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Comparative studies on chlorophyll concentration in some important plant families

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Abstract

Chlorophyll is a notable green photosynthetic pigment found in cyanobacteria, algae, and higher plants. Chlorophyll manifests antioxidant, anti-cancerous properties, It also has a role in the food and pharmaceutical industries. The main objective of this research investigation is to compare the total chlorophyll content in some important plant families (Apocynaceae, Asteraceae, Euphorbiaceae, Fabaceae) and to make a comparative study on the concentration of chlorophyll as this bioactive compound has great importance in food and pharmaceutical industries. In the present study, chlorophyll was extracted from the leaves of ten plants from each family that are locally available, using 80% acetone and quantified by the Arnon method (1949) respectively. The final results revealed that Euphorbiaceae exhibited an enormous amount of average total chlorophyll. However, *Catharanthus roseus* from Apocyanaceae exhibited the highest concentrations of total chlorophyll.

Keywords: Arnon, chlorophyll a, chlorophyll b, total chlorophyll, euphorbiaceae, pharmaceutics

Introduction

Chlorophyll is a green photosynthetic pigment, it was discovered in the year 1906. The chlorophyll molecule consists of a cyclic structure composed principally of 4 pyrole nuclei containing magnesium at its centre [1-5]. It is a necessary pigment for photosynthesis as it absorbs the light energy from sun and converts it into chemical energy in the plants [6-9]. Chlorophylls can be classified into 3 types -Chlorophylls, Bacteriochlorophylls and Chlorobium chlorophylls. Chlorophylls are further classified into Chl a, b, c, and d. Chl a is the marked photosynthetic pigment in higher plants, algae and cyanobacteria. It absorbs red light in the visible region at 680 nm. Chl b also absorbs red light in the visible region at 660 nm. Chl c is well described in microbes, alga, and absorbs light in red region (450–640 nm). However, Chl d is reported in cyanobacteria whose habitats are that areas that lack visible light. Hence, they absorb light in the IR region between 700 and 730 nm. Recent, studies reported a new chlorophyll called as Chl f [10, 11]. Bacteriochlorophylls are found in anaerobic group of bacteria. They are further classified into Chl a, b, c, d, e, g, all the members of this group have absorption maximum in Infrared region [10, 12]. Chlorobium chlorophylls (Cchl) are reported in the green sulphur bacteria [10]. Generally, the standard ratio of the chlorophyll a and b in higher plants is approximately 3:1 ratio. However, it has been identified that this ratio of Chl a to Chl b varies depending on genetic and environmental conditions [9, 10]. Although there are several methods for extraction and quantification of chlorophyll concentration in leaves. Acetone is the most commonly used solvent in chlorophyll extraction as it helps in giving very prominent absorption peaks [5, 10, 13, 14, 15]. Chlorophyll is a registered food additive, and its products can be utilized as a photodynamic agent in various herbal treatments due to its anti-cancerous properties [16]. It helps in the growth and repair of tissues and neutralizing the pollution that we breathe. It increases the efficacy of magnesium and helps the blood in carrying the much-needed oxygen to all cells and tissues. They neutralize free radicals that do damage to healthy cells along with other vitamins like A, C and E [17]. A regular and recommended intake of chlorophyll keeps the circulatory and digestive systems much healthier [1]. The concentration of chlorophyll may vary in different region and season-wise [18]. It may change with different environmental stress and with pollution [19, 20-23].

The objective of the present study is to make a comparative study and find the plant species and family/ies that show high concentration of chlorophyll as it has huge importance in culinary, herbal treatments and pharmaceutical industries. The families chosen for this study are Apocynaceae, Asteraceae, Euphorbiaceae and Fabaceae due their local availability. The comparative study of aforementioned plant families has not been discussed earlier, hence the study was undertaken with the above mentioned objective.

