Khulna University
Life Science School
Forestry and Wood Technology Discipline

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Title: Chlorophyll extraction of few Mangrove species (*Heriteria fomes, Exceoecaria agallocha, Bruguiera sexangula*)

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Programme: Bachelor of Science in Forestry

Chlorophyll Extraction of Few Mangrove Species (*Heritiera fomes, Excoecaria agallocha, Bruguiera sexangula*)

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KHULNA-9208
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2018

Chlorophyll Extraction of Few Mangrove Species (*Heritiera fomes, Excoecaria agallocha, Bruguiera sexangula*)

B.Sc. Thesis

By

Dolamoni Biswas

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Chlorophyll Extraction of Few Mangrove Species (*Heritiera fomes, Excoecaria agallocha, Bruguiera sexangula*)

COURSE TITLE: PROJECT THESIS

COURSE NO: FWT-4114

This thesis work has been prepared and submitted to Forestry and Wood Technology Discipline, Khulna University, Khulna, Bangladesh for the fulfillment of the 4-years professional B.Sc.(Hons.) degree in forestry.

Submitted To

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Dedicated to…….

My

Beloved Parents

Declaration

I, hereby, declare that the results submitted in this thesis are entirely the authors own investigations and this work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidate for any degree to any other university or institution.

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APPROVAL

The style and format of the project thesis submitted to Forestry and Wood Technology Discipline Khulna University, Khulna, Bangladesh, in partial fulfillment of the requirements for the 4 years' professional BSc. (Hons.) degree in Forestry has been approved.

Md. Sharif Hasan Limon

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Acknowledgement

At the very beginning, I would like to express my gratitude to Almighty God for his blessings upon me for the successful completion of this thesis paper. I am very much grateful to my parents for their continuous encouragement.

I gratefully acknowledgement to my deepest sense of gratitude to my undergraduate thesis supervisor Md. Sharif Hasan Limon, Professor, Forestry and wood technology discipline ,Khulna University, Khulna for the kind supervision and continuous support of my thesis work. Otherwise I could not come up with this endeavor.

I would also like to give my sincere gratitude to Dr. Mahmood Hossain, Professor, Forestry and wood technology discipline, Khulna University, Khulna for providing us chemical and other laboratory instruments and allow us working in his laboratory.

I also want to give my special thanks to S M Rubaiot Abdullah. Associate Professor, Forestry and wood technology discipline, Khulna University, for guiding us and his cordial support to my work.

I want to give my special thanks to my friend Mahfuza Ara Shoshi and my co-partner friend Banasri Sarker for their cordial support.

Finally I do express my thanks to all of my well-wisher.

Abstract

Chlorophyll is the green molecule in plant cells that carries out the bulk of energy fixation in the process of photosynthesis. It is actually not a single molecule but a family of related molecule. It is basically two types, named chlorophyll a and chlorophyll b. Each form of chlorophyll absorbs slightly different wavelengths of light. Extraction of chlorophyll is not so easy. There are different methods using for the extraction of chlorophyll such as using acetone, ethanol, DMF, DMSO and so on. Among them Using DMSO has been successfully used for its different advantages.

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Chapter.1

1. Introduction

1.1 Background of the study

Chlorophylls are broadly distributed in green fruits and vegetables as the primary photosynthetic pigments, and are responsible for the color of green plants. In nature, chlorophyll a and b predominate in higher plants, and the ratio of chlorophyll a to chlorophyll b in the chloroplast is 3:1. Structurally, chlorophylls are composed of a por-phyrin ring substituted with a centrally bound magnesium(Mg2+)atom and a highly hydrophobic esterified phytol tail (C_2OH_{39}).. The structural deference between chlorophylls a and b is that chlorophyll a has a –CH3 group on C7, whereas chlorophyll b has a –CHO group (Von Elbe & Schwartz, 1996; Ferruzzi& Blakeslee, 2007). Chlorophyll is the most important organic molecules for photosynthesis, which allows plants to absorb solar radiation and transfer the light energy to the reaction centre of the photo systems. Meanwhile chlorophyll concentration is a kind of indicator of photosynthetic capacity, nutritional stress, developmental stage and productivity (Yang et al., 2010; Moran et al., 2000). According to previous studies, spectroscopy analysis technology has been used to estimate the chlorophyll concentration of leaves not only in the laboratory but also in the field (Casa et al., 2015; Vincini et al., 2014; Jin et al., 2014).

