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EXTRACTION AND QUANTIFICATION OF PIGMENTS FROM INDIAN TRADITIONAL MEDICINAL PLANTS: A COMPARATIVE STUDY BETWEEN TREE, SHRUB AND HERB

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ABSTRACT: Chlorophyll and carotenoids are important photosynthetic pigments found in higher plants, cyanobacteria and algae. Carotenoids are responsible for bright red, yellow and orange color in many fruits and vegetables. These two pigments play an important role in plant health. Chlorophyll and carotenoids are reported to contain anticancer properties. Chlorophyll and carotenoids protect skin and help in normal blood clotting, hormonal balance and in deodorization. Chlorophyll and carotenoids have healing effects on oxidation and inflammatory conditions and has great antioxidant properties also. The primary objective of this comparative study is to find out the plant which contains high amount of chlorophyll and carotenoids as these two bioactive compounds has great applications in herbal medicine. In the present course of study, chlorophyll and carotenoids were extracted from the leaves of 21 medicinal plants using 80% acetone and quantified by Arnon method (1949) and Lichtenthaler and Wellburn method respectively. The results revealed that among the 21 medicinal plants under this study, *Cocos nucifera* (Tree) contains highest amount of total chlorophyll and total carotenoids and can be used as an easy source for the extraction of these important pigments.

INTRODUCTION: Chlorophyll is a green photosynthetic pigment and has a long hydrophobic phytol chain in its structure [1, 2]. At the center of pigment ring there is a magnesium ion. This pigment was discovered in 1906, and for the first time magnesium had been detected in any living tissue [3]. Two major categories of chlorophyll exist in the photosystems of higher plants or angiosperms and they are chlorophyll a and chlorophyll b [4, 5, 6].

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Chlorophyll in certain cases is synthesized from succinyl - CoA or from glycine. But actually the immediate precursor for chlorophyll a and b is protochlorophyllide [7]. Chlorophyll a and chlorophyll b differ mainly in the composition of a side chain or structurally (in chlorophyll a it is - CH_3, in chlorophyll b it is –CHO). Generally the standard ratio of the chlorophyll a and chlorophyll b in higher plants or in angiosperms is approximately 3:1 or close to this ratio [8].

Green photosynthetic pigments chlorophyll absorbs light in the red (640 - 700 nm) and the blue - violet (400 - 500 nm) areas of the visible spectrum [9, 6]. Green light (~550 nm) is not absorbed but reflected giving chlorophyll its unique identifying coloration [8, 10, 11, 12]. There are several methods are for extraction and quantification of chlorophyll

concentrations in leaves. However, use of acetone as solvent becomes a useful one because acetone helps in giving very prominent chlorophyll absorption peaks that help in correct chlorophyll determination [13, 14]. Chlorophyll or chlorophyll products can be utilized as a photodynamic agent [4] in different herbal treatment modalities. Low chlorophyll concentration can also be used as environmental stress and pollution indicator [15].

Carotenoids or tetraterpenoids are types of pigments that are produced by plants, algae, several bacteria and fungi [16, 17]. These important pigments can be produced from oils, fats and other organic metabolic building blocks by plants, algae, several bacteria and fungi [17, 18]. Generally tetraterpenoids from foods are accumulated in the fatty tissues of animals [16]. There are more than 600 carotenoids in nature and these are divided into two major categories *viz.* xanthophylls (contains oxygen) and carotenes (purely hydrocarbons and contains no oxygen) [16, 19].

All of these bioactive molecules are derived from tetraterpenoids *i.e.* these compounds are produced from 8 different organic molecules (isoprene) and consists of 40 carbon atoms [16]. Carotenoids mainly absorb light in the 400 - 550 nm regions (violet to green light) of the visible spectrum [20]. Hence, carotenoids are yellow, orange, or red colored in nature [16, 17]. These pigments are the major and dominant pigment in autumn season.

