ESTIMATION OF CHLOROPHYLL a, CHLOROPHYLL b AND TOTAL CHLOROPHYLL IN MEDICINAL PLANTS

Project submitted to University of Kerala in partial fulfillment of the requirement for the Degree of Bachelor of Science in Chemistry.

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2022

DECLARATION

We hereby declare that this project work titled "**ESTIMATION OF CHLOROPHYLL a, CHLOROPHYLL b AND TOTAL CHLOROPHYLL IN MEDICINAL PLANTS** " is a bonafide record of the project work carried out under the supervision and guidance of ***Dr. Sunita Kurur, Head of the Department, Department of Chemistry, All Saints' College, Thiruvananthapuram,*** and that no part of the project report has been submitted by us for any other degree, diploma, or similar titles of any other university.

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CERTIFICATE

This is to certify that this project report titled **"ESTIMATION OF CHLOROPHYLL a, CHLOROPHYLL b AND TOTAL CHLOROPHYLL IN MEDICINAL PLANTS"** submitted by SANDRA DIXON , SANGEETHA .A ,SNEHA .L,VIDYA .S, YEMEEMA SAJI is a bonafide record of project work carried out by them under my supervision and guidance and that no part of the report has been presented for any other Degree, Diploma or any other similar title of any other University.

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ACKNOWLEDGEMENT

We record our deep sense of gratitude and indebtness to our respectful Teacher and Guide **Dr. Sunita Kurur**, Head of the Department of Chemistry All Saints' College, Thiruvananthapuram, for her sustained help, inspiring guidance and valuable suggestion to materialize this effort.

We would like to express our sincere thanks to the lab assistants for their help and support to do this project. We would like to express our heartfelt gratitude to our friends who accompanied us during the project and had given their goodwill and constructive ideas for doing this project.

Last but not the least we thank God Almighty for his benevolence and blessing.

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CONTENTS

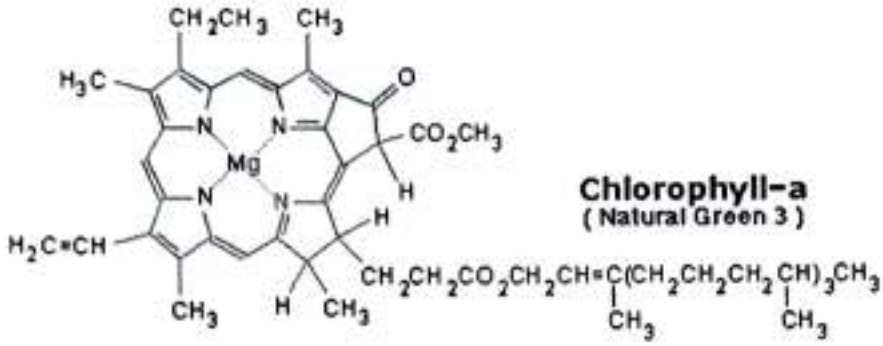
INDRODUCTION

The presence of pigments in plant tissues gives colour to them, which is different depending on variety and species. Chlorophyll is the natural compound present in green plants that gives them their colour. It helps plants to absorb energy from the sun as they undergo the process of photosynthesis. There are two types of chlorophyllin plants: chlorophyll a and chlorophyll b. All plants contain either one of these two varieties. They are both fat soluble compounds that have antioxidant properties.

CHLOROPHYLL A

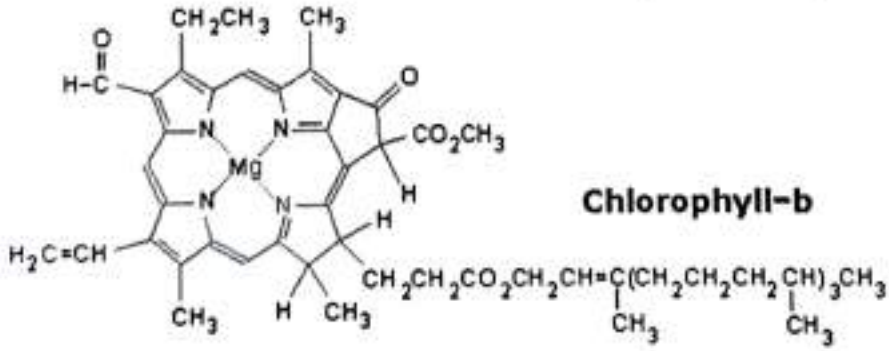
Chlorophyll a is a specific form of chlorophyll used in oxygenic photosynthesis. It absorbs most energy from wavelengths of violet-blue and orange-red light and it is a poor absorber of green and near green portions of the spectrum. Chlorophyll does not reflect light but chlorophyll containing tissues appear green because green light diffusively reflected by structures like cell walls becomes enriched in the reflected light.

The molecular structure of chlorophyll a consists of a porphyrin ring whose four nitrogen atoms surround a central magnesium atom, has several other attached side chains and a hydrocarbon tail. Side chains are attached to the porphyrin ring of the various chlorophyll molecules. Different side chains characterize each type of chlorophyll molecule and alters the absorption spectrum of light. The only difference between chlorophyll a and chlorophyll b is that chlorophyll b has an aldehyde instead of a methyl group at the C-7 position. (Raven P H, 2000)



CHLOROPHYLL B

Chlorophyll b is a form of chlorophyll. Chlorophyll b helps in photosynthesis by absorbing light energy. It is more soluble than chlorophyll a in polar solvents because of its carbonyl group. Its colour is green and it primarily absorbs blue light. In land plants the light harvesting antennae around photosystem 2 contain the majority of chlorophyll b. Hence in shade-adapted chloroplasts which have an increased ratio of photosystem 2 to photosystem 1 there is a higher ratio of chlorophyll b to chlorophyll a. This is adaptive as increasing chlorophyll b increases the range of wavelengths absorbed by the shade chloroplasts.



