CHAPTER IV

LIGHT HARVESTING COMPLEXES

AS

SENSITIZER

1. Light harvesting complexes

Light harvesting complexes (LHCs) are essential components of photosynthesis in plants, algae, and cyanobacteria. They are responsible for capturing and transferring light energy to the reaction centers, where it is converted into chemical energy.

1.1. Isolation of LHCs

The isolation of LHCs is crucial for studying their structure, function, and interactions with other components of the photosynthetic machinery. Below listed are the few methods adopted by past researchers:

1.1.1. Solubilization with Detergents

One of the most commonly used methods for LHC isolation is solubilization with detergents (Bell et al. 2015). This method involves solubilizing the thylakoid membranes, which contain the LHCs, with a mild detergent such as dodecyl maltoside or digitonin. The solubilized LHCs can then be purified using chromatographic techniques such as size-exclusion chromatography or ion-exchange chromatography (Connelly et al. 1997).

The solubilization process can be optimized by varying the detergent concentration, temperature and pH. However, it is important to note that excessive detergent can lead to denaturation or aggregation of the LHCs. (Ridder et al. 2005) Moreover, the choice of detergent is critical, as different detergents can have varying effects on the stability and activity of the LHCs (Barros et al. 2009). For example, non-ionic detergents such as Triton X-100 can cause dissociation of the LHCs, while ionic detergents such as sodium dodecyl sulfate (SDS) can denature the complexes.

1.1.2. Isolation with Organic Solvents

Another method for LHC isolation is extraction with organic solvents such as acetone or methanol. This method involves grinding the plant tissue in a solvent, followed by centrifugation to remove the insoluble debris. The resulting supernatant contains the LHCs, which can be further purified using chromatography.

The advantage of this method is that it is simple and does not require the use of detergents. However, it can lead to the loss of some LHCs due to their insolubility in organic solvents. Moreover, the extraction process can cause damage to the LHCs, leading to their denaturation or aggregation. (Schmid et al. 1997)

1.1.3. Isolation with Chloroform-Methanol

A modification of the organic solvent extraction method is the chloroform-methanol extraction method. This method involves grinding the plant tissue in a chloroform-methanol mixture, followed by centrifugation to remove the insoluble debris. The resulting supernatant contains the LHCs, which can be further purified using chromatography. The advantage of this method is that it is more efficient than the organic solvent extraction method, as it can extract a wider range of LHCs. Moreover, the chloroform-methanol mixture can stabilize the LHCs and prevent their denaturation or aggregation. (Ezban et al. (2014)

1.1.4. Isolation with Non-Aqueous Solvents

A more recent method for LHC isolation is extraction with non-aqueous solvents such as hexane or heptane. This method involves grinding the plant tissue in a non-aqueous solvent, followed by centrifugation to remove the insoluble debris. The resulting supernatant contains the LHCs, which can be further purified using chromatography. The advantage of this method is that it can extract a wider range of LHCs than the detergent or organic solvent extraction methods.

1.1.5. Isolation with Aqueous Two-Phase Partitioning

Aqueous two-phase partitioning is a method that uses two immiscible aqueous solutions to separate the LHCs from other proteins in the thylakoid membranes. This method involves mixing a polymer such as polyethylene glycol (PEG) with a salt such as potassium phosphate, resulting in the formation of two phases. The thylakoid membranes are then added to the mixture, and the LHCs partition into one of the two phases based on their hydrophobicity. The advantage of this method is that it is gentle and does not require the use of detergents or organic solvents. Moreover, it can selectively isolate specific LHCs based on their hydrophobicity.

1.1.6. Isolation with Immunoprecipitation

Immunoprecipitation is a method that uses antibodies to selectively isolate specific LHCs from the thylakoid membranes. This method involves incubating the thylakoid membranes with an antibody that recognizes a specific LHC, followed by the addition of protein A or protein G beads. The beads bind to the antibody-antigen complex, allowing for the isolation of the LHCs. The advantage of this method is that it is selective and can isolate specific LHCs with high purity. However, it requires the generation of specific antibodies for each LHC of interest.