Mangrove plants are well known for their productivity and faster carbon sequestration than any other tropical forests. They are inherently plastic and can change their structure at the root, leaf and stand levels in response to salinity in order to exclude salt from the xylem stream, maintain leaf hydraulic conductance, avoid cavitation and regulate water loss (e.g. suberization of roots and alterations of leaf size, succulence and angle, hydraulic anatomy and biomass partitioning). Although mangrove tree species generally prefer exposed habitats, there are considerable differences in light requirement among species during the course of forest succession (Putz and Chan 1986,Tanouchi et al 2000). Chlorophyll is a 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. Chlorophyll content varies within a species and among the species coupled with other environmental factors. The mangrove plants of the Sundarbans are least explored. Laboratory extraction of chlorophyll from mangrove plants is not available which could otherwise been helpful to identify productive efficiency of a particular species and subsequently the ecosystem quantitatively.

1.2 Objectives of the study

- To review different literature about chlorophyll extraction methods.
- To extract chlorophyll from *Heritiera fomes*, *Excoecaria agallocha*, *Bruguiera sexangula*.

Chapter.2

2. Literature Review

2.1. General description of chlorophyll

Chlorophyll is a green compound found in leaves and green stems of plants. Initially, it was assumed that chlorophyll was a single compound but in 1864 Stokes showed by spectroscopy that chlorophyll was a mixture. If dried leaves are powdered and digested with ethanol, after concentration of the solvent, 'crystalline' chlorophyll is obtained, but if ether or aqueous acetone is used instead of ethanol, the product is 'amorphous' chlorophyll.(Smith,E.L,1941)

The word chlorophyll first used in 1819.The noun chlorophyll derives from the Greek words khloros meaning "pale green" and phyllon meaning "a leaf." Plants use chlorophyll to trap energy from the sun. Without this energy, plants would be unable to initiate the process of photosynthesis, which converts water and carbon dioxide into starches that plants can use for food.

Chlorophyll is a light-absorbing pigment. It absorbs blue and red wavelengths of light and for this reason it gets its green color. The green wavelengths are reflected, giving that unmistakable color to plants.

Chlorophyll molecules are contained inside chloroplast which is the food producers of the cell found in all green parts of a plant. Inside the chloroplasts there are thylakoid membranes, which contain photo systems. Photo systems are made of a group of light-harvesting complexes, which is just a fancy term for pigment molecules and proteins. The chlorophyll molecules are arranged in and around the photo systems, and this allows them to transfer the light energy into the center of the photo system.

There is an interesting fact about chlorophyll is that its molecular structure is similar to hemoglobin, which is a critical part of human blood. The only exception is their central atom which is iron for hemoglobin and magnesium for chlorophyll. Due to this unique quality, liquid chlorophyll performs the same function in the body as the hemoglobin.(Hardison, R.C.,1996)

2.2 History of chlorophyll

- Chlorophyll was first isolated and named by Joseph BienaimeCaventou and Pierre Joseph Pelletier in 1817.
- The presence of magnesium in chlorophyll was discovered in 1906, and was the first time that magnesium had been detected in living tissue.
- After initial work done by German chemist Richard Willstatter spanning from 1905 to 1915, the general structure of chlorophyll a was elucidated by Hans Fischer in 1940.

- 1960, when most of the stereochemistry of "chlorophyll a" was known, Robert Burns Woodward published a total synthesis of the molecule.
- In 1967, the last remaining stereochemical elucidation was completed by Ian Flemingand in 1990 Woodward and co-authors published an updated synthesis

2.3 Functions of chlorophyll

- Chlorophyll in the Biosynthesis of Sugars

Plants use both forms of chlorophyll to collect the energy from light. Chlorophyll is concentrated in the thylakoid membranes of chloroplasts. Chloroplasts are the organelles in which photosynthesis takes place. The thylakoids are small sacs of membrane, stacked on top of each other. Embedded in these membranes are a variety of proteins that surround chlorophyll. These proteins work together to transfer the energy from light, through chlorophyll, and into the bonds of ATP – the energy transferring molecule of cells. ATP can then be used in the Calvin cycle or dark cycle, to create sugars.