IMPORTANTS OF CHLOROPHYLL IN MEDICINAL PLANT

Chlorophyllin is semi-synthetic, water soluble, and not fat soluble. When you ingest chlorophyll, it moves around your body in micelles, which are molecular groups that contain fat. Studies in rodents show that chlorophyll can reduce the occurrence of cancerous tumours.

It was found that chlorophyll can form close bonds to carcinogenic chemicals called aflatoxins. When they bind the chlorophyll helps to block the absorption of the aflatoxins in the intestines. Chlorophyll also helps to prevent damage done to genes by harmful aflatoxins. Taking 100 milligram doses of chlorophyllin three times a day for four months decreased aflatoxin damage to DNA by up to 55%. Chlorophyll has antioxidant properties, even though they are not as strong as those found in other nutrients like vitamin C and vitamin E. Natural chlorophyll has no side effects and so far, only has benefits for humans.

MEDICINAL PLANTS

At present plant-based medicines are widely employed in various public health practices throughout the globe as they are safe and cost effective and efficiently combat various deadly diseases and help in maintaining good health. Herbal medicines are very commonly used in Unani, Ayurveda, Sidda, folk and other traditional practices of healthcare management. According to the estimation by the World Health Organization about 80% of people on the globe are still dependant on traditional herb-based medications due to their low-cost easy accessibility and likely negligible side effects in comparison to allopathic medicines. Certainly, many of the leading active drug molecules of plants and their derivatives used presently in allopathic medicine are mainly due to the understanding of traditional medical practices for curing diseases. Modern drug discovery research is governed by natural plant-based compounds and their products followed by synthetic chemical drugs. Currently natural products are considered as a major source of medicaments and hence they are extensively used by pharmaceutical industries. This has led towards increased global demand for medicinal plants in the modern era of natural medicine, leading to exploration and exploitation of new plant sources for their medicinal properties

COLEUS AMBOINICUS

Coleus amboinicus is a semi – succulent perennial plant in the family Lamiaceae with a pungent oregano like flavour and odour. Coleus amboinicus is considered to be native to parts of Africa the Arabian Peninsula and India. This herb has therapeutic and nutritional properties attributed to its natural phytochemical compounds which are highly valued in the pharmaceutical industry. Besides it has horticultural properties due to its aromatic nature and essential oil producing capability. It is widely used in folk medicine to treat conditions like cold, asthma, constipation, headache, cough, fever and skin diseases. The leaves of the plant are often eaten raw or used as flavouring agents, or incorporated as ingredients in the preparation of traditional food. The literature survey revealed the occurrence 76 volatiles and 30 non- volatile compounds belonging to different classes of phytochemicals such as monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids, phenolics, flavonoids, esters, alcohols and aldehydes. Studies have cited numerous pharmacological properties including antimicrobial, anti-inflammatory, antitumor, wound healing, anti-epileptic, larvicidal, antioxidant and analgesic activities. Also, it has been found to be effective against respiratory, cardiovascular, oral, skin, digestive and urinary diseases. (Paton A J *et. al.*, 2019)

NEEM

Azadirachta indica commonly known as neem is a tree in the mahogany family Meliaceae. It is one of species in the genus Azadirachta and is native to the Indian subcontinent and most of the countries in Africa. It is typically grown in tropical and semi-tropical regions. Neem trees also grow on islands in southern Iran. Its fruits and seeds are the source of neem oil. The bark, leaves and seeds are used to make medicine. Less frequently, the root, flower and fruit are also used. Neem leaf is used for leprosy, eye disorders, bloody nose, intestinal worms, stomach upset, loss of appetite, skin ulcers, diseases of the heart and blood vessels,

fever, diabetes, gum disease and liver problems. The leaf is also used for birth control and to cause abortions. The bark is used for malaria, stomach and intestinal ulcers, skin diseases, pain and fever. The flower is used for reducing bile, controlling phlegm and treating intestinal worms. The fruit is used for haemorrhoids, intestinal worms, urinary tract disorders, bloody nose, phlegm, eye disorders, diabetes, wounds and leprosy. Neem twigs are used for cough, asthma, haemorrhoids, intestinal worms, low sperm levels, urinary disorders and diabetes. People in the tropics sometimes chew neem twigs instead of using toothbrushes but this can cause illness; neem twigs are often contaminated with fungi within 2 weeks of harvest and should be avoided. The seed and seed oil are used for leprosy and intestinal worms. The stem, root, bark and fruit are used as a tonic and astringent. Some people apply neem directly to the skin to treat head lice, skin diseases, wounds and skin ulcers, as a mosquito repellent and as a skin softener. It was found that neem leaves have the highest chlorophyll content followed by curry leaves. (Henry Yule and A C Burnell, 1996)