1.1.7. Isolation with Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE)

Blue native polyacrylamide gel electrophoresis (BN-PAGE) is a method that separates the LHCs based on their size and charge. This method involves solubilizing the thylakoid membranes with a mild detergent such as digitonin, followed by electrophoresis on a polyacrylamide gel containing a blue dye. The blue dye binds to the LHCs, allowing for their visualization and isolation. The advantage of this method is that it can isolate intact LHC complexes with their associated pigments and cofactors. Moreover, it can separate the LHCs based on their size and charge, allowing for their identification and characterization. (Croce et al. 2002)

1.1.8. Isolation with Affinity Chromatography

Affinity chromatography is a method that uses a ligand to selectively bind to a specific LHC, allowing for its isolation. This method involves immobilizing the ligand on a chromatography column, followed by the addition of the thylakoid membranes. The LHCs that bind to the ligand are retained on the column, while the other proteins are washed away. The advantage of this method is that it is selective and can isolate specific LHCs with high purity. However, it requires the generation of specific ligands for each LHC of interest.

In conclusion, there are several methods for the isolation of light harvesting complexes from plants, each with its advantages and limitations. The choice of method depends on the specific LHC of interest, the downstream application and the available resources.

The method followed in this work was isolation using centrifugation and purification using DEAE column chromatography. The method requires preparation of buffers, which is a solution to maintain the pH during the process.

2. Experimental

The experimental process is basically meant to isolate the components in the plant extract called thylakoids. Thylakoids are a group of chlorophyll and other pigments that are involved in capturing light energy and transferring it to the reaction centre. Parts of this electron transport chain system are designated as Photosystem I and Photosystem II with some interlinks. Physically, thylakoids are membrane-bound compartments inside the chloroplasts of plant cells where the light-dependent reactions of photosynthesis take place. Photosystem I (PSI) is located in the thylakoid membrane of the chloroplasts. Light-harvesting complexes (LHCs) are also located in the thylakoid membrane. LHCs are associated with both photosystem I and photosystem II and play a crucial role in the absorption of light energy and its transfer to the reaction centres, where the process of photosynthesis begins. The experimental method is aimed at isolation of PSI.

2.1. PREPARATION OF BUFFERS:

Buffer A: 0.3M sucrose, 15mM NaCl, 30mM tricine, pH 7.8 NaOH

Buffer B: 5mM EDTA and 5mM tricine, pH 7.8 NaOH

Buffer C: 0.3M sucrose and 30mM tricine, pH 7.8 NaOH

Fresh spinach (Spinacia oleracea L.) leaves were stored at 4^0C overnight after washing. The leaves were then homogenized in homogenizing buffer A. The paste obtained was filtered through 4 layered cheese-cloths and centrifuged at 2000g for 2min. Pellet containing chloroplasts were then washed two times with the hypotonic buffer B and centrifuged at 2000g for 2min. Unstacked membrane were obtained by centrifugation at 25000g and then suspended in suspension buffer C. The obtained thylakoids were then solubilized in triton-X 100 (4.8% w/v). After stirring it for 15min, sample was centrifuged again at 15000g for 15 mins. The complex was then further purified, using DEAE-cellulose column chromatography.

2.2. DEAE-CELLULOSE COLUMN PREPARATION:

DEAE-cellulose dry resin was suspended in 5 volumes of distilled water and allowed to settle for 45 min. The settled volume of the resin was then measured. This is the column volume (CV) to be used for measurement of the amount of solution. The suspension was filtered using Whatman filter paper. The resin was suspended in 2CV of buffer-A (0.1M NaOH, 0.5M NaCl) for 10min and then slurry was filtered. The slurry was continued to be washed with another 2CV of buffer A. The step was repeated using buffer-B (0.5M NaCl), C (0.1M HCl, 0.5M NaCl), and distilled water simultaneously. Washing was continued with distilled water 5-10CV until the pH of effluent was 5 or greater. The slurry was suspended in 2CV of 1M NaCl and the pH was adjusted to 7.8 using the NaOH and stored at 0-5^0C. The resin was again washed with 5 CV of distilled water. This resin was resuspended with 2CV of 1X starting buffer for 15min. Small amount of suspension was filtered to check the pH. If the pH is within 0.15 units of the buffer, the resin is ready to use.