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Materials and Methods

Collection of plants

Forty commonly available plants were selected for this study (Table 1). These plants belonged to four different plant families i.e., Apocyanaceae, Asteraceae, Euphorbiaceae and Fabaceae. Plants belonging to Apocyanaceae are *Allamanda blanchetii* (Purple allamanda), *Allamanda cathartica* (Yellow allamanda), *Catharanthus roseus*: pink and white (Madagascar periwinkle), *Cascabela thevetia* (Yellow oleander), *Nerium oleander* (Nerium), *Plumeria alba* (White frangipani), *Plumeria pudica* (Wild plumeria), *Plumeria rubra* (Red frangipani), *Tabernaemontana divaricata* (Pin wheel flower); Those belonging to Asteraceae were *Ageratum conyzoides* (Billy goat weed), *Artemisia pallens* (Dhavanam), *Calypocarpus vialis* (Straggler daisy), *Dendranthema X grandiflorum*: Pink and white (Chrysanthemum), *Emilia sonchifolia* (Lilac tassel flower), *Parthenium hysterophorus*

(Congress grass), *Sphagneticola trilobata* (Trailing daisy), *Synedrella nodiflora* (Cinderella weed) *Tridax procumbens* (Coat buttons); Those belonging to Euphorbiaceae were *Acalypha indica* (Indian copperleaf), *Codiaeum variegatum* (Croton), *Croton sparsiflorus* (Ban tulasi), *Euphorbia hirta* (Asthma plant), *Euphorbia milli* (Crown of thorns), *Jatropha curcas* (Physic nut), *Jatropha integerrima* (Peregrina), *Phyllanthus niruri* (Gale of the wind), *Ricinus communis* (castor), *Sauropus androgynus* (Vitamin plant); Those belonging to Fabaceae were *Albizia saman* (Monkey pod tree), *Cajanus cajan* (Pigeon pea), *Clitoria ternatea* (Asian pigeon wings), *Delonix regia* (Royal poinciana), *Leucaena leucocephala* (River tamarind), *Milletia pinnata* (Pongam oil tree), *Peltophorum pterocarpum* (Copper pod), *Phaseolus vulgaris* (Common bean), *Saraca asoca* (Ashoka tree), *Senna siamea* (Cassod tree). These plants were procured from Bengaluru, Karnataka, India.

Table 1: List of Plants with Their Respective Scientific, Common Names and Families

Sl. No	Botanical name	Common name	Family
1.	<i>Allamanda blanchetii</i>	Purple allamanda	Apocyanaceae
2.	<i>Allamanda cathartica</i>	Yellow allamanda	Apocyanaceae
3.	<i>Catharanthus roseus</i> (pink)	Madagascar periwinkle	Apocyanaceae
4.	<i>Catharanthus roseus</i> (white)	Madagascar periwinkle	Apocyanaceae
5.	<i>Cascabela thevetia</i>	Yellow oleander	Apocyanaceae
6.	<i>Nerium oleander</i>	Nerium	Apocyanaceae
7.	<i>Plumeria alba</i>	White fragipani	Apocyanaceae
8.	<i>Plumeria pudica</i>	Wild fragipani	Apocyanaceae
9.	<i>Plumeria rubra</i>	Red fragipani	Apocyanaceae
10.	<i>Tabernaemontana divaricata</i>	Pin wheel flower	Apocyanaceae
11.	<i>Ageratum conyzoides</i>	Billygoat weed	Asteraceae
12.	<i>Artemisia pallens</i>	Dhavanam	Asteraceae
13.	<i>Calypocarpus vialis</i>	Straggler daisy	Asteraceae
14.	<i>Dendranthema X grandiflorum</i>	Pink	Asteraceae
15.	<i>Dendranthema X grandiflorum</i>	White	Asteraceae
16.	<i>Emilia sonchifolia</i>	Lilac tassel flower	Asteraceae
17.	<i>Parthenium hysterophorus</i>	Congress grass	Asteraceae
18.	<i>Sphagneticola trilobata</i>	Trailing daisy	Asteraceae
19.	<i>Synedrella nodiflora</i>	Cinderella weed	Asteraceae
20.	<i>Tridax procumbens</i>	Coat buttons	Asteraceae
21.	<i>Acalypha indica</i>	Indian copper leaf	Euphorbiaceae
22.	<i>Codiaeum variegatum</i>	Croton	Euphorbiaceae
23.	<i>Croton sparsiflorus</i>	Ban thulasi	Euphorbiaceae
24.	<i>Euphorbia hirta</i>	Asthma plant	Euphorbiaceae
25.	<i>Euphorbia milli</i>	Crown of thorns	Euphorbiaceae
26.	<i>Jatropha curcas</i>	Physic nut	Euphorbiaceae
27.	<i>Jatropha integerrima</i>	Peregrina	Euphorbiaceae
28.	<i>Phyllanthus niruri</i>	Gale of the wind	Euphorbiaceae
29.	<i>Ricinus communis</i>	Castor	Euphorbiaceae
30.	<i>Sauropus androgynus</i>	Vitamin plant	Euphorbiaceae
31.	<i>Albizia saman</i>	Monkeypod tree	Fabaceae
32.	<i>Cajanus cajan</i>	Pigeon pea	Fabaceae
33.	<i>Clitoria ternatea</i>	Asian pigeon wings	Fabaceae
34.	<i>Delonix regia</i>	Royal poinciana	Fabaceae
35.	<i>Leucaena leucocephala</i>	River tamarind	Fabaceae
36.	<i>Milletia pinnata</i>	Pongam oil tree	Fabaceae
37.	<i>Peltophorum pterocarpum</i>	Copper pod	Fabaceae
38.	<i>Phaseolus vulgaris</i>	Common bean	Fabaceae
39.	<i>Saraca asoca</i>	Ashoka tree	Fabaceae
40.	<i>Senna siamea</i>	Cassod tree	Fabaceae