Carotenoids or tetraterpenoids mainly play two important roles in higher plants and algae. These are a) they absorb light for use in photosynthesis, and b) they give protection to chlorophyll from photo damage [16, 18]. These bioactive molecules can show the Vitamin A activity. In case of higher plants or angiosperms, Lutein and xanthophyl are the most available carotenoids and its significant role in preventing eye problems and hence they are presently under various scientific investigations [16, 21].

Due to the masking effect of green photosynthetic pigments, common carotenoids pigments, available in mature leaves, are often not visible [22]. When green color of photosynthetic pigments is absent or not prominent due to physiological reason of plants as in autumn foliage, the yellow and orange coloration of the carotenoids are getting predominant. The concentration of chlorophyll and carotenoids may vary in different region and season-wise [23]. It may change with different environmental stress and with pollution [24, 25, 26, 27, 28]. The main focus of this present comparative study is to find out the plants which contain highest amount of total chlorophyll and total carotenoids as these two bioactive molecules has huge importance and applications in herbal treatment or in pharmaceutical industry. For this study three categories of plant, such as tree, shrub and herb [23] were taken.

MATERIALS AND METHODS:

Collection of Plants: Twenty-one medicinal plants were selected for this study. These medicinal plants are categorized in three types as Tree, Shrub and Herb. These are Tree: *Azadirachta indica* (Neem), *Moringa oleifera* (Sajne), *Ficus religiosa* (Asathha), *Polyalthia longifolia* (Devdaru), *Cocos nucifera* (Narkel), *Alstonia scholaris* (Chhatim), *Dalbergia sissoo* (Shisu) Shrub: *Cassia alata* (Dadmari), *Bougainvillea spectabilis* (Baganbilas), *Tecoma stans* (Chandraprabha), *Tabernaemontana divaricata* (Tagar), *Lantana camara* (Lanthan), *Glycosmis pentaphylla* (Ashseora), *Corchorus capsularis* (Pat) and Herb: *Ocimum sanctum* (Tulsi), *Tridax procumbens* (Bishalyakarani), *Euphorbia hirta* (Borokerui), *Cleome rutidosperma* (Nil Hurhure), *Heliotropium indicum* (Hatishur), *Commelina benghalensis* (Kanshira), *Acalypha indica* (Muktajhuri). These traditional medicinal plants were collected from West Bengal, India.

Extraction of Chlorophyll [8, 10, 11] (Arnon, 1949): 100 mg of finely cut fresh leaves were taken and grinded with 15 - 20 ml of 80% acetone. It was then centrifuged at 8000 rpm for 8 min. The supernatant was transferred and the procedure was repeated till the residue becomes colorless. The volume make up has been done up to 50 ml. The absorbance of the solution was taken at 470 nm, 645 nm and 663 nm against the solvent (80% acetone) blank. The process was followed for all the plant samples.

Estimation of Chlorophyll Content [8, 10, 26]: 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(A663) – 2.69 (A645)]*V/1000*W

Chlorophyll b (mg/gm tissue): [22.9(A645) – 4.68 (A663)]*V/1000*W

Total Chlorophyll (a + b) (mg/gm tissue): [20.21 (A645) + 8.02(A663)]*V/1000*W

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

Estimation of Carotenoids (Lichtenthaler and Wellburn Method [14, 29, 30, 31]): The amount of Carotenoids was estimated by using Lichtenthaler and Wellburn method. The same chlorophyll extract was measured at 470 nm in spectrophotometer to estimate the Total Carotenoid (xanthophylls + carotene) content. Total Carotenoids (mg / gm tissue):

C x + c = (1000A470 – 1.82Ca – 85.02Cb) / 198

Where, A = Absorbance at respective wave length, Ca= Chlorophyll-a, Cb= Chlorophyll-b

RESULTS AND DISCUSSIONS: Plants are the important source of food, shelter and obviously of life. For safe life, health and prevention of diseases plants are highly necessary [8]. Chlorophylls and carotenoids are the two important bioactive molecules in plants. These two bioactive compounds have great applications in herbal medicine [4, 19].