PHYLLANTHUS URINARIA

Phyllanthus urinaria commonly called chamber bitter, gripeweed, shatterstone, stonebreaker or leafflower is a species of suffruticose and herb in the family Phyllanthaceae. Phyllanthus urinaria is an annual perennial herbal species found in tropical Asia, America, China and the Indian Ocean islands. It is used in folk medicine as a cure to treat jaundice, diabetes, malaria and liver diseases. Phytochemical investigations reveal that the plant is a rich source of lignans, tannins, flavonoids, phenolics, terpenoids and other secondary metabolites. Pharmacological activities include anticancer, hepatoprotective, antidiabetic, antimicrobial and cardioprotective effects. The whole plant, roots, fruits and leaves of phyllanthus urinaria is used for the treatment of various complications in different regions of the world. In India Phyllanthus urinaria is considered a very good diuretic and the crushed plant is used as a fish poison. (Wehtje G R, *et. al.*, 1992)

CATHARANTHUS ROSEUS

Catharanthus roseus commonly known as bright eyes, cape periwinkle, graveyard plant, Madagascar periwinkle, old maid, pink periwinkle, rose periwinkle is a species of flowering plant in the family Apocynaceae. It is native and endemic to Madagascar, but grown elsewhere as an ornamental and medicinal plant. It is a source of the drugs vincristine and vinblastine, used to treat cancer. It was formerly included in the genus Vinca as vinca rosea. In traditional medicine, the periwinkle has been used for relieving muscle pain, depression of the central nervous system also used for applying to wasp stings and to heal wounds. Its application ranges widely from the prevention of diabetes to treatment of stomach ache. The plant is exploited and studied as a medicinal plant as it was found to produce more than 100 monoterpenoid indole alkaloids that contain the two major vital cytotoxic dimeric alkaloids that are used for cancer chemotherapy treatment, also many alkaloids have a medicinal role. The compounds include the anti-cancer compounds. (Markgr, 1972, Moudi M, *et. al.*, 2013)

CLITORIA TERNATEA

Clitoria ternatea commonly known as Asian pigeonwings, bluebellvine, blue pea, butterfly pea, cordofan pea, Darwin pea, blue ternate is a plant species belonging to the family Fabaceae. It is a traditional ayurvedic medicine which has been used for centuries as a memory enhancer, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizing and sedative agent. A wide range of secondary metabolites including triterpenoids, flavanol glycosides, anthocyanins and steroids has been isolated from Clitoria ternatea linn. Its extracts possess a wide range of pharmacological activities including antimicrobial, antipyretic, anti-inflammatory, analgesic, diuretic, local anaesthetic, antidiabetic, insecticidal, blood platelet aggregation-inhibiting and for use as a vascular smooth muscle relaxing property. This plant has a long use in traditional Ayurvedic medicine for several diseases. (Oguis G K *et. al.*, 2019)

DATURA STRAMONIUM

Datura stramonium known by the common name thorn apple, jimsonweed, devil's snare is a species of flowering plant in the nightshade family Solanaceae. Its likely origin was in Central America, and it has been introduced in many world regions. It is an aggressive invasive weed in temperate climates across the world. It has frequently been employed in traditional medicine to treat a variety of ailments. It has also been used as a hallucinogen taken entheogenically to cause intense sacred or occult visions. It is unlikely ever to become a major drug pf abuse owing to effects upon both mind and body frequently perceived subjectively as being highly unpleasant, giving rise to a state of profound and long-lasting disorientation or delirium with a potentially fatal outcome. It contains tropane alkaloids which are responsible for the psychoactive effects, and may be severely toxic. Some medicinal uses of the plant are its anti-inflammatory property of all parts of the plant, stimulation of the central nervous system, respiratory decongestion, treatment of dental and skin infections, alopecia and in the treatment of toothache. Consumption of any part of the plant may result in a severe anticholinergic reaction that may lead to toxicity and occasionally cause diagnostic difficulties. Cases of poisoning have been reported after eating the berries. Death may occur from heart failure after ingesting 125 seeds, because the seed contain the highest concentration and has a rapid onset of action, thus may be potentially useful as an alternative to atropine for the treatment of the muscarinic symptoms of organophosphate toxicity and some of central anticholinergic effects.

BLACK NIRGUNDI

Nirgundi also called a five leaved chaste tree, is a potent ayurvedic plant, that possesses noteworthy therapeutic properties and heals several ailments including asthma, muscle spasms and anxiety. It is scientifically termed Vitex negundo. Nirgundi's natural habitats are chiefly

in the southern parts of Asia and Africa, being widely cultivated in the tropical environments of China, India, Indonesia, Tanzania and Madagascar. It is a deciduous shrub, usually 2 to 8 meters in height, with a brown bark and green leaves that hold five leaflets. The flowers are white or blue in colour and upon developing, give rise to succulent, oval shaped, purple fruits or drupes, with a fleshy pulp and seed in the interior. The roots, leaves, flowers, fruits and bark of the nirgundi plant are utilized in herbal concoctions in the form of oils, pastes, juices and powders, to cure disorders ranging from widely prevalent fevers to the very rare leprosy. Today this magical herb is being naturalized and propagated worldwide, including America and Australia, so the global population can reap the excellent advantages that nirgundi offers, for overall well-being. A vast array of beneficial plant- based compounds with antioxidant and anti-inflammatory traits are present in nirgundi. These include flavonoids and phenols which display cardioprotective qualities for heart wellness, besides terpenoids and organic fatty acids that are laden with calming and analgesic properties to relieve mental stress, joint pain and muscle aches. Nirgundi is also bestowed with alkaloids such as nishindine, vitricine which confer useful anticancer and antimicrobial effects on the body, thus combating tumour growths and stomach infections.