Extraction of Chlorophyll [4, 5, 13, 24] (Arnon, 1949)

100 mg of finely cut fresh leaves were taken and ground with 15 - 20 ml of 80% acetone. The leaf extract was then centrifuged at 8000 rpm for 8 min. The supernatant was transferred and the procedure was repeated till the residue became colourless. The volume was made up to 50 ml and the

extract was stored under darkness until the absorbance was measured, to prevent damage to the chlorophyll pigments. The absorbance of the supernatant was measured at 645 nm and 663 nm against the solvent (80% acetone) blank. The above mentioned process was repeated for all the plant samples.

Estimation of Chlorophyll Content [4, 5, 13, 22]

The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equation (Arnon, 1949):

Chlorophyll a (mg/gm tissue): $[12.7(A_{663}) - 2.69 (A_{645})] * V/1000*W$

Chlorophyll b (mg/gm tissue): $[22.9(A_{645}) - 4.68 (A_{663})] * V/1000*W$

Total Chlorophyll (a + b) (mg/gm tissue): $[20.21 (A_{645}) + 8.02(A_{663})] * V/1000*W$

A = Absorbance of specific wavelength; V = Final volume of Chlorophyll extract in 80% Acetone; W = Fresh weight of Tissue extract

Results**Table 2:** Comparison of Pigment Quantity and Ratio between Chlorophyll - A and Chlorophyll - B and Total Chlorophyll of 40 Common Plants