In this study, fresh leaves of twenty-one medicinal plants (already reported with traditionally medicinal importance) comprising tree, shrub, and herbs, were used for extraction and quantification of the main photosynthetic pigments *viz.* chlorophyll and carotenoids **Table 1**.

TABLE 1: COMPARISON OF PIGMENT QUANTITY AND RATIO BETWEEN CHLOROPHYLL - A AND CHLOROPHYLL - B AND TOTAL CHLOROPHYLL AND TOTAL CAROTENOIDS OF 21 MEDICINAL PLANTS

Type	Botanical Name	Local Name	Family	Chl-a (mg/gm tissue)	Chl-b (mg/gm tissue)	Chl- a and Chl-b ratio	Total Chl (mg/gm tissue)	Total Carotenoids (mg/gm tissue)	Total Chl and Total Carote-noids Ratio
Tree	*Azadirachta indica*	Neem	Meliaceae	2.411±0.031	0.775±0.019	3.109:1	3.186±0.049	2.059±0.027	1.548:1
	Moringa oleifera	Sajne	Moringaceae	1.318±0.011	0.624±0.016	2.111:1	1.942±0.021	1.039±0.007	1.869:1
	Ficus religiosa	Ashwattha	Moraceae	2.051±0.024	1.070±0.046	1.916:1	3.119±0.066	1.406±0.013	2.219:1
	Polyalthia longifolia	Devdaru	Annonaceae	1.104±0.019	0.592±0.028	1.864:1	1.696±0.048	0.855±0.024	1.985:1
	Cocos nucifera	Narkel	Arecaceae	3.009±0.035	1.930±0.039	1.559:1	4.939±0.072	2.759±0.038	1.790:1
	Alstonia scholaris	Chhatim	Apocyanaceae	1.345±0.013	0.770±0.023	1.747:1	2.115±0.019	0.613±0.006	3.450:1
	Dalbergia sissoo	Sishu	Fabaceae	1.916±0.015	0.860±0.017	2.228:1	2.776±0.021	0.295±0.014	9.410:1
Shrub	*Cassia alata*	Dadmari	Fabaceae	2.034±0.025	0.791±0.016	2.570:1	2.825±0.038	2.039±0.034	1.386:1
	Tabernaemontana divaricata	Tagar	Apocynaceae	1.148±0.009	0.334±0.032	3.438:1	1.482±0.036	0.811±0.014	1.828:1
	Tecoma stans	Chandaprabha	Bignoniaceae	2.411±0.008	0.917±0.018	2.630:1	3.327±0.025	1.749±0.011	1.901:1
	Bougainvillea spectabilis	Baganbilas	Nyctaginaceae	1.619±0.011	0.580±0.020	2.791:1	2.199±0.030	1.107±0.012	1.987:1
	Lantana camara	Lanthan	Verbenaceae	1.589±0.014	0.624±0.021	2.546:1	2.213±0.035	0.833±0.011	2.657:1
	Glycosmis pentaphylla	Ashseora	Rutaceae	2.272±0.014	1.520±0.015	1.495:1	3.792±0.023	1.468±0.012	2.583:1
	Corchorus capsularis	Pat	Malvaceae	1.363±0.015	0.529±0.017	2.577:1	1.891±0.013	0.599±0.019	3.157:1
Herb	*Cleome rutidosperma*	Nil / Beguni Hurhure	Cleomaceae	1.205±0.009	0.433±0.005	2.784:1	1.638±0.013	2.460±0.032	0.666:1
	Heliotropium indicum	Hatishur	Boraginaceae	3.041±0.025	0.985±0.016	3.088:1	4.025±0.019	1.922±0.022	2.094:1
	Ocimum sanctum	Tulsi	Lamiaceae	0.844±0.008	0.357±0.006	2.361:1	1.201±0.012	0.676±0.013	1.777:1
	Euphorbia hirta	Boro-keruie	Euphorbiaceae	1.997±0.044	1.056±0.082	1.891:1	3.052±0.124	1.535±0.023	1.989:1
	Tridax procumbens	Bishalyakarani	Asteraceae	0.989±0.005	0.435±0.013	2.272:1	1.424±0.017	0.724±0.007	1.967:1
	Commelina benghalensis	Kanshira	Commelinaceae	1.471±0.015	0.671±0.021	2.193:1	2.142±0.024	0.978±0.015	2.190:1
	Acalypha indica	Muktajhuri	Euphorbiaceae	1.015±0.016	0.709±0.016	1.432:1	1.724±0.022	0.513±0.014	3.361:1