PEPEROMIA PELLUCIDA

Peperomia pellucid is an annual, shallow-rooted herb, usually growing to a height of about 15 to 45 cm it is characterized by succulent stems, shiny, heart-shaped, fleshy leaves and tiny, dot-like seeds attached to several fruiting spikes. It has a mustard like odour when crushed. The family Piperaceae comprises about a dozen genera and around 3000 species. It has been used for treating abdominal pain, abscesses, acne, boils, colic, fatigue, gout, headache, frenal disorders and rheumatic joint pain. Indians use the whole plant to stop haemorrhages. The roots are used to treat fevers and the aerial parts are used as dressing wounds. It is also used topically for skin disorders such as acne and boils. It is used to decrease uric acid levels, which is a cause

of arthritis and gout. It can be used as a decoction or eaten raw as a salad. (Ziba P I, *et.al.*, 2001).

CHROMOLAENA ODORATA

Chromolaena odorata is a tropical and subtropical species of flowering shrub in the sunflower family. it is native to the Americas, from Florida and Texas in the United States south through Mexico and the Caribbean to South America. It has been introduced to tropical Asia, West Africa, and parts of Australia. In particular, the several parts of this herb have been used to treat wounds, burns and skin infections. Furthermore, it has also been shown to possess anticancer, antidiabetic, anti-hepatotoxic, anti-inflammatory, antimicrobial and antioxidant properties. Its phytochemical components are alkaloids, flavonoids, flavanone, essential oils, phenolics, saponins, tannins, and terpenoids. The other important constituents of this plant are Eupolin, chromomoric acid, quercetagin and quercetin all of which contribute to its remedial properties.

OCIMUM TENUIFLORUM

Ocimum tenuiflorum, commonly known as holy basil, tulsi is an aromatic perennial plant in the family Lamiaceae. It is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics. Tulsi is called the queen of all herbs, it is used widely in Ayurvedic and naturopathic medicines which helps in the healing of the human body in a natural manner. Not only do tulsi leaves benefit people, but their flowers too. Tulsi, help you get rid of many health problems ranging from fever to kidney stones. Ayurvedic texts have also categorised the wonder herb as a stimulant, antipyretic and aromatic in nature. (Staples George, *et. al.*, 1999)

OBJECTIVE

Chlorophyll is a green pigment found in plants, herbs and algae. Plants use chlorophyll for getting nutrients through the process of photosynthesis and it is also used as nutrient supplement due to its health benefits. It helps to clean toxin in the body and fight infections.

Taking into consideration the health benefits of chlorophyll the objective of present work is to estimate chlorophyll a and chlorophyll b contents in some medicinal plants commonly found in Kerala using UV-visible spectroscopic method, helping in proper choice of food supplements.

REVIEW

Chlorophyll is a pigment present in photosynthetic organisms like plants, algae etc. It has light harvesting centres where the sunlight is absorbed at different frequencies. There are different types of chlorophyll based on the biochemical properties. There is a higher ratio of chlorophyll b to chlorophyll a. Chlorophyll benefits the plants in a unique and distinctive ways. The chlorophyll was extracted from the leaves of ten medicinal plants and characterized by UV-Visible spectroscopy. Concentration of chlorophyll a and b was calculated using Arnon method. Chlorophyll was first isolated and named by Joseph BienaiméCaventou and Pierre Joseph.

Gitelson A.A and et al conducted an experiment between leaf chlorophyll content and algorithms for non-destructive chlorophyll content in higher plant leaves. The method was leaf samples are collected from 6 different regions of China having wide range of colour and chlorophyll content, especially 2 years old Satsuma trees. The leaves are collected and placed in NMR reflectance spectra by using wavelength field -based spectrometer with probe. Thus, they obtained spectra range from 1100 nm to 2500nm with 6nm resolution. Thus, they calculated chlorophyll content in leaves of trees which change their colour during October. (Gitelson A. A. *et al.*, 2003)

Siwach and Gill, in 2014 found the chlorophyll content is high in adult leaves in comparison to young leaves. This is based on the fact that adult leaves are more matured than young leaves. James et al studied the mesophyll content and found young leaves have more mesophyll content, so they appear as blue grey colour while, adult leaves have low mesophyll content and they appear greener and reached conclusion adult leaves have more chlorophyll content than young. (Siwach.*et al.*, 2014)

According to US Environmental Protection agency, A personal computer can be used to calculate the chlorophyll content. The advantage is, it makes the analysis easier and reduces errors. They done the work based on the method explained by Nelson D.H in 1960 using IBM compatible PC with an RS 232C board and used Beckman DU-6 software and spreadsheet software lotus 123. After completing analysis using Spectrometer the file contain absorbance data is transferred to Lotus 123 and calculated final chlorophyll results. They transferred result to LMS after review. Thus accuracy chlorophyll content result was obtained by their work. (Nelson D.H., 1960)

