



ESTIMATION OF PIGMENTS IN LEAVES OF MEDICINAL PLANTS

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ABSTRACT

The Chlorophyll is the basic photosynthetic pigment of plants which is required to prepare food material in the form carbohydrates. The pigments in plant varies from organs and species to species. The estimation of amount of chlorophyll pigment was carried out in five different medicinal plants, such as *Azadirachta indica*, *Aegle marmelous*, *Pongamia pinnata*, *Santalum album*, *Ocimum sanctum*. Determination of chlorophyll content was made on the basis of values of absorbance on spectrophotometer. The amount of chlorophyll - a ranged from 2.30 mg/gm to 5.24 mg/gm of leaf tissues, chlorophyll - b ranged from 1.36 mg/gm to 2.31 mg/gm of leaf tissues. The total chlorophyll ranged from 4.08 mg/gm to 7.55 mg/gm of leaf tissues. The maximum amount of total chlorophyll was recorded in leaves of *Pongamia pinnata* and minimum in *Azadirachta indica*.

KEYWORDS: Chlorophyll, *Azadirachta indica*, *Aegle marmelous*, *Pongamia pinnata*, *Santalum album*, *Ocimum sanctum*.

INTRODUCTION

The research in plant science has increased all over the world to highlight the potential of medicinal plants used in various traditional system of medicine. The decoction or extract of medicinal plants are used in the treatment of different diseases to animals and human beings. There are many plants on earth planet used for preparation of different kinds of ayurvedic herbal medicines and cosmetic products. The leaves of medicinal plants contain photosynthetic pigment chlorophyll which possess anticancer and antioxidant properties. It is used against normal blood clotting and to check hormonal balance. The synthesis of chlorophyll pigment depends on penetration and perception of sun light.^[1] The pigments are functionally important molecules in photosynthetic plants. The proportion of pigment is dynamic in plastids. The traits of plant depends upon the presence of different forms of pigment. The green coloured pigment constitutes chlorophyll - a, chlorophyll -b, xanthophylls and carotenoids. The Leaf chlorophyll contents are parameters used as indicators in photosynthetic mechanism and metabolism of plant. The chlorophyll is an antioxidant compound stored in the chloroplast of leaves, stems, and flowers.^[2] The chlorophyll pigment plays an important role preparation of carbohydrates in presence of sunlight, water and carbon dioxide. The medicinal properties of plants depends on the concentration of chlorophyll pigment. A regular and recommended intake of natural plant pigments in the form of herbal extract keep the digestive

system healthier and provides mineral nutrients to human body. Considering the medicinal importance of pigments attempts were made to determine the amount of chlorophyll pigments in leaves of some medicinal plants.

MATERIALS AND METHODS

Collection of sample

The twigs of fresh leaves of *Azadirachta indica*, *Aegle marmelous*, *Pongamia pinnata*, *Santalum album* and *Ocimum sanctum* were collected in the month of January and February 2020 from the campus of Govt. Vidarbha Institute of Science and Humanities, Amravati, which is located at 20.93° N and 77.75°E in Vidarbha region of Maharashtra state, in India. The samples were brought to the laboratory. The fresh, healthy and green leaves were plucked and used for analysis of chlorophyll pigment.

Pigment extraction from leaves

The chlorophyll pigment was extracted with the help of organic solvent, acetone.^[3,4] The fresh leaves were taken in a glass petriplate, they were washed with tap water to remove debris and dust particles from leaf surface. The leaves were blotted to dry and cut into fine pieces. 10gm. of leaf tissues were weighed and kept in clean mortar. The leaf tissues were grinded in mortar containing 20 ml of 80 % acetone and 0.5gm MgCO₃ powder. A crush of leaves were made with the pestle and it was then homogenized uniformly. The leaf extract was then centrifuged at 5000 rpm for 5 minutes. The supernatant was transferred to volumetric flask and made final

volume 100 ml with 80% acetone. It was stored in cool place for further analysis.

Measurement of absorbance of pigment

The absorbance of chlorophyll supernatant was measured on spectrophotometer at 663 nm and 645 nm. The sample of each plant were taken separately in a clean glass cuvette and absorbance was measured against 80% acetone as blank.

Estimation of chlorophyll pigment

The estimation of pigment was made on the basis of values of absorbance obtained at two different wavelengths on spectrophotometer. The values were put in the formula and amount of chlorophyll - a, chlorophyll -b and total chlorophyll were determined.

Chlorophyll - a = $12.7 \times A_{663} - 2.69 \times A_{645}$ (gm / tissue)

Chlorophyll - b = $22.9 \times A_{645} - 4.68 \times A_{663}$ (gm / tissue)

Total chlorophyll (a + b) = $20.2 \times A_{645} + 8.02 \times A_{663} \times 0.1$ (gm / tissue)

Here, A = Absorbance of a sample at wavelength,

The amount of chlorophyll content was calculated in miligrams per gram of leaf tissues. The values were recorded in observation table.