± signifies the standard deviation from mean

From **Table 1**, we observed that chlorophyll a content is ranging from maximum 3.041 mg/g tissue (*Heliotropium indicum*, Herb) to minimum 0.844 mg/g tissue (*Ocimum sanctum*, Herb). Chlorophyll b content is ranging from maximum 1.930 mg/g tissue (*Cocos nucifera*, Tree) to minimum 0.334 mg/g tissue (*Tabernaemontana divaricata*, Shrub).

Total chlorophyll content is ranging from maximum 4.934 mg/g tissue (*Cocos nucifera*, Tree) to minimum 1.201 mg/g tissue (*Ocimum sanctum*, Herb). Total carotenoid content is ranging from maximum 2.759 mg/g tissue (*Cocos nucifera*, Tree) to 0.295 mg/g tissue (*Dalbergia sissoo*, Tree). From **Table 1**, maximum chlorophyll a and chlorophyll b ratio observed 3.438:1 (*Tabernaemontana divaricata*, Shrub) and minimum chlorophyll a and chlorophyll b ratio observed 1.432:1 in (*Acalypha indica*, Herb). From **Table 1**, maximum total chlorophyll and total carotenoid ratio observed is 9.410:1 (*Dalbergia sissoo*, Tree) and minimum ratio observed is 0.666:1(*Cleome rutidosperma*, Herb).