Verh Internat and Verein Limnol, in their work of determination of photosynthetic pigment of algal plants shows the method using ESS spectroscopy. The algal sample is taken in a centrifuge tube and added 10 ml of acetone into it and placed in dark box. A microtip is inserted in tube and sonified for 20 sec and the tip is washed with 1ml acetone. The extract is made up by 13.0 ml acetone and placed in dark box at a 4°C cold room overnight. The clear extract is separated and placed in spectrometer and absorbance is noted. They reached a conclusion that if 750nm, 663nm 645nm, 630nm they obtained uncorrected chlorophyll and 750, 665, 663, 645 and 630 they obtained corrected and uncorrected chlorophyll. (Internat.V. *et al.*, 1956)

0.25g of leaf samples is macerated with 10ml of 80% acetone using a pestle and mortar. Extract is centrifuged for 10 minutes. The supernatant solution is transferred into a 25ml volumetric flask made up to 25 ml using 80% acetone. Colour intensity of the green pigment is read at 645nm and 663nm for chlorophyll a, chlorophyll b and total chlorophyll content respectively using spectrophotometer. The amount of chlorophyll present in the medicinal leaf samples was estimated by the method of Arnon. Absorbance was measured at 645nm and 663nm in a spectrophotometer. The chlorophyll content was determined by using Arnon's

(1949) equation Chlorophyll a = 12.7 (A 663) – 2.69 (A 645),Chlorophyll b = 22.9 (A 645) – 4.68 (A 663 ,Total chlorophyll = 20.2 (A 645) + 8.02 (A 663) (Arnon D I, 1949)

Inskeep W. P and Bloom P R measured the chlorophyll content in medicinal plants using 80% acetone and N. N DIMETHYLFORMAMIDE. They dissolved 1 gm sample of chlorophyll in DMF and passed. Into tubes containing acetone and absorbance is obtained using Beckmann's model 25 double beam spectrometer and quartz cuvettes and obtained wavelength at 618 nm to 665 nm. Thus, they calculated chlorophyll A and chlorophyll B. (Inskeep W. P and Bloom P. R., 1985)

Estimation of chlorophyll content in common household Medicinal leaves and their utilization to avail health benefits of chlorophyll was performed by Arathi K and V. Suneetha based on the method explained by S. Sadasivan and A. Manickam in 1991 on (bio chemical methods). 1g of finely cut fresh leaves with addition of 80% of acetone, centrifuged, and transferred until become colourless. The absorbance of the extract solution is calculated. The conclusion was, among the Tulsi, Neem, Mint and Curry leaves, Curry leaves have highest chlorophyll content and highest source of Chlorophyll, followed by Neem. (Arathy K and Suneetha V., 2011)

Chlorophyll content in some antiallergic medicinal plants was performed by Pawar S. G, Kamble V. M using Hiscox and Israelstam method without maceration. (Hiscrox J. D. *et. al.*, 1979) 100g leaves were washed with DW and chopped and taken in a test ube containing 10ml DWSO and incubated in water bath at 60°c for 15 min and absorbance was measured using UV double beam spectrophotometer. They concluded p. Pinnata have highest chlorophyll content among the samples and high growth potential. (Pawar S., 2015)

Optical method for estimating chlorophyll Content was performed by Madain Perez-patrico and Jorge Luis using reflectance and transmittance parameter using low-cost camera

and simple optical arrangements. This is an accurate method and it found to be useful in current industrial plantation procedures. (Péréz.M. *et al.*, 2018)

Kadam V. B, Slave S B, Sonvane M. D, Adhire P. P calculated chlorophyll content in some medicinal plants of genius Terminalia of Marathwada region in Maharashtra in (2014) using 80% Of acetone and method proposed by Duxbury and yestsch in 1956. They recorded optical densities of leaves at 480, 510, 645 and 663 nm and reached a conclusion that during summer season the leaves have high content of chlorophyll A than chlorophyll B and during winter the leaves have high content of chlorophyll B than chlorophyll A. (Kadam V. B. *et al.*, 2014)

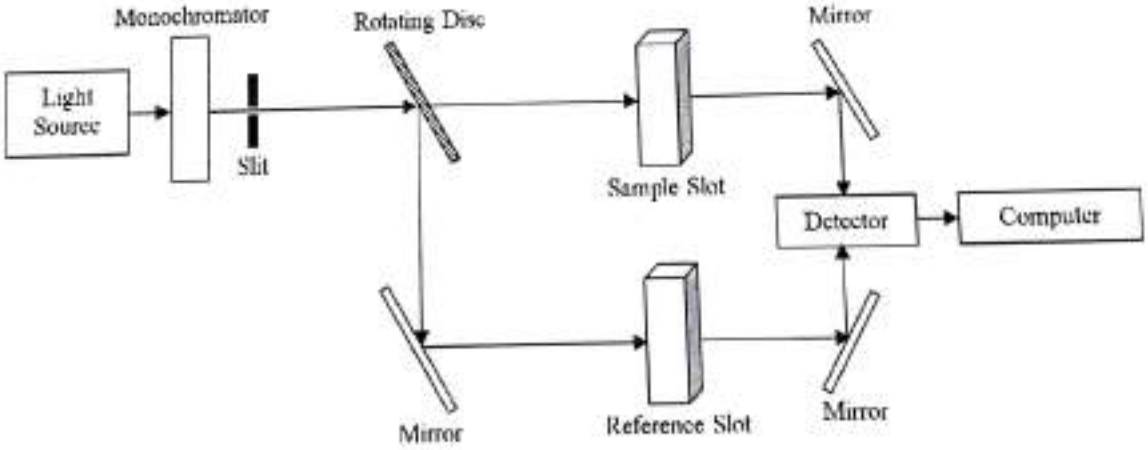
MATERIALS AND METHODS

The various types of samples were collected from the gardens and analysed for their chlorophyll a and chlorophyll b content. The medicinal plants samples collected and subjected to analysis of chlorophyll a and chlorophyll b content in the samples.

