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Estimation of plant pigments concentration from tulsi (*Ocimum sanctum* Linn.): a six months study

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Abstract
Chlorophyll and Carotenoids are two very important plant pigments and are essential for living organisms. Chlorophyll used as food additive. Carotenoids give color to fruits and vegetables. These two photosynthetic pigments act as antioxidant and have the ability to fight against several types of cancer and are also prescribed in case of skin and eye problems.
The objective of this research investigation is to observe the specific time period when the concentrations of these two pigments is highest, and accordingly to propose the plant for pharmaceutical and food industry to extract these pigments for herbal product preparations for mankind.
One widely used medicinal plant *Ocimum sanctum* (Tulsi) from the family Lamiaceae was chosen for the study. In the present course of study, fresh leaves from the plant were collected for six months tenure (May, 2017 to October, 2017) and these two pigments quantified based on standard methods.
Present study showed that total Chlorophyll and total Carotenoids content is highest in October i.e.in autumn season and amount of pigments concentration was lowest in July i.e. in rainy season.
From the result it can be concluded that autumn season would be month of choice for collection and extraction of these two bioactive pigments for pharmaceutical product preparation.

Keywords: chlorophyll, carotenoids, herbal products, *Ocimum sanctum*, seasonal changes

Introduction
In South Asian countries natural atmosphere changes with the climate and it is happening in different season by many meteorological factors (temperature, precipitation, rainfall and humidity etc.). Due to this factors different physiological changes happens in case of plants. Among them plant pigment fluctuations are most important.Chlorophyll and Carotenoids play a huge role in photosynthetic process as well as in plant defence from extensive solar radiations [1,2,3].
Chlorophyll is a bioactive molecule that absorbs sunlight and use sits energy to synthesize carbohydrates from $CO_2$and water [4, 5]. This process is called as photosynthesis and this is the basisfor sustaining the physiological mechanism of all plants. Green photosynthetic pigment chlorophyll has the capacity to transfer the radiant energy of sunlight into the chemical energy of organic carbon matters in the cell [4, 6].Chlorophyll hastetrapyrole ring with a magnesium ion which is centrally located [7].It has a long hydrophobic phytol chain in its structure actually that determines whether chlorophyll moleculeis fat soluble and insoluble in water [8, 9, 10]. Mainly, two different types of chlorophyll i.e. chlorophyll a and chlorophyll b are found in higher plants. 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. Chlorophyll absorbs light in the red (640-700 nm) and the blue-violet (400-500 nm) portions of the visible spectrum. Green light (~550 nm) is not absorbed but reflected giving chlorophyll its characteristic coloration [5, 7].
There are several method sare available for extraction and estimation of chlorophyll concentrations in plant leaves. However, use of 80 % acetone as solvent becomes beneficial one. Because acetone helps to give very prominent chlorophyll absorption peaks to get accurate chlorophyll determination [11, 12, 13].
Carotenoids provide bright coloration to fruits and vegetables and serve as antioxidants, and it is an important source for vitamin A activity. Carotenoids absorb light in the blue region of the spectrum and play significant role in the organization of photosynthetic membranes. These important pigments can be produced from oils, fats and other organic metabolic building blocks by plants, algae and microbes. Variations of leaf carotenoids depend upon the seasonal climatic changes.Natural pigments isolated from marine algae have huge applications in the field of food, cosmetics and herbal medicine. Variation in the photosynthetic capacity is related with the potential to transport electrons in photo systems, and also to the variations in

specific leaf area and solar reflectance, seasonal changes as well as total chlorophyll content and chlorophyll a/b ratio.The main focus of this present research study is to find out the specific month where we can observe highest and lowest amount of total chlorophyll and total carotenoids in case of the experimental plant [14, 15,16, 17].

In this present research investigation *Ocimum sanctum* Linn. (synonym: *Ocimum tenuiflorum*) has been chosen as experimental plant because this aromatic perennial herbaceous plant has vast importance from ancient times for its ethno medicinal values. Basically this plant is native to India and thus it would be easily available for pharmaceutical industries to prepare herbal formulation using these two pigments [18, 19].