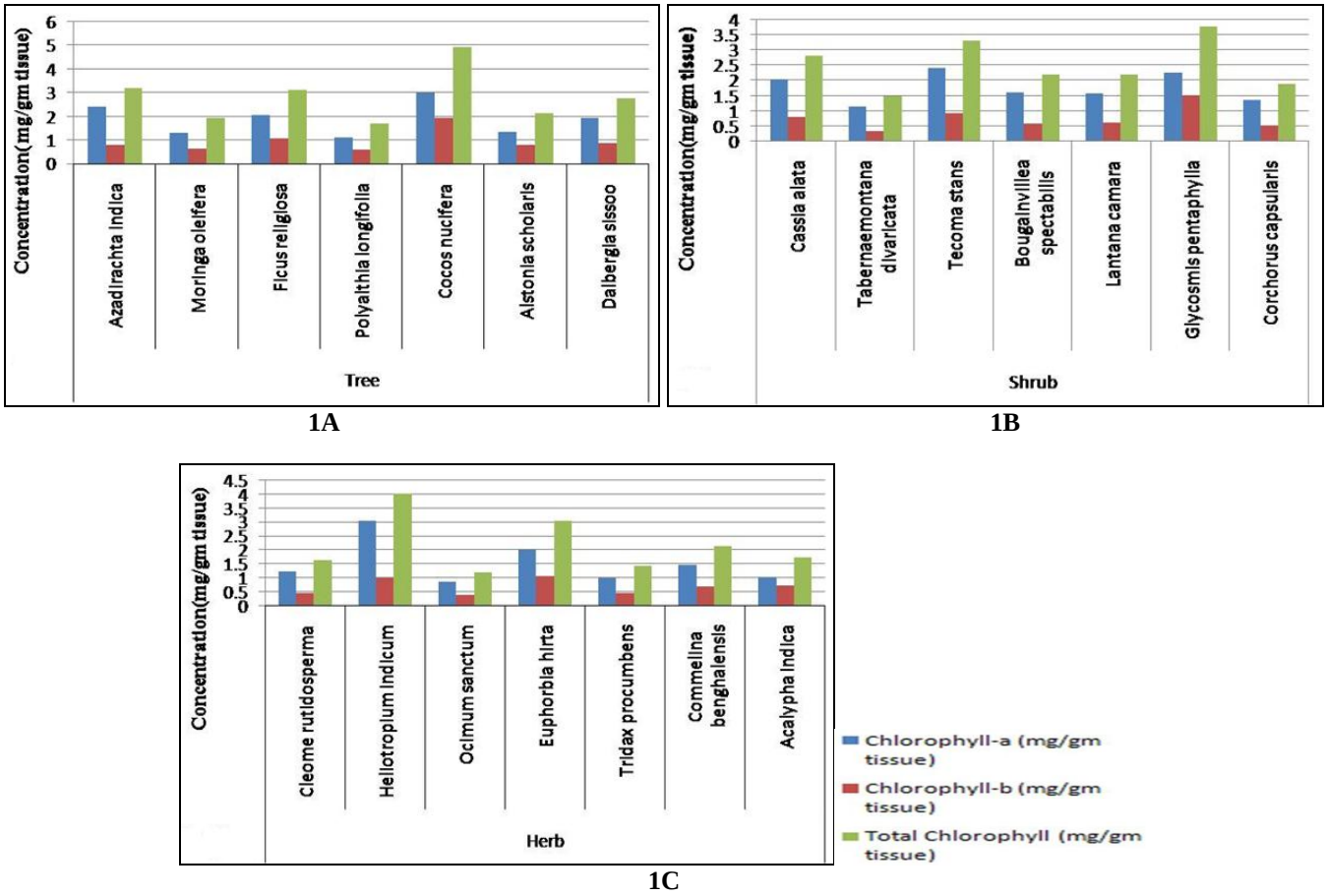


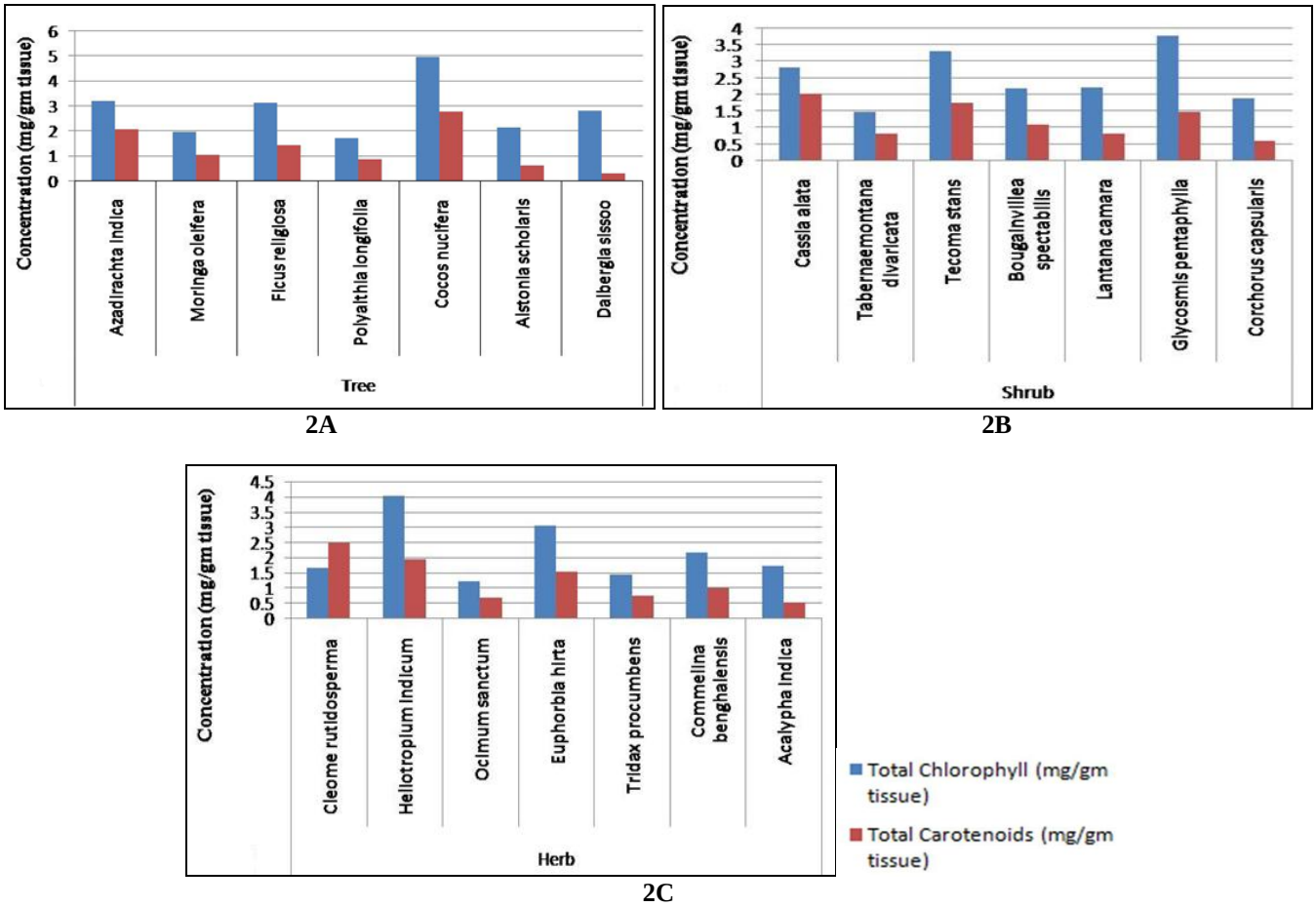
FIG. 1: CONCENTRATIONS OF CHLOROPHYLL A, CHLOROPHYLL B AND TOTAL CHLOROPHYLL (mg/g TISSUE): COMPARISON OF MEDICINAL TREES (1A), SHRUBS (1B) AND HERBS (1C)

