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ESTIMATION OF CHLOROPHYLL PIGMENTS AND STUDIES ON THEIR MEDICINAL PROPERTIES OF SELECTED 10 MEDICINAL PLANTS

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ABSTRACT

Leaves are a mandatory organ for higher plants which are autotraphs supply own energy needs to convert the radiant energy into chemical energy. Leaf chlorophyll content provides valuable information about physiological status of plants and mainly serves as to food production by photosynthesis. Chlorophylls are virtually essential pigments for the conversion of light energy to stored chemical energy. The absolute amounts of the pigments as well as their ratio are important physiological characteristics of the leaf, whole plant, and plant communities. Chlorophyll bears antioxidant properties which can be used in herbal medicinal drug discovery. Our objective was to estimate the chlorophyll a, b and total chlorophyll from the selected10 medicinal plants namely Acalypha indica, Croton sparsiflorus, Caesalpinia pulcherima, Cloeme viscose, Euphorbia hirta, Indicofera dinctoria, Phyllanthus nirurii, Phaseolus vulgaris, Ricinus communis, Vicia faba and phyllanthus nirurii shows the highest amount of total chlorophyll content among the other selected medicinal plants The chlorophyll content can directly determine photosynthetic potential and primary production . In addition, chlorophyll, which is a potential cancer preventative agent that has drawn significant attention recently. It is also used as a food-coloring agent and known as natural green 3(chlorophyllin), and it has the E number E141. Chlorophyll also gives an indirect estimation of the nutrient status because much of leaf nitrogen is incorporated in chlorophyll pigments mainly chl a chl b pigments.

KEYWORDS: Chlorophyll, Herbal medicine, Light energy, Pigments, Photosynthesis.

INTRODUCTION

Leaves are the most important vital plant organ that grown from the twigs, especially green colour (chlorophyll) and mainly serves as to food production by photosynthesis. Leaf chlorophyll content provides valuable information about physiological status of plants. Chlorophylls are virtually mandatory pigments for the conversion of light energy to stored chemical energy and also essential pigments of higher plant assimilatory tissues responsible for variations of color from dark green to yellow. Chlorophyll is an antioxidant compounds which are present and stored in the chloroplast of green leaf plants and mainly it is present in the green area of leaves, stems, flowers and roots.[1,2]

Chlorophyll is green pigment responsible for the colour of leaves. Its presence in leaves is crucial for photosynthesis. Chlorophyll is responsible to absorbance of green colour and other green colour portions of the spectrum. Fortunately they absorb most powerfully is the blue colour of the electromagnetic spectrum also the red colour. However the chlorophyll content has medicinal qualities. The chlorophyll is also plays important role in plant physiology and it can be act as nutrition in decline blood sugar conditions, detoxification, digestion, excretion and decreasing allergens.[2] However in using modern technique like satellite remote sensing technology being used for analysis of leaf chlorophyll concentration can also be measured. Variation in leaf chlorophyll content can provide information about the physiological condition of a leaf or plant.

Present paper records the chlorophyll content of 10 selected medicinal plant leaves. This work was an experimental study and objective of study was to analyze chlorophyll a, b and total chlorophyll content in selected medicinal plants. However the chrlophyll is very important macromolecule which indicates performance of photosynthesis and energy utilization rate. Also it gives us energy in the form of food or plant material. Chrlophyll bears antioxidant properties which can be used in a medicinal drug discovery. Biological activities

attributed to chlorophyll derivatives consistent with cancer prevention include antioxidant and antimutagenic activity. In tumour or cancer therapy chlorophyll or chlorophyll derivatives can be utilized as a photodynamic agent.[3] Chlorophyllin, chlorophyll derivative, is used as a food additive and alternative medicine. As a food-coloring agent, chlorophyllin is known as natural green 3 and has the E number E141. The major food groups contributing to dietary intake of copper complexes of chlorophylls and chlorophyllins are sugar confectionery, desserts, sauces and condiments, cheese and soft drinks. As alternative emedicine, Chlorophyll has positive effects on inflammation, oxidation, and wound healing.