Study Site

Kolkata is the capital of West Bengal. Study site was Dum Dum which is part of North Kolkata. Dum Dum is geographically situated between22.6168^0 N of latitude and 88.4275^0 E of longitude with 9.14m above mean sea level in semiarid of eastern India. Climate of Kolkata is relatively moderate and moist except in the winter season. This region is tropical, sub-humid and distinct seasonally. It is characterized by 5seasonsi.e.summer, rainy, winter, spring and autumn. The average annual rainfalls of this metropolitan city is about 1800mm and with about 62% precipitation following during the monsoon months of June to September and an average there are 30 rainy days in an year in the district [4, 20].

Materials and Methods

Collection of Plants

Traditional medicinal plant *Ocimum sanctum* (Tulsi) was selected for this study. It was collected from Dum Dum which is part of Kolkata, West Bengal, India.

Extraction of Chlorophyll (Arnon, 1949) [7, 11, 12, 15, 17]

100 mg of finely cut fresh *Ocimum sanctum* leaves were taken and grinded with approximately 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 color less. 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) as reference.

Estimation of chlorophyll content [11, 12, 15, 17, 21]

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) [15, 17, 22, 23, 24, 25]

The concentration of Carotenoids was estimated by using well-known Lichtenthaler and Well burn method. The 80% acetone extract was measured at 470 nm in spectrophotometer to quantify the total carotenoid (xanthophylls + carotene) concentrations. Total Carotenoids (mg / gm tissue):

$$C_{x+c} = (1000A470 - 1.82C_a - 85.02C_b) / 198$$

Where, A = Absorbance at respective wave length,

C_a = Chlorophyll-a, C_b=Chlorophyll-b

Results and Discussions

Plants are the huge source of basic livelihood. For safety and prevention of diseases plants are important. Chlorophylls and carotenoids are the two important bioactive molecules in plants. These two bioactive compounds have great applications in herbal medicine [7]. Chlorophyll also shows high anti-oxidant activity [26].

In this study, fresh leaves of a traditional herb *Ocimum sanctum* (already reported with traditionally impact in social importance) were used for extraction and quantification of the main photosynthetic pigment i.e. chlorophyll and carotenoids.

Calculated Data

***Ocimum sanctum* (Tulsi)**

Table 1: Pigment concentration expressed in mg/gm tissue.

Month	Chlorophyll-a	Chlorophyll-b	Chl-a:Chl-b	Total Chlorophyll	Total Carotenoids	Total Chlorophyll :Total Carotenoids
May'17	0.902±0.0096	0.307±0.0154	2.94:1	1.210±0.0186	0.697±0.0167	0.71:1
June'17	0.953±0.0085	0.371±0.0095	2.56:1	1.323±0.0095	0.646±0.0089	2.04:1
July'17	0.754±0.0258	0.215±0.0316	3.51:1	0.969±0.0571	0.376±0.0149	2.57:1
August'17	1.23±0.0109	0.399±0.0152	3.08:1	1.627±0.0252	0.821±0.0075	1.98:1
September'17	1.0114±0.0076	0.3113±0.0165	3.25:1	1.3224±0.0176	0.5652±0.0079	2.34:1
October'17	1.912±0.0044	0.534±0.0149	3.58:1	2.446±0.0155	1.291±0.0049	1.89:1

± signifies the standard deviation from mean.

From table 1, we observed that, *Ocimum sanctum* contains highest amount of plant pigments in October month. The pigments parameters i.e. chlorophyll-a, chlorophyll-b, total chlorophyll and total carotenoids is found to be maximum in the autumn season. From the same table we observed that the lowest concentration of pigments i.e. chlorophyll-a, chlorophyll-b, total chlorophyll and total carotenoids were found in July month i.e. in rainy season. From the table 1 we also observed that the chlorophyll-a and chlorophyll-b standard ratio is maintained in case of this medicinal plant which is 3:1 or close to this [7, 15]. The highest chlorophyll-a and chlorophyll-b ratio is observed in October and it is 3.58:1. The lowest chlorophyll-a and chlorophyll-b ratio is observed in June and it is 2.56:1.