In the method adopted absorbance of samples were measured using a UV-visible spectrometer and the concentration of the samples were calculated from it.

Principles of determination of concentration using UV- Visible spectrophotometer

Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed or emitted when the molecules or atoms or ions of a sample move from one energy state to another energy state. UV-Visible spectroscopy is a type of absorption spectroscopy in which light of the ultra-violet region (200-800 nm) is absorbed by the molecule which results in the excitation of the electrons from the ground state to a higher energy state. A schematic diagram of A schematic diagram UV visible spectrometer is shown in figure below.



Spectrophotometer techniques are mostly used to measure the concentration of solutes in the solution by measuring the amount of the light that is absorbed by the solution in a cuvette placed in the spectrophotometer. The measurement of concentration using UV-Visible spectrophotometer is based on the principle of Beer Lambert Law

Beer Lambert Law

The amount of energy absorbed or transmitted by a solution is proportional to the solutions absorptivity and the concentration of the solute in sample terms, a more concentrated solution absorb more light than a more dilute solution does.

Mathematical statement of beer lambert law $A=\varepsilon lc$

Where, A= absorbance, ε= molar absorptivity or molar extinction coefficient (amount of energy absorbed per mole of substance dissolved), l= path difference (thickness of the solution), c=concentration of the solution in molarity

Principle of Estimation of chlorophyll

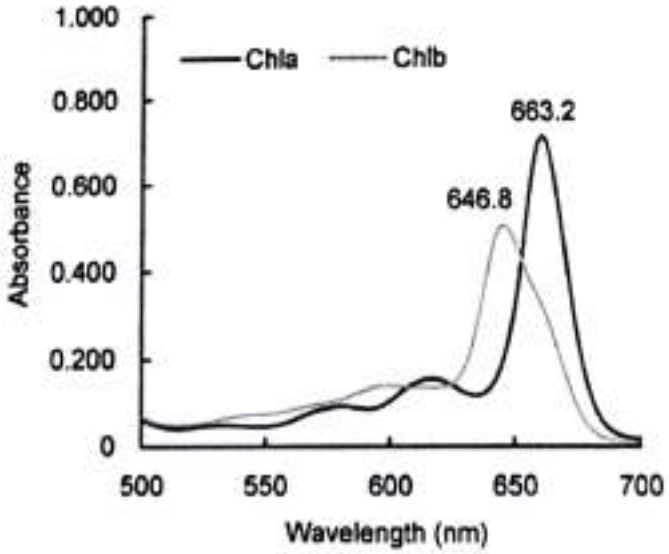
The amount of chlorophyll present in the leaf samples was estimated by the method of Arnon (1949). Absorbance was measured at 645nm and 663nm in a spectrophotometer. The chlorophyll content was determined by using the following formula.

Arnon's (1949) equation:

Chlorophyll a = 12.7 (A 663) – 2.69 (A 645)

Chlorophyll b = 22.9 (A 645) – 4.68 (A 663)

Total chlorophyll = 20.2 (A 645) + 8.02 (A 663)



Spectrum of chlorophyll

Collection of plants

SL NO	BOTANICAL NAME	COMMON NAME	FAMILY
1.	Coleus amboinicus	Indian mint	Lamiaceae
2.	Azadirachta indica	Neem	Meliaceae
3.	Phyllanthus urinaria	Chamber bitter	Phyllanthaceae
4.	Catharanthus roseus	Bright eyes	Apocynaceae
5.	Clitoria ternatea	Butterfly pea	Fabaceae
6.	Datura stramonium	Jimsonweed	Solanaceae
7.	Vitex negunda	Karinochi	Lamiaceae
8.	Peperomia pellucida	Mashithand	Piperaceae
9.	Ocimum tenuiflorum	Holy basil	Lamiaceae
10.	Chromolaena odorata	Communist pacha	Asteraceae

➢ **Samples analysed for chlorophyll a and chlorophyll b**

Coleus amboinicus (Indian mint)

Azadirachta indica (Neem)

Phyllanthus urinaria (chamber bitter)

Catharanthus roseus(Bright eyes)

Clitoria ternatea (Butterfly pea)

Datura stramonium (jimsonweed)

Vitex negunda (karinochi)

Peperomia pellucida (Mashithand)

Ocimum tenuiflorum (Holy basil)

Chromolaena odorata (communist pacha)

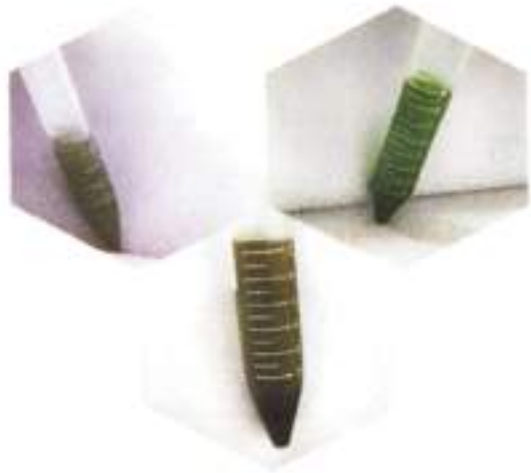
MATERIALS REQUIRED : Leaf samples, Acetone(80%), Volumetric flask, Funnel, Beaker, Spectrophotometer, Pestle and mortar.