Chlorophyll and chlorophyllin can form complexes structures with certain chemicals causing cancer such as aflatoxin-B1found in powders and extracts of many spices, herbs and higher plants or some heterocyclic amines found in cooked meat, or polycyclic aromatic hydrocarbons found in tobacco smoke.[4-8] The formations of these complex structures may interfere with gastro intestinal absorption of potential carcinogens, and the amounts of carcinogenic substances in susceptible tissues may be reduced.[9] A number of the studies on cancer preventative effects of chlorophyll derivatives have been done.[9,10-20] Chlorophyllin may inhibit the growth of calcium oxalate dihydrate; being considered to be a primary phase in calcium oxalate stone formation.[21-25] Therapeutic properties of chlorophyll can be summarized as followings.[15]

- Stimulating immune system.
- Benefit against sinusitis, fluid buildup, and skin rashes.
- Ability to help combat anemia.
- Eliminating molds in the body.
- Purifying the blood and the organism, cleaning it of toxins.
- Ability to help prevent cancer and is being used in cancer therapy.
- Cleaning the intestines.
- Ability to help to rejuvenate and energize the body.
- Detoxification of the liver.
- Ability to normalize blood pressure.
- Combating bad odors, bad breath as well as body odor; due to the magnesium salts that it contains.

Spectrophotometry

Chlorophyll exhibit two major light absorption bands, one on the blue side of the visible spectrum (>460nm) and one in red (630-670nm). As carotenoids, co-extracted with chlorophylls, also have strong absorption maxima in the blue, spectrophotometry measurements are limited to the red absorption bands. owing to overlapping of the main absorption bands and of secondary maxima in the range 630- 670nm, spectrophotometric procedures have been developed to determine the three chlorophylls in the same extract.

Literature Survey

Bentley Glass (1961) has stated that "Life is a photo chemical phenomenon." The ability of green plants to absorb radiant energy of sun sand to convert it into chemical energy formthe basis of all life. This conversion of light energy into chemical energy is archived with the help of pigments that are present within the chloroplast of plant cells. Main photosynthetic pigments are chlorophylls, carotinoids and phycobilins.

Blackman (1901) was the first person of discover that photosynthesis. Has a distinct dark phase in addition to the light phase and measured the temperature coefficient of photosynthesis. The temperature coefficient of a reaction is the ratio of the rate of the reaction at a given temperature to the rate at a temperature 10^0 lower.

The intermittent light experiment Warburg (1919) found that if light was supplied to a plant in short intense flashes separated by brief dark periods the photosynthetic yield per second of illumination was higher than light of the same intensity was given in a continuous manner.

Pristely (1772) discovered that photosynthesis was the reverse of respiration. The green pigment chlorophyll was isolated early in the nineteenth century and later the hypothesis that photosynthesis led to the storage of chemical energy was proposed by Rabinowitch and Govindjee, 1969.

In 1954 Arnon demonstrated that chloroplast isolated from spinach leaves assimilate CO_2 with illuminated. When sunlight strikes a chlorophyll molecule, the chlorophyll molecule absorbs light. The chlorophyll molecules could fluoresce, re-emitting the light. However, if all the chlorophyll molecules fluoresce, then the energy absorbed by the chlorophyll is lost and cannot be used to drive photosynthesis. Instead, the excited chlorophyll molecules transfer energy to chloroplasts to initiate the chemical reactions involved in photosynthesis. Hence the chloroplast quench the fluorescent emission of chlorophyll, trapping the light energy so that it can be used to drive the chemical reactions of photosynthesis.

Jeffrey and Mantoura, 1997 reported that chlorophyll a is the principal pigment in plants. In converting light energy to chemical energy, it allows photosynthesis, i.e., light –induced carbon fixation (primary production) to take place. As a biomass indicator of aquatic micro-algae that support food webs in the sea, it is probably the most frequently measured biochemical parameter in oceanography.

Boffey S.A. stated that the photosynthetic pigment are present in algae of the chlorophyll, plant and cyanobacteria. It is measured by both spectrophotometry and fluorometry indicating the abundance of photosynthetic organism in salt water and fresh water. The level of chlorophyll in fresh water is an important

factor in determining the water quality. Chlorophyll a is the common chlorophyll pigment TSWEET isolated chlorophyll from the yellow pigments by filtration through sugar, CaCo3 and other adsorbents. The different pigments were adsorbed in different coloured zones and so formed his well-known chromatogram.

Sukran Dere(1998) was found that the level of chlorophyll a in fresh water form *cladophoraglomerata* was rather than high in comparison with Ulvarigita L, codiumtomentosum and Cladostephusverticillatus Ag. The chorophyll a level was also found higher in Ulvarigit.

Indira priyadarsini et al., 2015 estimated the chlorophyll content of Tridox procumbens grown in normal and polluted region and reported that the chlorophyll content in normal and polluted regions is 2.99 mg/g respectiely.

Faisal and Anis et.al. (2006) reported higher amount of chlorophyll a and chlorophyll b in micro propagated plants of Psoraleacorylifolia compared to chlorophyll a and chlorophyll b in seedlings. The amount of chlorophyll a and chlorophyll b in normal leaf was less when compared to the regenerated leaf.