From the **Fig. 1A**, we observed that highest chlorophyll a containing tree is *Cocos nucifera* (3.009 mg/g tissue) and lowest chlorophyll a containing tree is *Polyalthia longifolia* (1.104 mg/g tissue). From the **Fig. 1B** it is observed that highest chlorophyll a containing shrub is *Tecoma stans* (2.411 mg/g tissue) and lowest chlorophyll a containing shrub is *Tabernaemonatana divaricata* (1.148 m/g tissue). **Fig. 1C** showed that the highest chlorophyll a containing herb is *Heliotropium indicum* (3.041 mg/g tissue) and lowest chlorophyll a containing herb is *Ocimum sanctum* (0.844 mg/g tissue). Among the total 21 medicinal plants highest chlorophyll a containing plant is *Heliotropium indicum* (3.041 mg/g tissue) and the lowest chlorophyll a containing plant is *Ocimum sanctum* (0.844 mg/g tissue).

From the **Fig. 1A**, we observed that highest chlorophyll b containing tree is *Cocos nucifera* (1.930 mg/g tissue) and lowest chlorophyll b containing tree is *Polyalthia longifolia* (0.592 mg/g tissue). From the **Fig. 1B**, we observed that highest chlorophyll b containing shrub is *Glycosmis pentaphylla* (1.520 mg/g tissue) and lowest chlorophyll b containing shrub is *Tabernae-monatana divaricata* (0.333 mg/g tissue). **Fig. 1C** showed that the highest chlorophyll b containing herb is *Euphorbia hirta* (1.056 mg/g tissue) and lowest chlorophyll b containing herb is *Ocimum*

sanctum (0.357 mg/g tissue). Among the total 21 medicinal plants highest chlorophyll b containing plant is *Cocos nucifera* (1.930 mg/g tissue) and the lowest chlorophyll b containing plant is *Tabernaemonatana divaricata* (0.333 mg/g tissue).