RESULTS AND DISCUSSION

The plants are important sources of food for all living organism. They are beneficial for maintenance of good health and prevention from diseases. The five different medicinal plants were screened for determination of amount of chlorophyll pigment in leaves (Table 1). The *Azadirachta indica* is a perennial large tree with well developed branches. The branches bears medium sized elongated to oblong leaves. The leaves are green, glossy, and smooth with serrate margin. They have medicinal property and protect liver from damage. The extract of leaves is used as blood purifier. The leaves are also used as insect and pest repellent. The *Aegle marmelous* is a medium sized tree. It is considered as a sacred and holy tree. The leaf of this plant is divided into three leaflets. The leaflets are green coloured. The extract of leaflets is used as laxative and to relieve inflammation of the mucous membrane. The decoction of leaves is used in the treatment of chronic fever.

Table 1: List of medicinal plants used for estimation of chlorophyll pigment.

Sr. No.	Name of the Plant	Family	Vernacular name
1.	<i>Azadirachta indica</i> L.	Meliaceae	Bitter Neem
2.	<i>Aegle marmelous</i> L.	Rutaceae	Bel
3.	<i>Pongamia pinnata</i> L.	Fabaceae	Karanj
4.	<i>Santalum album</i> L.	Santalaceae	Chandan
5.	<i>Ocimum Sanctum</i> L.	Lamiaceae	Tulsi

The *Pongamia pinnata* is a medium sized evergreen tree. It is grown as shade tree due to its dense canopy. The leaves of this plant are broad, green, glossy and smooth. They are simple and petiolate. The extract of leaves is used to relieve pain from inflammation and constipation. *Santalum album* is a small evergreen tree. It produces dark green, simple and ovate to lanceolate leaves. The decoction of leaves is used against dandruff, lice, skin inflammation and sexually transmitted diseases. The *Ocimum sanctum* is highly aromatic perennial herb or under shrub. The leaves of this plant are simple, hairy, green and strongly scented. The leaves possess an antioxidant property and yields an aromatic essential oil. The extract of leaves is having antibacterial and antifungal property. The decoction of leaves is

stimulative and used in making herbal tea. It promotes hair growth.

The values of chlorophyll-a, chlorophyll -b and Total chlorophyll were calculated as per the formula and tabulated (Table 2). It is found that the amount of chlorophyll - a ranges from 2.30 to 5.24 mg / gm of tissue and chlorophyll- b from 1.36 to 2.31 mg /gm of tissue. The total chlorophyll content ranged from 4.05 to 7.55 mg /gm of tissue. The highest amount of total chlorophyll was found in *Pongamia Pinnata* (7.55 mg/gm of tissues). The minimum amount of total chlorophyll was noted in *Azadirachta indica* (4.08 mg/gm of tissues).

Table 2: Estimation of chlorophyll pigment in medicinal plants.

Sr. No.	Name of the plant	Chlorophyll - a (mg / gm of tissue)	Chlorophyll - b (mg / gm of tissue)	Total Chlorophyll (mg / gm of tissue)
1.	<i>Azadirachta indica</i> L.	2.30	1.78	4.08
2.	<i>Aegle marmelous</i> L.	4.12	2.10	6.22
3.	<i>Pongamia pinnata</i> L.	5.24	2.31	7.55
4.	<i>Santalum album</i> L.	4.89	2.18	7.07
5.	<i>Ocimum Sanctum</i> L.	3.14	1.36	4.5

The variation in total chlorophyll content was found in five medicinal plants. The variation may be due to some abiotic environmental factors such as sunlight, temperature, moisture and mineral nutrients in the soil. The mesophyll tissues and sunlight play a crucial role in the functioning of chlorophyll pigment during process of photosynthesis,^[5] The intensity of sunlight affects on chlorophyll content.^[6,7] The deficiency of micronutrients in soil may also affect on the concentration of chlorophyll molecules in chloroplast. The amount of chlorophyll - b was found less than chlorophyll - a. The research on separation of chlorophyll pigment in different plants are made by techniques of chromatography, such as paper chromatography, TLC and HPLC The chlorophyll - a is primary pigment with dark green colouration. The chlorophyll - b shows light green colouration. Both the chlorophyll - a and Chlorophyll - b. are an essential pigments of photosystems The xanthophyll is yellow coloured and carotene shows orange colouration. These pigments are accessory pigments in chlorophyll The presence of xanthophyll and carotene along with water constitutes the spectral characters of plant.^[8,9] The separation of accessory pigments may depends upon the size of molecules that are separating along with solvents system.

CONCLUSION

The concentration of chlorophyll content varies species to species in different plants. The variation may be due to dust pollution, geographical situation and with respect to fluctuations in season. The abiotic factors of environment affects on the pigment synthesis The rate of photosynthesis in leaves depends upon the amount of basic pigment and accessory pigments. The chlorophyll - b, xanthophyll and carotene are the supporting pigments for photosynthesis. The infection of plants due to various pathogenic microorganisms in soil or water affects and reduces the synthesis of pigment. The amount of chlorophyll content may be considered as one of the criteria for measurement of healthy and diseased condition of plant.

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