Kousar et al., (2007) extracted estimated and determined chlorophyll and different pigment in black gram leaves using different methods, the main pigments are chlorophyll a, b and phaeophytin. It was studied at different wavelengths.

Jose Francisco (2008) estimated the chlorophyll concentration in leaves of tropical wood species from Amazonian forest using portable chlorophyll meter. Non-destructive optical methods have been developed for estimation and measurement of chlorophyll concentrations in leaves.

MATERIALS AND METHODS

Collection of plant materials:

In the present study, 10 medicinal plants namely *Acalypha indica, Croton sparsiflorus, Caesalpinia pulcherima, Cloeme viscose, Euphorbia hirta, Indicofera dinctoria, Phyllanthus nirurii, Phaseolus vulgaris, Ricinus communis and Vicia faba* were selected for the experimental purposes. These species are mostly preferred to grow in tropical region. Healthy and uninfected plant species were collected at their stage of maturity and care was also taken during sampling of plant leaves to avoid mechanical injuries. Fresh leaf samples were wash thoroughly in tap water followed by distilled water in the laboratory, kept to dry in room temperature and analyzed for the determination of chlorophyll ch-a and ch-b and total chlorophyll.

Analytical procedure

Accurately weighted 0.5g fresh plant leaf sample was taken and homogenized in tissue homogenizer with 10ml of 80% ice-cold acetone. Homogenized sample mixture was centrifuge for 3000rpm for 15min. Collect the supernatant. If the pellet is green in colour add 10ml of 80% ice cold acetone and again homogenized. Repeat the above procedure until non green pellet is obtained. Collect the supernatant and measure the total volume of extract. The solution mixture was analyzed for chlorophyll-a, chlorophyll–b content in spectrophotometer and spectral absorbance for chlorophyll-a, chlorophyll-b read at the wave length of 663_{nm} and 645_{nm} respectively using acetone as blank

Calibrate at zero absorbance using a blank of 80% Acetone measure absorbance of blank and samples at 645 and 663 nm no longer than 20 minutes after extraction procedure completed. A blank of pure Acetone will be included in each run. The absorbance of this blank will be subtracted from the absorbance readings of each sample before any calculation have been made.

RESULT AND DISCUSSION

There are bunch of different equations for calculating amounts of chlorophylla, b and total chlorophyll. In the present study, Arnon's [1949] equations was choosed for the calculation of chlorophyll pigments extracted by 80% acetone.

Arnon's Equation		
1.	Chlorophyll-a[µg/mg]	$0.0127\times A_{663}$- $0.00269\times A_{645}$
2.	Chlorophyll-b[µg/mg]	$0.0229\times A_{645}$- $0.00468\times A_{663}$
3.	Total chlorophyll[µg/mg]	$0.0202\times A_{645}$- $0.00801\times A_{663}$

A= Absorbance

The presence of amount of chlorophyll pigments varying from species to species. The variations of amount of chlorophyll pigment mainly depending on the environmental conditions and climatic changes of the particular area. Chlorophylls are pigments that give color to vegetables and several fruits, where they play key roles in photosynthesis. Chlorophylls cannot be synthesized by animal tissues, though animal cells can chemically modify them for assimilation. Thus, these molecules must be obtained from foods. Several reports have demonstrated that plant pigments play important roles in human health. In fact, the potential health benefit of a diet rich in chlorophylls have been indicated in recent studies reporting their role as agents preventing some diseases.

The present study clearly focused that *phyllanthus nirurii* shows the highest amount of total chlorophyll content among the other selected medicinal plants. Fresh leaf juice with coconut milk is given as an appetizer to children. The total chlorophyll content indicating the medicinal value to human health benefits. Leaf decoction of *phyllanthus nirurii* are used in gonorrhea, jaundice and troubles of joints and urinary tract. While heating

chlorophyll easily decomposed to form a chlorophyll derivative like chlorophyllin. Chlorophyll is a good source of antioxidant nutrients. Antioxidant nutrients such as vitamins A, C and E help to neutralize harmful molecules (free radicals) in the body that can cause damage to healthy cells. Many studies support that chlorophylls and its derivatives have antioxidant properties[27-30] but some studies shown that chlorophyll was responsible for a pro-oxidant effect on the oxidation of oils.[31-33] The pro-oxidant and antioxidant properties of chlorophylls and its derivatives depend on the presence of light, when in dark medium chlorophylls and its derivatives act as antioxidant otherwise pro-oxidant.

Table. 1.