The series of proteins that transfer energy from light and channel it into the synthesis of sugars are known as photosystems. The entire process, both light and dark cycles together, is known as photosynthesis, and occurs in plants, algae, and some bacteria. These organisms take in carbon dioxide, water and sunlight to produce glucose. They can use this glucose .In the process of cellular respiration to create ATP or they can combine the glucose into more complex molecules to be stored.(Wolff, et.al, 1960).

- Chlorophyll in the production of oxygen

A by-product of photosynthesis is oxygen. Plants can use this oxygen in cellular respiration, but they also release excess oxygen into the air. This oxygen allows many non-plants to undergo respiration as well, thereby supporting life on Earth. The oxygen is produced in the first part of the light cycle of photosynthesis. Plants split water molecules to produce electrons, hydrogen ions, and diatomic oxygen. The electrons supply the electron transport chain that drives ATP production. The oxygen is released into the air. In this way, all the oxygen we breathe is produced.

2.4 Types of chlorophyll

There are actually 2 main types of chlorophyll, named

I. Chlorophyll A
II. Chlorophyll B

Chlorophyll A

The green pigment which is responsible for the absorption of light, providing energy for oxygenic photosynthesis is called chlorophyll A. It is found in all plants, green algae, and cyanobacteria. In chlorophyll A, the most effectively absorbing wavelengths of the spectrum are 429 nm and 659 nm, which are responsible for violet-blue and orange-red colors, respectively. Chlorophyll A reflects blue-green color, which is responsible for the green color of most of the land plants. Chlorophyll A is the most important pigment in photosynthesis, which serves as the primary electron donor in the electron transport chain of photosynthesis. On the other hand, it transfers the light energy trapped in the antenna complex into the photo systems P680 and P700, where the specific chlorophylls are present in the thylakoid membrane of the chloroplast. Chlorophyll A consists of a chlorine ring, where four nitrogen atoms surround a magnesium ion. Several side chains and hydrocarbon tails are also attached to the chlorine ring. The C-7 position of the chlorine ring is attached to a methyl group in chlorophyll A.

Chlorophyll B

The green pigment which is responsible for collecting light energy and passing into chlorophyll A during photosynthesis is Chlorophyll B. It is found in plants and green algae. In chlorophyll B most effectively absorbing wavelengths of spectrum are 455nm and 642nm which are responsible for violate and red colors respectively. Chlorophyll B reflects a yellow green color. In land plants most of chlorophyll B is found light trapping antenna in photo system p-680.The structure of chlorophyll B is mostly similar to Chlorophyll A.

2.5 Benefits of chlorophyll

Because of chlorophyll, all life on Earth is possible. The first benefit of chlorophyll is sugar, produced through the process of ATP which is driven by chlorophyll. Plants, as primary producers, produce the basis of the food chain. All other organisms in the food chain rely on the sugars plants create to sustain life. While the top predators in a food chain may never eat a single plant, they most certainly eat herbivores. These herbivores only eat plants, and grow and create muscle by digesting and utilizing plant nutrients. The accumulation of these nutrients in nature would not be possible without chlorophyll. The second benefit realized by all organisms is oxygen. While chlorophyll does not produce oxygen directly, chlorophyll and the complex of proteins it is associated with transfer electrons to molecules like ATP and NADPH, which can hold energy in bonds. The need for electrons to drive this process causes water molecules to be split, creating oxygen. This oxygen is released into the atmosphere. Plants, algae, and cyanobacteria, produce all of the oxygen in the atmosphere. All other animals, and most plants, need this oxygen to survive.

2.6 Uses of Chlorophyll

- Chlorophyll is vital to the survival of both the plants and animal kingdoms due to its critical "light harvesting" role in photosynthesis.
- Chlorophyll is used as a coloring agent due to its selective absorbance of light of certain wavelengths and consequent green color.(Hosikian, et al 2010)
- The derivatives of chlorophyll are also used widely in pharmaceutical products.

Since chlorophyll stimulates tissue growth, it prevents the advancement of bacteria and speeds up the wound healing process. Chlorophyll is similar in chemical structure to hemoglobin and, as such, is predicted to stimulate tissue growth in a similar fashion through the facilitation of a rapid carbon dioxide and oxygen interchange. Because of this property, chlorophyll is used not only in the treatment of ulcers and oral sepsis but also in proctology.(Chow, H.C, Serlin. R, 1975).

2.7 Susceptibility of Chlorophyll

Stability of chlorophyll depends on storage and processing conditions such as PH, temperature, Oxygen, metals and enzymes. Depending on the conditions, there could be changes in molecular structure of chlorophyll causing discoloration that is an indicator of low quality.