From the **Fig. 1A**, it is observed that highest total chlorophyll containing tree is *Cocos nucifera* (4.939 mg/g tissue) and lowest total chlorophyll containing tree is *Polyalthia longifolia* (1.696 mg/g tissue). From the **Fig. 1B**, it is observed that highest total chlorophyll containing shrub is *Glycosmis pentaphylla* (3.792 mg/g tissue) and lowest total chlorophyll containing shrub is *Tabernaemonatana divaricata* (1.482 mg/g tissue). **Fig. 1C** showed that the highest total chlorophyll containing herb is *Heliotropium indicum* (4.025 mg/g tissue) and lowest total chlorophyll containing herb is *Ocimum sanctum* (1.201 mg/g tissue). Among the total 21 medicinal plants highest total chlorophyll containing plant is *Cocos nucifera* (4.939 mg/g tissue) and the lowest total chlorophyll containing plant is *Ocimum sanctum* (1.201mg/g tissue).

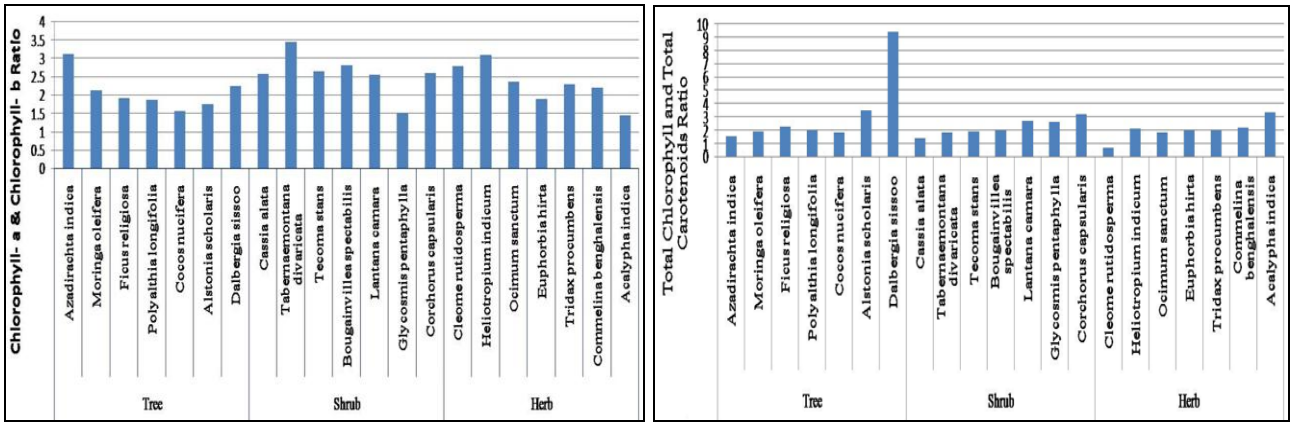


2A **2B**

2C

FIG. 2: CONCENTRATION OF TOTAL CHLOROPHYLL AND TOTAL CAROTENOIDS (mg/g TISSUE): COMPARISON OF MEDICINAL TREES (2A), SHRUBS (2B) AND HERBS (2C)

From the **Fig. 2A**, it is observed that highest total carotenoids containing tree is *Cocos nucifera* (2.759 mg/g tissue) and lowest total carotenoids containing tree is *Dalbergia sissoo* (0.295 mg/g tissue). From the **Fig. 2B**, we observed that highest total carotenoids containing shrub is *Cassia alata* (2.039 mg/g tissue) and lowest total carotenoids containing shrub is *Corchorus capsularis* (0.599 mg/g tissue). **Fig. 2C** showed that the highest total carotenoids containing herb is *Cleome rutidosperma* (2.460 mg/g tissue) and lowest total carotenoids containing herb is *Acalypha indica* (0.513 mg/g tissue). Among the total 21 medicinal plants highest total carotenoids containing plant is *Cocos nucifera* (2.759 mg/g tissue) and the lowest total carotenoids containing plant is *Dalbergia sissoo* (0.295 mg/g tissue).