To determine concentration [µg/ml] of chl-a, chl-b,and total chlorophyll by extracting solvent acetone in spectrophotometer.				
S. No.	**Plants name**	**Chl-a**	**Chl-b**	**T.Chl**
1.	Acalypha indica	0.0081	0.0101	0.018
2.	Croton sparsiflorus	0.0069	0.0079	0.0146
3.	Caesalpinia Pulcherima	0.0056	0.0109	0.0164
4.	Cleome viscose	0.0022	0.0108	0.0129
5.	Euphorbia hirta	0.0019	0.0136	0.0153
6.	Indigofera dinctoria	0.008	0.0092	0.0152
7.	Phyllanthus nirurii	0.0053	0.0192	0.0245
8.	Phaseolus vulgaris	0.0097	0.0011	0.0084
9.	Ricinus communis	0.0013	0.0023	0.0035
10.	Vicia faba	0.0092	0.0136	0.0226

Chl-a= chlorophyll-a, Chl-b= chlorophyll-b T.chl= Total chlorophyll

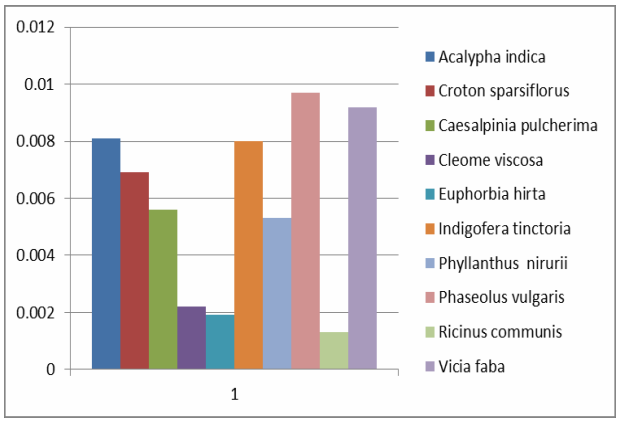

Plate. 1. Amount of chlorophyll-a in selected medicinal plants.

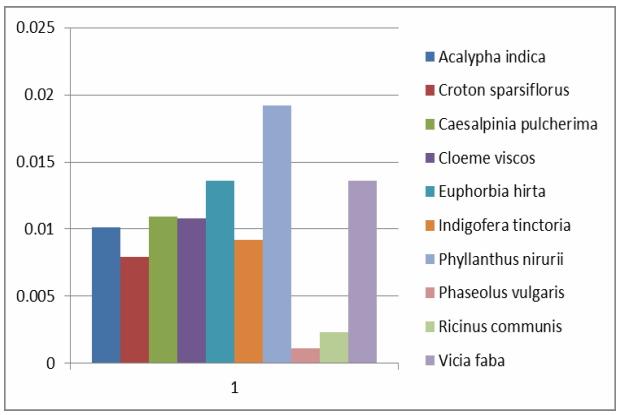

Plate. 2. Amount of chlorophyll-b in selected medicinal plants.

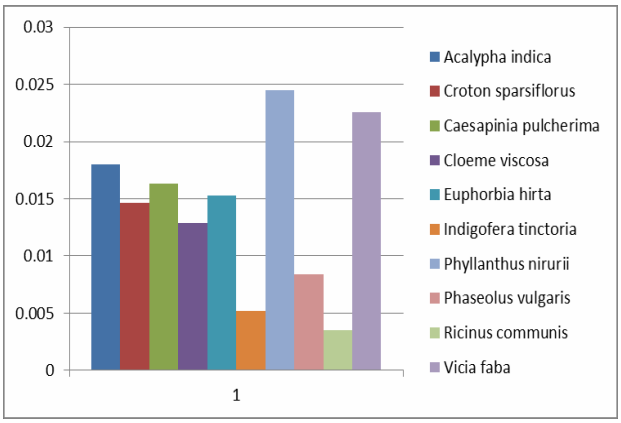

Plate. 3. Amount of total chlorophyll in selected medicinal plants.

In almost all the selected medicinal plants, the total chlorophyll content was high. However the structure of mesophyll plays a very important role in the photosynthetic process of leaves through functioning of the internal light.[34,35]

CONCLUSION

From the findings of the present study we conclude that most of the medicinal plants showed higher total chlorophyll content.(*Phyllanthus nirurii, Vicia faba and Acalypha indica)* Cholorophyll content can be used as measurement of healthiness of plant canopy and the rate of photosynthesis as well. This study will be helpful to do research in chlorophyll content analysis of various medicinal plants and their uses. Also the effect of medicinal properties of chlorophyll and chlorophyll derivatives on herbal drug discovery is an important point of study. In future, clear understanding of human health benefits of chlorophyll, will be more important in

order to create new pave of chlorophyll and chlorophyll derivatives based herbal medicines especially anticarcinogenic ailments.

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