2.7.1 Temperature

Chlorophyll is susceptible to heat treatment which causes some structural changes. Mild heat treatment result with the formation of chlorophyll isomers. Increased temperature or long periods of heat treatments result in the formation of pyroderivatives of chlorophyll. High temperature in a short time might be an effective approach to preserve chlorophyll.

2.7.2 pH

Chlorophyll is susceptible to low pH conditions. Chlorophyll degradation with the acidic conditions is the result of degradation of chlorophyll to pheophytin. This reaction is called pheophytinization.(Yilmaz C, Gokmen V, 2016)

Chapter 3

3. Description of the methods used for chlorophyll determination

3.1 Different Methods using for chlorophyll Determination

There are different methods which are used for the extraction and determination of chlorophyll from leaf. Some of them are described bellow

Method No: 1 Using Acetone

Samples should be collected on the day of lab, kept out of bright light, and refrigerated until just before coming to lab. Filtered samples for chlorophyll analysis may be stored up to a few weeks if they are kept indesiccators and frozen. The following procedures should be conducted in dim light.

- After shaking the sample, measure out 50 ml in a graduated cylinder and place in a small plastic bottle. Preserve with Lugol's solution. Pour the sample into one of the settling chambers provided. This sample can be used later for identifying algal species.
- Assemble the filtering apparatus using a Whatman GF/C filter. Shake raw water sample and quickly pour at least 50ml into a graduated cylinder. Note volume, and then pour into the filtering apparatus. Filter at low suction pressure. More sample can be added as long as the final volume filtered is recorded (the entire sample should be filtered if the filter paper does not clog first). While there is still about 5 ml of water left in the filter tower, add 10 ml of $MgCO_3$ suspension. Make sure that cover the filter evenly and that shake the suspension immediately before use.
- If sample is to be analyzed later, carefully fold filter in half and place in pre-cut foil labeled with: date, lake, depth, volume filtered. Place foil in container filled with desiccant in freezer.
- Acetone should be used only under a fume hood.
- Remove the filter containing the algae sample and place in a cold tissue grinder tube.
- Add 4-5 ml of 90% alkaline acetone (keep acetone on ice).
- Grind the sample filter vigorously for approximately 30 seconds while keeping the tube on ice. Then rearrange the filter if it has been compressed to the bottom of the tube.
- Grind sample for another 30 seconds. Dump contents of grinding tube into a 15ml graduated centrifuge tube.
- Rinse pestle and grinding tube with 90% acetone into the graduated centrifuge tube.
- Centrifuge tube for 15 minutes at medium speed.
- Record volume of extract in the centrifuge tube.
- Fill cuvette with extract and read absorbance at 750 (turbidity blank), 665,663, 645, and 630 nm on a spectrophotometer.

- Use acetone as a reference.
- Add 25 µl of 2N HCl to the extract, mix thoroughly and wait at least 1 minute.
- Reread absorbance at the previous wavelengths.
- Rinse out cuvette with acetone and shake dry prior to use with the next sample.

Method No: 2 Using Ethanol

- After shaking the sample, measure out 50 ml in a graduated cylinder and place in a small plastic bottle. Preserve with Lugol's solution. Pour the sample into one of the settling chambers provided. This sample can be used later for identifying algal species.
- Assemble the filtering apparatus using a Whatman GF/C filter. Shake raw water sample and quickly pour at least 50ml into a graduated cylinder. Note volume, and then pour into the filtering apparatus. Filter at low suction pressure. More sample can be added as long as the final volume filtered is recorded (the entire sample should be filtered if the filter paper does not clog first). While there is still about 5 ml of water left in the filter tower, add 10 ml of $MgCO_3$ suspension. Make sure that cover the filter evenly and that shake the suspension immediately before use
- If sample is to be analzyed later, carefully fold filter in half and place in pre-cut foil labeled with: date, lake, depth, volume filtered. Place foil in container filled with desiccant in freezer.
- Remove the filter and place it in a test tube with 15 ml 90% ethanol.
- Carefully heat the tube in a water bath (a beaker on a hot plate) to boiling (78 degree C). Allow to boil for 1 - 2 minutes.
- Place a rubber stopper lid into the test tube and thoroughly mix the contents of the tube on a vortex mixer.
- Remove the filter and centrifuge the sample at half speed for 5 minutes and transfer about 10 ml of the supernatant to the spectrophotometer cuvette.
- Zero the spectrophotometer using 90% EaOH at 665 nm, then read the transmittance of the sample at 665 nm (Eb665) and 750 nm (Eb750). The measurement at 750 nm is a correction for turbidity.
- Acidify the sample by adding 0.1 ml of 2N HCl (or 0.01 ml of 4N HCl per ml of extract) directly to the cuvette. Mix well, and after 5 minutes read the transmittance at 665 nm (Ea665) and 750 nm (Ea750).
- Using the same procedure, acidify a cuvette of 90% EtOH to see if the blank must be corrected.(Wellburn, A. R, 1994)