3A 3B

FIG. 3: COMPARISON OF RATIO BETWEEN CHLOROPHYLL A AND CHLOROPHYLL B (3A) AND TOTAL CHLOROPHYLL AND TOTAL CAROTENOIDS (3B) AMONG 21 MEDICINAL PLANTS

The results showed in **Fig. 3A** that the highest chlorophyll a and chlorophyll b ratio containing tree is *Azadirachta indica* (3.109:1) and lowest chlorophyll a and chlorophyll b ratio containing tree is *Cocos nucifera* (1.559:1). From the same **Fig. 3A** we observed that highest chlorophyll a and chlorophyll b ratio containing shrub is *Tabernaemontana divaricata* (3.438:1) and lowest chlorophyll a and chlorophyll b ratio containing shrub is *Glycosmis pentaphylla* (1.495:1).

Fig. 3A also showed that the highest chlorophyll a and chlorophyll b ratio containing herb is *Heliotropium indicum* (3.088:1) and lowest chlorophyll a and chlorophyll b ratio containing herb is *Acalypha indica* (1.432:1). Among the total 21 medicinal plants highest chlorophyll a and chlorophyll b ratio containing plant is *Tabernaemontana divaricata* (3.438:1) and the lowest chlorophyll a and chlorophyll b ratio containing plant is *Acalypha indica* (1.432:1).

From the **Fig. 3B**, we observed that highest total chlorophyll and total carotenoids ratio containing tree is *Dalbergia sissoo* (9.410:1) and lowest total chlorophyll and total carotenoids ratio containing tree is *Azadirachta indica* (1.548:1). From the same **Fig. 3B** it is observed that highest total chlorophyll and total carotenoids ratio containing shrub is *Corchorus capsularis* (3.157:1) and lowest total chlorophyll and total carotenoids ratio containing shrub is *Cassia alata* (1.386:1).

Fig. 3B also showed that the highest total chlorophyll and total carotenoids ratio containing herb is *Acalypha indica* (3.361:1) and lowest total chlorophyll and total carotenoids ratio containing herb is *Cleome rutidosperma* (0.666:1). Among the total 21 medicinal plants highest total chlorophyll and total carotenoids ratio containing plant is *Dalbergia sissoo* (9.410:1) and the lowest total chlorophyll and total carotenoids ratio containing plant is *Cleome rutidosperma* (0.666:1).

CONCLUSION: The results clearly showed that, out of these 21 traditionally important medicinal plants total chlorophyll content is maximum in *Cocos nucifera*, tree (4.939 mg/g tissue) and is minimum in *Ocimum sanctum*, herb (1.201 mg/g tissue). The results also clearly indicated that among the total 21 medicinal plants highest amount of total carotenoids is present in *Cocos nucifera*, tree (2.759 mg/g tissue) and the lowest amount of total carotenoids is detected in *Dalbergia sissoo*, tree (0.295 mg/g tissue).

So, *Cocos nucifera* (tree) is the plant with highest amount of total chlorophyll and total carotenoids enriched leaves among these 21 traditionally used Indian medicinal plants. *Cocos nucifera* (tree) can be used for extracting chlorophyll and carotenoids for pharmaceutical industry purposes as these two photosynthetic pigments or bioactive compounds have huge medicinal applications.

The results showed that the ratio of chlorophyll a and chlorophyll b found in case of four medicinal plants species is close to 3:1; which goes well with earlier reports [8]. The four plant species are *Azadirachta indica*, tree (3.109:1) and *Heliotropium indicum*, herb (3.088:1), *Bougainvillea spectabilis*, shrub (2.791:1) and *Cleome rutidosperma*, herb (2.784:1).

The concentration of chlorophyll and carotenoids may vary with region, season and leaf conditions. Plant pigments concentration can vary depending on different species as well as by local environmental, bio-geological and bio-geochemical factors. So in this context further study is recommended.

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CONFLICT OF INTEREST: The authors are declaring that there are no conflicts of interest regarding the publication of this research article.

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