Sl. No	Botanical name	Chl-a (mg/g tissue)	Chl-b (mg/g tissue)	Total chl (mg/g tissue)
1.	<i>Allamanda blanchetii</i>	2.818	2.180	4.998
2.	<i>Allamanda cathartica</i>	7.58	4.458	12.038
3.	<i>Catharanthus roseus</i> (pink)	3.91	2.503	6.413
4.	<i>Catharanthus roseus</i> (white)	24.44	9.303	33.743
5.	<i>Cascabela thevetia</i>	1.648	1.276	2.924
6.	<i>Nerium oleander</i>	2.733	0.416	3.149
7.	<i>Plumeria alba</i>	1.74	1.111	2.851
8.	<i>Plumeria pudica</i>	2.341	2.927	5.268
9.	<i>Plumeria rubra</i>	6.865	2.378	9.243
10.	<i>Tabernaemontana divaricata</i>	4.417	4.594	9.011
11.	<i>Ageratum conyzoides</i>	0.728	0.520	1.248
12.	<i>Artemisia pallens</i>	8.281	9.041	17.322
13.	<i>Calyptocarpus vialis</i>	8.050	11.011	19.061
14.	<i>Dendranthema X grandiflorum</i>	5.308	3.214	8.522
15.	<i>Dendranthema X grandiflorum</i>	4.996	3.112	8.108
16.	<i>Emilia sonchifolia</i>	5.037	5.527	10.564
17.	<i>Parthenium hysterophorus</i>	2.513	3.017	5.53
18.	<i>Sphagneticola trilobata</i>	1.455	0.315	1.77
19.	<i>Synedrella nodiflora</i>	3.563	2.134	5.697
20.	<i>Tridax procumbense</i>	4.366	3.89	8.256
21.	<i>Acalypha indica</i>	7.828	7.938	15.766
22.	<i>Codiaeum variegatum</i>	4.433	3.011	7.444
23.	<i>Croton sparsiflorus</i>	6.354	3.609	9.963
24.	<i>Euphorbia hirta</i>	4.251	1.566	5.817
25.	<i>Euphorbia milli</i>	6.445	5.643	12.088
26.	<i>Jatropha curcas</i>	8.657	2.638	11.295
27.	<i>Jatropha integerrima</i>	1.148	0.782	1.93
28.	<i>Phyllanthus niruri</i>	7.145	5.167	12.312
29.	<i>Ricinus communis</i>	6.278	2.922	9.2
30.	<i>Sauropus androgynus</i>	8.147	10.187	18.334
31.	<i>Albizia saman</i>	4.548	3.495	8.043
32.	<i>Cajanus cajan</i>	0.956	0.129	1.085
33.	<i>Clitoria ternatea</i>	1.936	2.134	4.07
34.	<i>Delonix regia</i>	1.724	1.248	2.972
35.	<i>Leucaena leucocephala</i>	3.357	2.553	5.91
36.	<i>Milletia pinnata</i>	5.588	6.414	12.002
37.	<i>Peltophorum pterocarpum</i>	3.052	4.221	7.273
38.	<i>Phaseolus vulgaris</i>	9.374	11.837	21.211
39.	<i>Saraca asoca</i>	2.018	2.783	4.801
40.	<i>Senna siamea</i>	0.610	1.527	2.137

From the Fig.1A, It was observed that the highest chlorophyll a containing Apocyanaceae family member was *Catharanthus roseus*(white) (24.44mg/g tissue) and the lowest was observed in *Cascabela thevetia* (1.648 mg/g tissue). From Fig.1B, it was observed that highest chlorophyll a containing Asteraceae family member was *Artemisia pallens* (8.281 mg/g tissue) and lowest was observed in *Ageratum conyzoides* (0.728 mg/g tissue). From Fig.1C, it was observed that highest chlorophyll

Results are tabulated in Table No. 2,3 and represented in the Column graphs. From Table 2, It was observed that chlorophyll a content is ranging maximum from 24.44 mg/g tissue (*Catharanthus roseus* (white), Apocyanaceae) to minimum 0.610 mg/g tissue (*Senna siamea*, Fabaceae). Chlorophyll b content was ranging from maximum 11.837 mg/g tissue (*Phaseolus vulgaris*, Fabaceae) to minimum 0.315 mg/g tissue (*Sphagneticola trilobata* Asteraceae). Total chlorophyll content was ranging from maximum 33.743 mg/g tissue (*Catharanthus roseus* (white), Apocyanaceae) to minimum 1.085 mg/g tissue (*Cajanus cajan*, Fabaceae). From Table 2, maximum chlorophyll a and chlorophyll b ratio was observed to be 7.410:1(*Cajanus cajan*, Fabaceae) and minimum chlorophyll a and chlorophyll b ratio was observed 0.399:1(*Senna siamea*, Fabaceae).

a containing Euphorbiaceae family member was *Jatropha curcas* (8.657 mg/g tissue) and lowest was observed in *Jatropha integerrima* (1.148 mg/g tissue). Fig.1D showed that the highest chlorophyll a containing Fabaceae family member was *Phaseolus vulgaris* (9.374 mg/g tissue) and lowest was observed in *Senna siamea* (0.610 mg/g tissue). Among the 40 plants highest chlorophyll a containing plant was *Catharanthus roseus* (white) (24.44 mg/g tissue) belonging to

Apocyanaceae family and lowest was observed in *Senna siamea* (0.610 mg/g tissue) belonging to Fabaceae.