Method No: 3Using Dimethylformamaid (DMF)

DMF is toxic and messy. We should wear rubber gloves and work under a fume hood.

- After shaking the sample, measure out 50 ml in a graduated cylinder and place in a small plastic bottle. Preserve with Lugol's solution. Pour the sample into one of the settling chambers provided. This sample can be used later for identifying algal species.
- Assemble the filtering apparatus using a Whatman GF/C filter. Shake raw water sample and quickly pour at least 50ml into a graduated cylinder. Note volume, and then pour into the filtering apparatus. Filter at low suction pressure. More sample can be added as long as the final volume filtered is recorded (the entire sample should be filtered if the filter paper does not clog first). While there is still about 5 ml of water left in the filter tower, add 10 ml of $MgCO_3$ suspension. Make sure that cover the filter evenly and that shake the suspension before use. If sample is to be analyzed later, carefully fold filter in half and place in pre-cut foil labeled with: date, lake, depth, volume filtered. Place foil in container filled with desiccant in freezer.
- Fold filter and place in a small amber vial with 7 ml of DMF. Make sure filter is submerged in solvent.
- Place in freezer for at least 12 hours
- Heat vials in degree C water bath for 15 minutes. Allow vials to cool before handling.
- Gently shake samples for 15-20 seconds. Pour contents of vial into a graduated centrifuge tube. Rinse filter inside vial and inside vial walls with DMF. Shake for 5 seconds. Pour into centrifuge tube. Repeat rinse.
- Record centrifuge tube volume and then centrifuge for 10 minutes at medium speed.
- Pour contents of tube into a cuvette and read on spectrophotometer or fluorometer.
- Add one drop of 10% HCl to the cuvette, mix and read again.
- Dispose of DMF waste in a labeled container.(Suzuki, R & Ishimaru T, 1990)

Calculations

Convert all transmittance to absorbance, to be used in the following equations:

- kChla µg/l = 29.6[(Eb665 - Eb750) - (Ea665 - Ea750)] * ev/(V * P)
- Phacophytin = (20.8 * Ea665 * ev/V) - Chla

Where,

- Ea= absorbance, acidified
- Eb = absorbance, base
- ev = volume of alcohol used in extraction, in ml
- V = volume of filtered sample, in liters

- P = path length (Note that this test assumes use of a 1 cm path length)

Method No 4:Using DMSO

DMSO is a hazardous substance. Before handling, ensure nitrile gloves, lab coat and safety glasses are worn. All work with DMSO must take place under a fume hood, as DMSO is an irritant if inhaled. DMSO is a C1 combustible liquid. It should not be used or stored near any source of ignition and should be stored well away from oxidising agents. DMSO is not a dangerous good.

- Add 1.0 mL DMSO to each Eppendorf containing maccrated leaf.
- Place eppendorf tube into matrix mill and mix. Extract at 30 Hz for 2 minutes.
- Centrifuge and remove supernatant.
- Add 1.0 mL of DMSO to pellet and re-extract.
- Centrifuge, remove supernatant and add to other 1.0 mL

Determination by using spectrophotometer

- Calibrate at zero absorptance using a blank of pure DMSO.
- Measure absorbance of blank and samples at 645 and 663 nm no longer than 20 minutes after extraction procedure completed.

A blank of pure DMSO will be included in each run. The absorbance of this blank will be subtracted from the absorbance readings of each sample before any calculations have been made. (Arnon DI, 1949,Hiscox JD, Israelstam,GF, 1979, Richardson AD, et al, 2002).

Calculations

There are a lot of different equations for calculating amounts of chlorophyll. Among them Arnon's (1949) equations for calculation of chlorophyll extracted in 90% acetone were proven by Hiscox&Israelstam (1979) to be virtually identical to chlorophyll extracted in DMSO.