Spectrophotometer

PROCEDURE:

Extraction of chlorophyll (Arnon, 1949)

0.25g of leaf samples is macerated with 10ml of 80% acetone using a pestle and mortar. Extract is centrifuged for 10 minutes. The supernatant solution is transferred into a 25ml volumetric flask made up to 25 ml using 80% acetone. Colour intensity of the green pigment is read at 645nm and 663nm for chlorophyll a, chlorophyll b and total chlorophyll content respectively using spectrophotometer.



RESULTS AND DISCUSSIONS

The experiment conducted was to determine the amount of chlorophyll a , chlorophyll b and total chlorophyll in various medicinal plants sample. The table given below shows the absorbance of ultraviolet and visible light by the spectrometer at two different wavelengths (645 and 663) of the various samples taken under study.

Sample Number	Absorbance at 645nm	Absorbance at 663nm
Sample 1	0.052	0.076
Sample 2	0.281	0.553
Sample 3	0.272	0.572
Sample 4	0.258	0.581
Sample 5	0.119	0.253
Sample 6	0.143	0.197
Sample 7	0.294	0.388
Sample 8	0.102	0.228
Sample 9	0.096	0.199
Sample 10	0.320	0.698

Table 1

Sample 1- COLEUS AMBONICUS

Sample 2- NEEM

Sample 3- PHYLLANTHUS URINARIA

Sample 4- WHITE FLOWER OF CATHARANTHUS ROSEUS

Sample 5- CLITORIA TERNATEA

Sample 6- JIMSON WEED

Sample 7- BLACK NIRGUNDI

Sample 8- PEPEROMIA PELLUCIDA

Sample 9- TULASI

Sample 10- CHROMOLAENA ODORATA

From table 1 obtained values the content of chlorophyll a and chlorophyll b is estimated using the proposed equation

Chlorophyll a : 12.7(A663)-2.69(A645)

Chlorophyll b : 22.9(A645)-4.68(A663)

Total Chlorophyll : 20.2(A645)+8.02(A663)

The amount of chlorophyll a , chlorophyll b and total chlorophyll present in various medicinal plants samples under study is calculated using the values of absorbance and the given formulas.

	Chlorophyll a (mg/g tissue)	Chlorophyll b (mg/g tissue)	Total chlorophyll (mg/g tissue)
Sample 1	0.82532	0.83512	1.65992
Sample 2	6.26721	3.84686	10.11126
Sample 3	6.53272	3.55184	10.08184
Sample 4	6.68468	3.18912	9.87122
Sample 5	2.89299	1.54106	4.43286
Sample 6	2.11723	2.35274	4.46854
Sample 7	4.13674	4.91676	9.05056
Sample 8	2.62122	1.26876	3.88896
Sample 9	2.26906	1.26708	3.53518
Sample 10	8.0038	4.06136	12.06196

Table 2

From the results as in table 2, the amount of chlorophyll a is highest in sample 10 (chloromolaena odoranta) with a value 8.0038mg/g which is significantly higher than that of other samples. The amount of chlorophyll a present in sample1 (Coleus Ambonicus) is too low when compared with others with a value 0.82532mg/g.

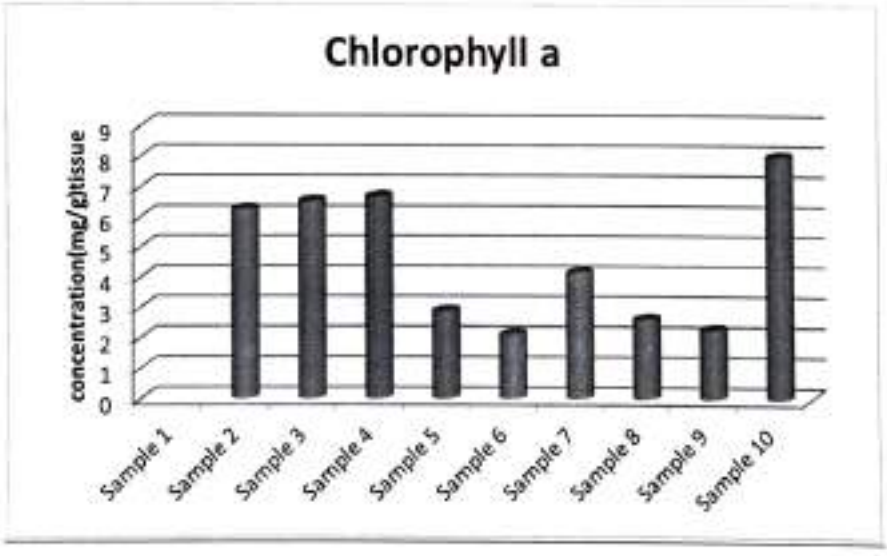
Similarly, in the case of chlorophyll b the highest value was noted in sample 7 (Black Nirgundi) which is 4.91676mg/g and followed by sample 10 (Chloromolaena Odoranta) with a value 4.06136mg/g. The values of chlorophyll b present in other samples are lower than this.