From the Fig.1A, it was observed that the highest chlorophyll b containing Apocyanaceae family member was *Catharanthus roseus*(white) (9.303 mg/g tissue) and the lowest was observed in *Nerium oleander* (0.416mg/g tissue). From Fig.1B, it was observed that highest chlorophyll b containing Asteraceae family member was *Calyptracarpus vialis* (11.011 mg/g tissue) and lowest was observed in *Sphagneticola trilobata* (0.315 mg/g tissue). From Fig.1C, it was observed that highest chlorophyll b containing Euphorbiaceae family member was *Sauropus androgynus* (10.187 mg/g tissue) and lowest was observed in *Jatropha integerrima* (0.782 mg/g tissue). Fig.1D showed that the highest chlorophyll b containing Fabaceae family member was *Phaseolus vulgaris* (11.837 mg/g tissue) and lowest was observed in *Cajanus cajan* (0.129 mg/g tissue). Among the 40 plants highest chlorophyll b containing plant was *Phaseolus vulgaris* (11.837 mg/g tissue) belonging to Fabaceae family and lowest was observed in *Sphagneticola trilobata* (0.315 mg/g tissue) belonging to Asteraceae.

From the Fig.1A, It was observed that the highest total chlorophyll containing Apocyanaceae family member was *Catharanthus roseus* (white) (33.743 mg/g tissue) and the lowest was observed in *Plumeria alba* (2.851 mg/g tissue).

From Fig.1B, it was observed that highest total chlorophyll containing Asteraceae family member was *Calyptracarpus vialis* (19.061 mg/g tissue) and lowest was observed in *Ageratum conyzoides* (1.248 mg/g tissue). From Fig.1C, it was observed that highest total chlorophyll containing Euphorbiaceae family member was *Sauropus androgynus* (18.334 mg/g tissue) and lowest was observed in *Jatropha integerrima* (1.93 mg/g tissue). Fig.1D showed that the highest total chlorophyll containing Fabaceae family member was *Phaseolus vulgaris* (21.211 mg/g tissue) and lowest was observed in *Cajanus cajan* (1.085 mg/g tissue). Among the 40 plants highest total chlorophyll containing plant was *Catharanthus roseus* (white) (33.743 mg/g tissue) belonging to Apocyanaceae family and lowest was observed in *Cajanus cajan* (1.085 mg/g tissue) belonging to Fabaceae.

From Table 3 & Fig.2, It was observed that the average chlorophyll a is maximum in the family Euphorbiaceae (6.0686 mg/g tissue) and minimum in the family Fabaceae (3.3163mg/g tissue). The maximum average chlorophyll b containing family was observed to be Euphorbiaceae (4.3463mg/g tissue) and minimum was observed in Apocyanaceae (3.1146mg/g tissue). From the same it was observed that the maximum average total chlorophyll was observed in the family Euphorbiaceae (10.4149mg/g tissue) and minimum was observed in Fabaceae (6.9504mg/g tissue).

Table 3: Comparison of Average Chlorophyll A, Chlorophyll B and Total Chlorophyll between 4 Families

Plant families	Average Total chlorophyll a (mg/g tissue)	Average Total chlorophyll b (mg/gm tissue)	Average Total chlorophyll (mg/gm tissue)
Apocyanaceae	5.8492	3.1146	8.9638
Asteraceae	4.4297	4.1781	8.6078
Euphorbiaceae	6.0686	4.3463	10.4149
Fabaceae	3.3163	3.6341	6.9504

Each value is the mean/average of 10 plants from each respective family.