Arnon's (1949) equations are as follows.

$$\text{Chla (g l-1)} = 0.0127\ A663 - 0.00269\ A645$$

$$\text{Chlb (g l-1)} = 0.0029\ A663 - 0.00468\ A645$$

$$\text{Total Chl (g l-1)} = 0.0202\ A663 + 0.00802\ A645$$

Chapter.4

4. Materials and method

4.1 Sample collection

We collected our sample from the mangrove area. Some samples were collected from BatiaghataUpazilla under Khulna district and some were collected from DakopUpazilla. The species that I used are-

a) Gewa (*Excoecaria agallocha*): This small tree species may grow up to 15 m high. Trees are either male or female. Male flower form drooping tassels, while female flowers appear as shorter spikes. The fruit is a small dark capsule. The milky latex of Excoecaria*agallocha*is very poisonous and powerfully irritant. Its contact with skin causes irritation and rapid blistering and slight contact with eyes can cause temporary blindness.

b) Kakra (*Bruguiera sexangula*): This is a mangrove shrub or tree usually growing up to 15m, occasionally 30m in height. The flowers have a pale yellow- green to pinkish-orange calyx with 12-14 lobes, 20-24 stamens and 10-12 creamy –orange, bi-lobed petals. The green, ciger –shaped viviparous propagule grows from within the calyx and is 5-12 cm long and 1-2 cm wide.

c) Sundri (*Heritiera fomes*): It is the dominant mangrove tree species of the Sundarbans. It is a medium sized evergreen tree growing to a height of 15 to 25 meters. The trunk has few large branches and the canopy is open. The roots are shallow and spreading and send up pneumatophores. The fruit carpels are up to 5cm long and 3.8cm wide.

Fig.4.1:*Excoecaria agallocha*

Fig.4.2:*Bruguiera sexangula*

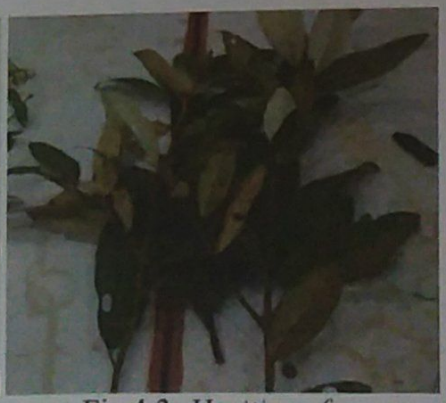

Fig.4.3: *Heritiera fomes*

Chemical:

Dimethylsulfoxide (DMSO): DMSO is an aprotic solvent with amphiphilic properties. It has been successfully used for the extraction of chlorophyll. It has been used as an industrial solvent since the mid-1800s. From about the mid-20th century, researchers have explored its use as an anti-inflammatory agent.

Instrument:

Spectophotometer: It measures the reflection or transmission properties of a material as a function of wavelength.

Method

- At first In laboratory we pre – heated the water bath at 65° C
- Then we take 7 ml of DMSO in a glass vials and put into the preheated water of water bath for 5 minutes.
- Then we take three disks from each leaf sample, weight them and put them into the glass vials.
- After the extractions, sample was removed from the water bath and then topped to 10 ml.
- Then the spectrophotometer was calibrated to zero absorbance using a blank of pure DMSO.
- Then we measured the absorbance of both blank and sample at 645 and 663 nm wavelengths.

Fig: Extracted chlorophyll

Time is a critical factor during chlorophyll extraction and it may vary with species. So, it is necessary to give trail to different time for extraction. There were three species and six treatment from every species.

For the proper measurement and result the glass vials were washed and dried before using.

Chapter.5

5. Result Discussion

Total chlorophyll for *H.fomes, E.agallocha and B sexangula* were found as 0.013573 gl/l, 0.009651 gl/l and 0.01043 gl/l respectively. (Table 1). Among these three species, *H.fomes* has the highest amount of total chlorophyll and the lowest amount was found for *E.agallocha*. There was significant variation of total chlorophyll among the species (Table 2 and Table 3, Fig-1). H.fomes is a shade tolerant species. As it is a climax species of Sundarban. So, the amount of chlorophyll is higher than other two species.