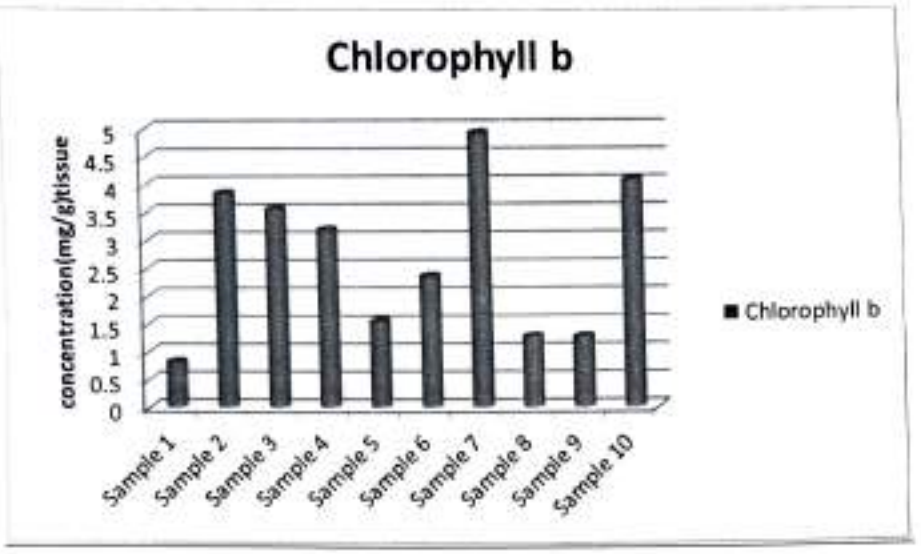
From the results the amount of total chlorophyll present in the samples under study; the highest amount is found in sample 10 (Chloromolaena Odoranta) with a value 12.06196mg/g it is found by sample 2 (Neem) with a value 10.11126mg/g and in sample 3 (Phyllanthus Urinaria) with a value 10.08184mg/gl. The total chlorophyll in other samples is lower than this.

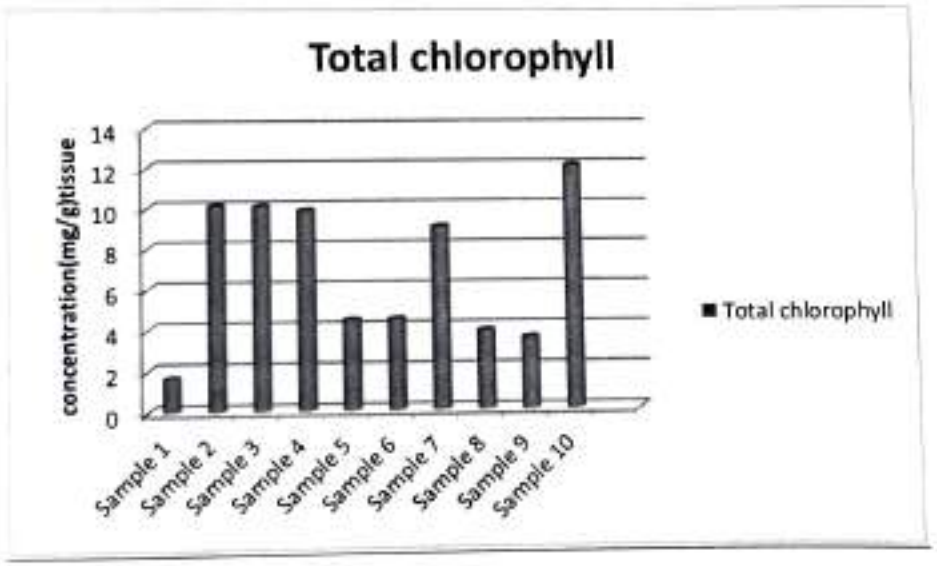
BAR GRAPHS

Y axis – Chlorophyll content in mg/g tissue

X axis – Samples




Chlorophyll b
concentration(mg/g)tissue
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6
Sample 7
Sample 8
Sample 9
Sample 10
Chlorophyll b


Total chlorophyll
concentration(mg/g)tissue
14
12
10
8
6
4
2
0
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6
Sample 7
Sample 8
Sample 9
Sample 10
Total chlorophyll

CONCLUSION

Chlorophyll is a pigment that absorbs and reflects specific wavelengths of light. Chlorophyll is found in the chloroplast of the cells. Chlorophyll a is the principal pigment involved in the photosynthesis and chlorophyll b is the accessory pigment which helps in collecting the energy for photosynthesis. Chlorophyll is used as a dietary supplement which helps in boosting immunity and to fight illness. It is also found to have certain cancer preventing properties and to treat haemoglobin deficiency disorders.

Chlorophyll benefits the body in a unique and distinctive ways. It helps to cleanse harmful toxins from the body and it also uses to fight infection. A recommended and regular intake of chlorophyll can keep the circulatory and digestive systems much healthier.

Considering the importance of these pigments as dietary supplement, a simple spectrophotometric technique was employed to estimate the content of these pigments in some selected medicinal plants. Results show that medicinal plant like Chromolaena Odoranta has high content of chlorophyll a, chlorophyll b and total chlorophyll. The chlorophylls were negligible in Coleus Ambonicus.

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