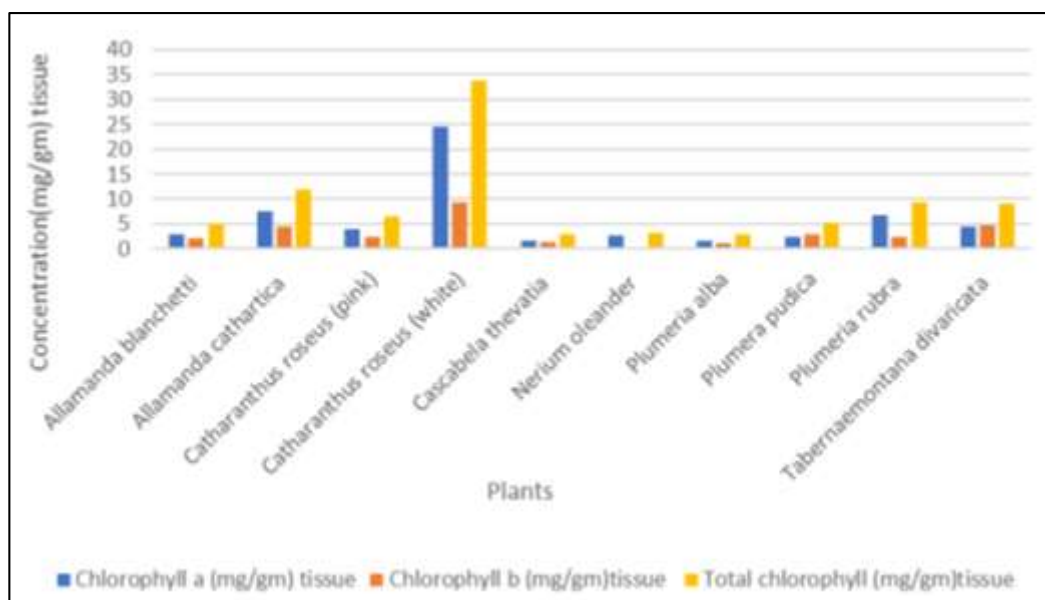


Fig 1A: Representation of chlorophyll pigments of family Apocyanaceae

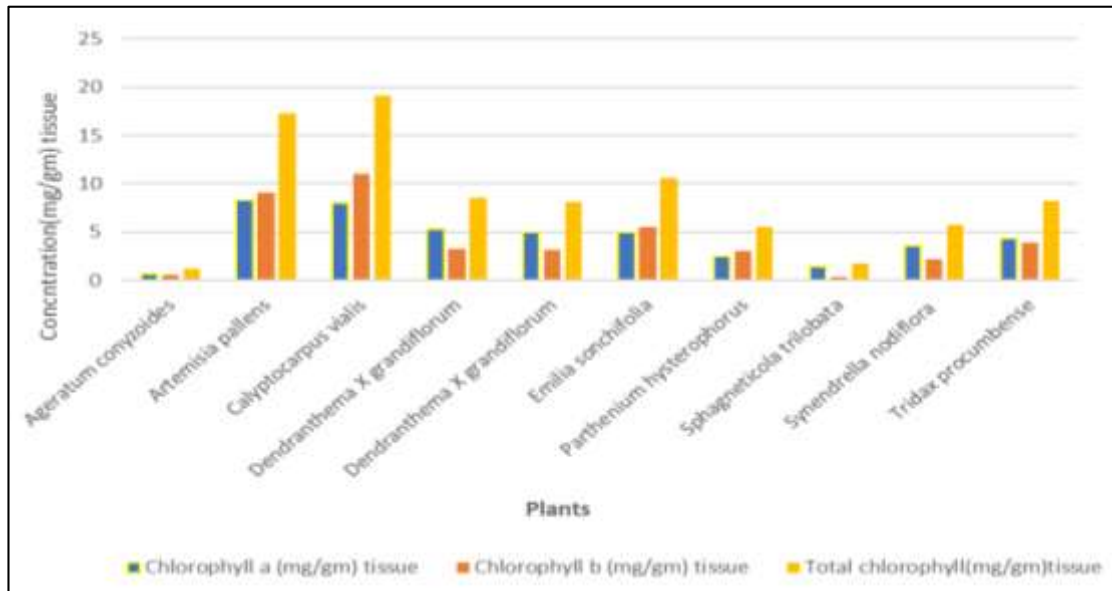


Fig 1B: Representation of chlorophyll pigments of family Asteraceae

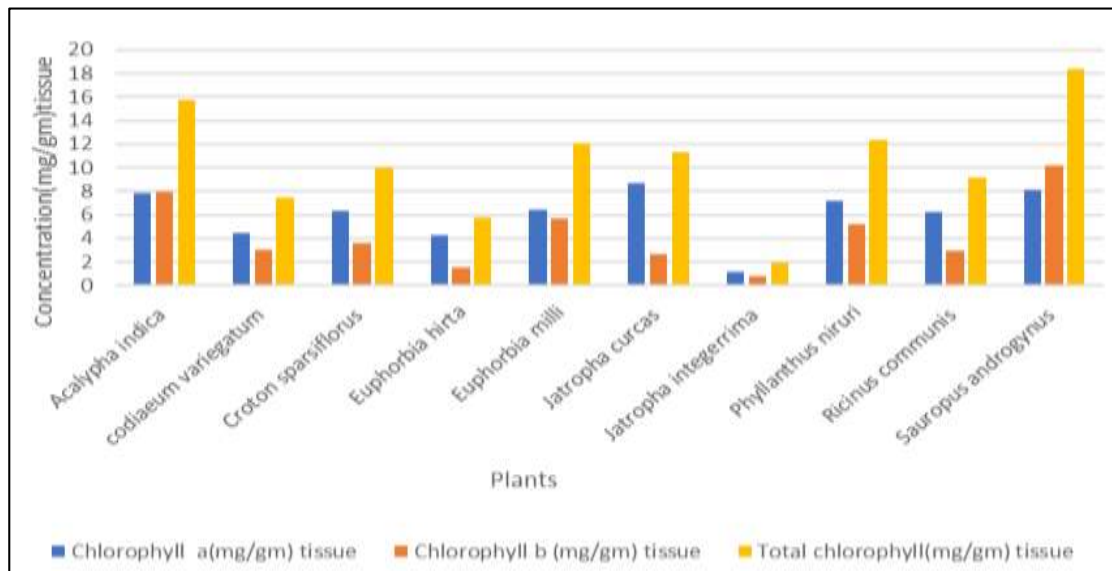


Fig 1C: Representation of chlorophyll pigments of family Euphorbiaceae

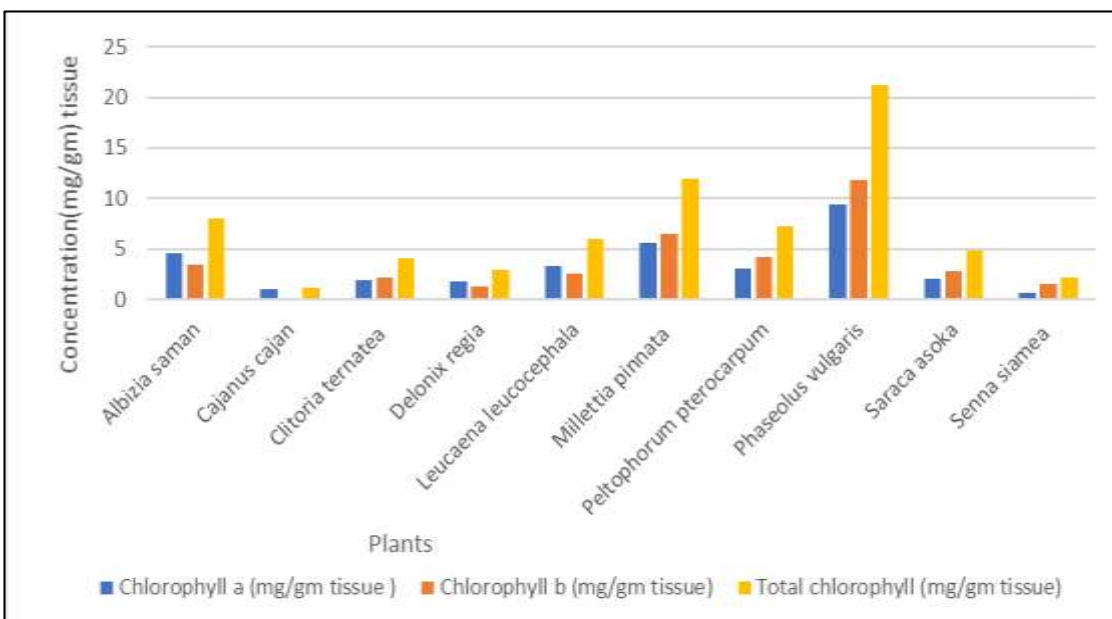


Fig 1D: Representation of chlorophyll pigments of family Fabaceae

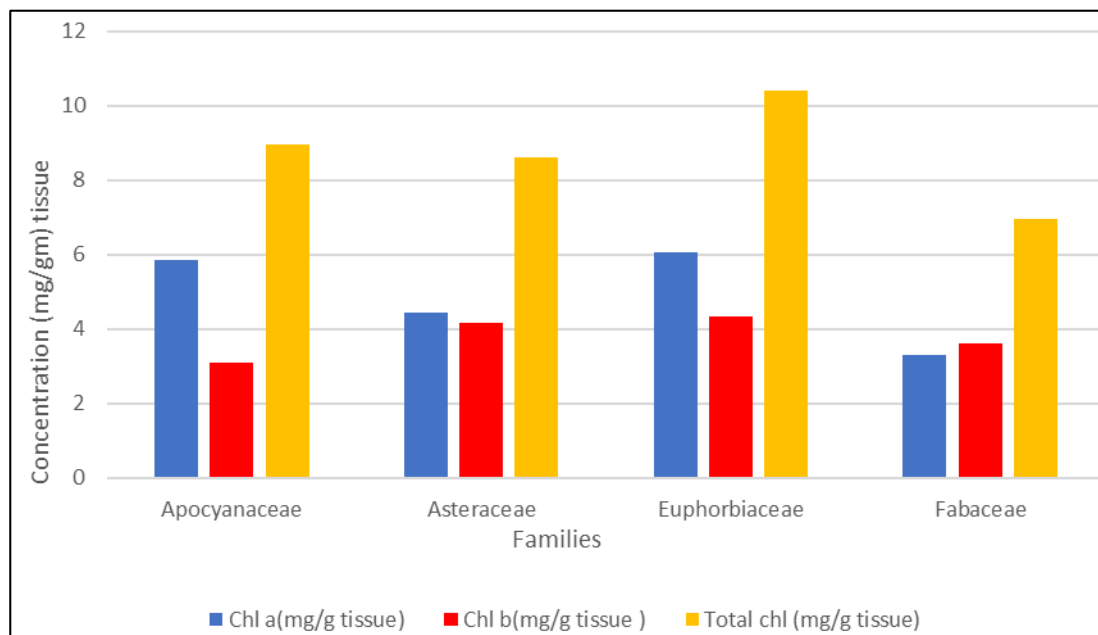


Fig 2: Representation of average chlorophyll a, chlorophyll b and total chlorophyll of Apocyanaceae, Asteraceae, Euphorbiaceae, Fabaceae.

Discussion

The results clearly indicate that, out of the 40 plants total chlorophyll content was maximum in *Catharanthus roseus* (white), Apocyanaceae (33.743 mg/g tissue) and minimum in *Cajanus cajan*, Fabaceae (1.085 mg/g tissue). However the family with average highest total chlorophyll content was found to be Euphorbiaceae followed by Apocyanaceae, Asteraceae and Fabaceae. Thus they can be used as a relatively inexpensive and very easily available source of chlorophyll instead of chlorophyll supplements for availing the health benefits of chlorophyll compound. All the plants mentioned in the research are habitual and possess many other pharmacological properties. However further research has to be carried out on the remaining plants of the Euphorbiaceae family to support this study.

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Conflicts

No known conflicts.

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