Table-1: Chlorophyll content in the leaves of *H.fomes,E.agallocha,B.sexangula*

Species	Chla(gl-1)	Chlb(gl-1)	Total Chl(gl-1)
H.fomes	0.009931	0.003645	0.013573
E.agallocha	0.007122	0.002306	0.009651
B.sexangula	0.008131	0.002531	0.01043

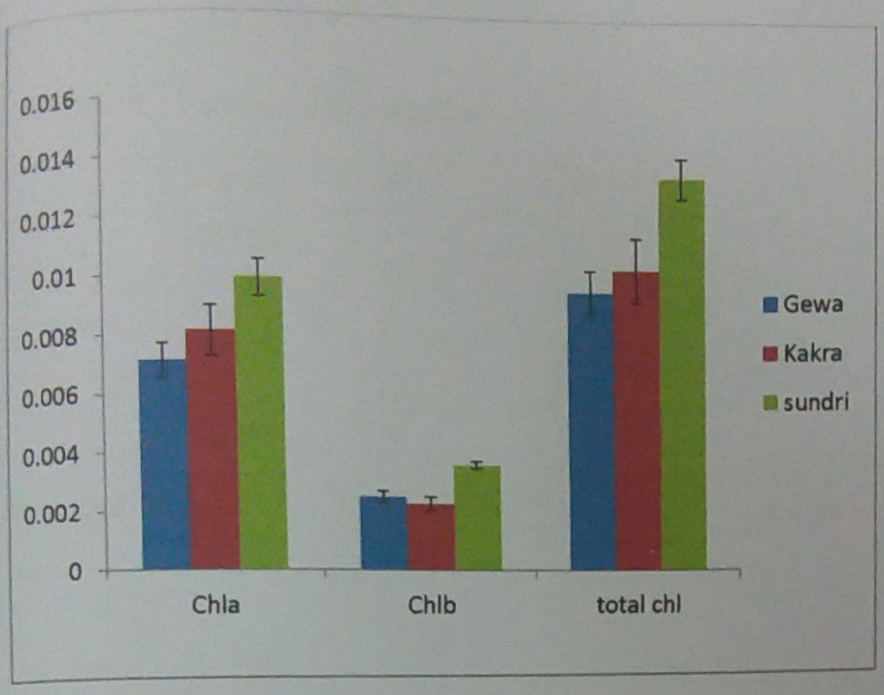

Fig-1:Chl a, Chl b and Total chlorophyll of *H.fomes, E.agallocha, B.sexangula*

Chlorophyll a and Chlorophyll b

Chlorophyll a was found to be the highest for *H.fomes* (0.009931 gl/l) and lowest for *E.agallocha* (0.007122 gl/l) .Whereas, chl b was found highest for *H.fomes* (0.003645 gl/l) and lowest for *E.agallocha* (0.002306 gl/l). One way analysis of variance found to be significantly different ($p<0.05$) (Table 2).The amount of chl a in *H.fomes* was significantly ($p<0.05$) (Table 2) different from *E.agallocha* and *B.sexangula*.

Table 2 : Variance Comparisons

	Source	DF	Adj SS	Adj MS	F-value	P-value
Chl-a	Species	2	0.000022	0.000011		
	Error	14	0.00004	0.000003	3.81	0.048
	Total	16	0.000062			
Chl-b	Species	2	0.000005	0.000003		
	Error	14	0.000003	0	12.01	0.001
	Total	16	0.000009			
Total Chl	Species	2	0.000046	0.000023		
	Error	14	0.000061	0.000004	5.23	0.02
	Total	16	0.000107			

Table 3: Tukey Pairwise Comparisons

	Species	N	Mean	Grouping	
Chl-a	*H.fomes*	5	0.009931	A	
	B.sexangula	6	0.008131	A	B
	E.agallocha	6	0.007122		B
Chl-b	*H.fomes*	5	0.003645	A	
	B.sexangula	6	0.002531		B
	E.agallocha	6	0.002306		B
Total chl	*H.fomes*	5	0.013573	A	
	B.sexangula	6	0.01043	A	B
	E.agallocha	6	0.009651		B

Chapter.6

6. Conclusion

The average amount of chl-a is greater than chl-b. Among the three species Sundri is a climax species of Mangrove. This is why the extraction amount of Sundri was better other than two species.

Chapter.7

7. Recommendation

Other Chemical such as DMF, acetone etc may be used. It may show different variations among the species.

Chapter.8

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