jan-Chang Chen, Chi Ming Yang shou Tsung Wu, Yuh- Lurung Chung, Albert Linton Charles and Chaur-Tzuhn Chen (2007): Leaf chlorophyll content and surface spectral reflectance of tree species along a terrain gradient in Taiwan's Kenting National Park. *Botanical studies.*, 48,71-77.
- 8) Johnson, E., D., (1926): A comparison of the juvenile and adult leaves of *Eucalyptus globulus*. *New Phytologist*, 25, 202-212.
- 9) Morikawa, C., K., Saigusa M., Nakanishi H., Nishizawa, N., K., and Mori, S., (2006): Overcoming Fe deficiency in guava (*Psidium guajava* L.) by co-situs application of controlled

- release fertilizers. *Soil Science and Plant Nutrition*, 52, 754–759.
- 10) Mirza, H., Kamrun N., Md. Mahabub A., 2, Roychowdhury R., Fujita M. (2013): Physiological, Biochemical, and Molecular Mechanisms of Heat Stress Tolerance in Plants. *Int. J. Mol. Sci.*, 14, 9643-9684.
 - 11) Penfold, A., R., and Willis J., L., (1956): The eucalypts: Botany, cultivation, chemistry, and utilization. Interscience Publishers, New York, NY,
 - 12) Philip, H., S., Shirly, M., D., Remote sensing: the quantitative approach. McGraw-Hill, New York, 1978, Pp. 226-227.
 - 13) Porra, R., J., Thompson, W., A., Kriedemann, P., E., (1989): Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim. Biophys. Acta*, 975, 384-394.
 - 14) Pryor, L., D., (1976): The biology of eucalypts. Edward Arnold, London.
 - 15) Richardson, A.D., S., P., Duigan, G., P., Berlyn, (2002): An evaluation of noninvasive methods to estimate foliar chlorophyll content. *New Phytologist*, 153, 185-194.
 - 16) Siwach P., Gill A. R., (2014): Micropropagation of *Ficus religiosa* L. via leaf explants and comparative evaluation of acetylcholinesterase inhibitory activity in the micropropagated and conventionally grown plants. *Biotech*, 4, 477–491.
 - 17) Singh, D., Singh B, Goel R. K. (2011): Traditional uses, phytochemistry and pharmacology of *Ficus religiosa*: a review. *J Ethnopharmacol*, 134, 3, 565–583.
 - 18) Smith, W., K., and Nobel, P., S. (1978): Influence of irradiation, soil water potential, and leaf temperature on leaf morphology of a desert broadleaf, *Encelia farinosa* Gray (Compositae). *American Journal of Botany*, 65, 429-432.
 - 19) Srichaikul, B., Bunsang, R., Samappito, S., Butkhup S., and Bakker, G., (2011): Comparative study of chlorophyll content in leaves of Thai *Morus alba* Linn. Species. *Plant Science Research*, 3, 17-20.