Graphs

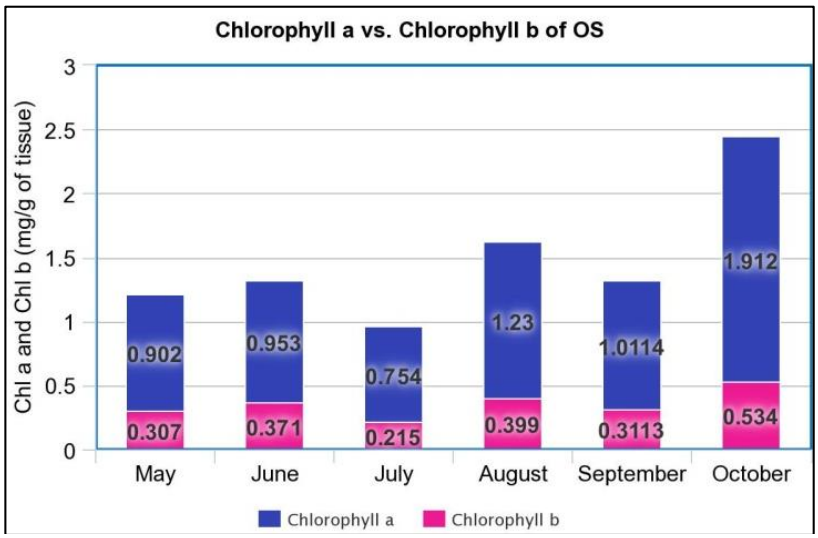

Fig 1

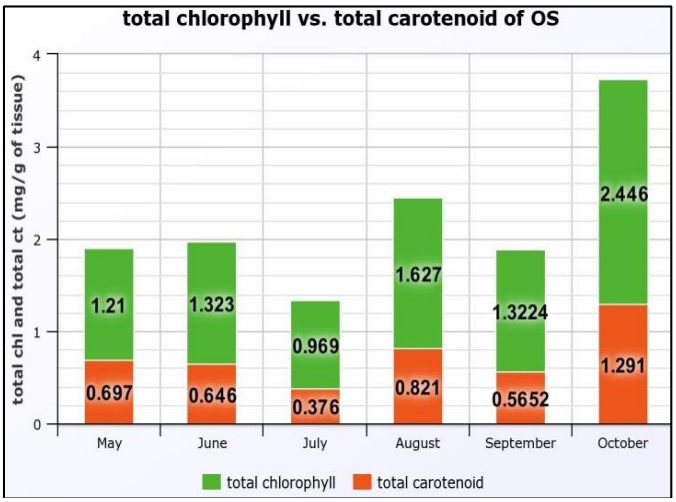

Fig 2

Figure 1, represents the comparative amount of chlorophyll-a and chlorophyll-b of *Ocimum sanctum* in a tenure of six months i.e. May, 2017 to October, 2017 which exactly covers three seasons (summer, rainy and autumn) in Kolkata, West Bengal. The highest amount of chlorophyll-a and chlorophyll-b observed in October month (autumn season) and the lowest amount of chlorophyll-a and chlorophyll-b found in July month (rainy season).

Figure 2, represents the comparative amount of total chlorophyll and total carotenoids of *Ocimum sanctum* plant in tenure of six months. The highest amount of total chlorophyll and total carotenoids observed in October month (autumn) and the lowest amount of total chlorophyll and total carotenoids found in July month (rainy season).

Conclusion

Highest amount of chlorophyll a, chlorophyll b, total chlorophyll and total Carotenoids contents were found for *Ocimum sanctum* (Tulsi) in October, 2017 and the lowest amount of chlorophyll a, chlorophyll b, total chlorophyll and total carotenoids contents are found in July, 2017. The total chlorophyll amount found in October was more than double in comparison with July month and total carotenoids amount found in October was more than triple in comparison with July month. So, we can extract these two important plant pigments in October month (Autumn season) for herbal products and other pharmaceutical or food products preparation purposes and for mankind, too. Meteorological or environmental condition may affect on the plant pigment contents. Temporal and seasonal conditions and biogeochemical situations can be the major factors for the fluctuation in plant pigment concentrations in medicinal plants [11]. In these contexts further research 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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