2.3. COLUMN CHROMATOGRAPHY:

The purification of **PS-I (Photosystem I)** from the solubilized thylakoid membranes involves loading the supernatant onto a DEAE-cellulose column prepared with a starting buffer containing 10mM Tris-HCl, 0.2% Triton X-100, 20% sucrose, whose pH is adjusted to 7.8 using NaOH. The flow rate is maintained at a constant rate of 1mL/min throughout the procedure. After loading the supernatant containing **PSI** onto the column using a peristaltic pump, the column is washed successively with different buffers to elute and collect the **PS-I** fractions for further analysis. The first wash involves washing the column with 1000ml of the starting buffer supplemented with 10mM NaCl. This step is followed by washing the column with 300ml of the starting buffer supplemented with 50mM NaCl. Finally, the column is washed with a linear gradient of NaCl ranging from 50-200mM. This is achieved by filling each chamber of the gradient apparatus with 200ml of the starting buffer supplemented with 50mM and then 200mM of NaCl, resulting in a gradual increase in NaCl concentration. During the washing steps, fractions (e.g., 5-7ml) are collected at regular intervals. The collected fractions can be analyzed for the presence of **PSI** using various techniques such as spectrophotometry.

All the LHC extracts from various plant species were investigated for their UV-Vis absorbance characterstics. The energy levels were calculated using Tauc's Plot. The multiplicity of levels indicate the presence of several components in the LHCs. This can provide a mechanism for absorbtion of multiple wavelengths of radiation across the UV-Vis spectrum. This can also provide intermediate energy states to the electrons for a path which inhibits the direct recombination of electrons.

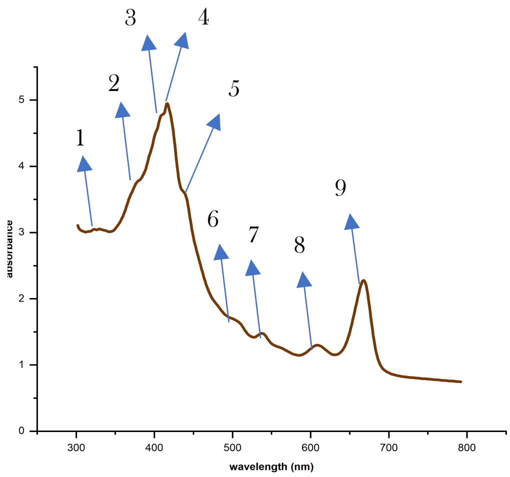
The relevant discussion has been provided in the next chapter.

The UV-Vis characteristics along with the Tauc's plot of the samples are given below.

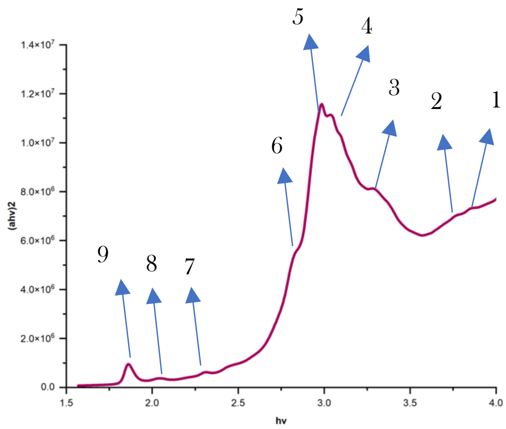
Plant Specie

Calotropis Gigantea

Aakdo (Crown flower)



UV- Vis Characterstics



Tauc's plot

Peak No.	Wavelength (nm)
1	320.74
2	378.43
3	408.31
4	416.53
5	438.60
6	502.53
7	537.34
8	608.46
9	670.95

Peak wavelengths

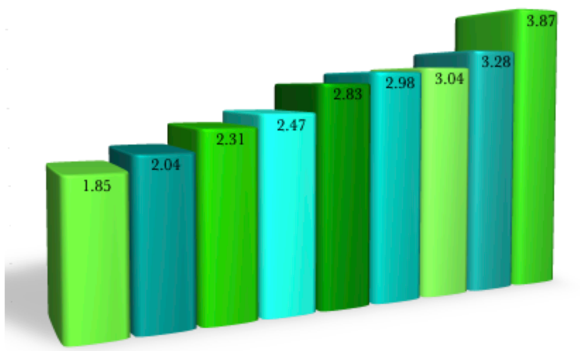
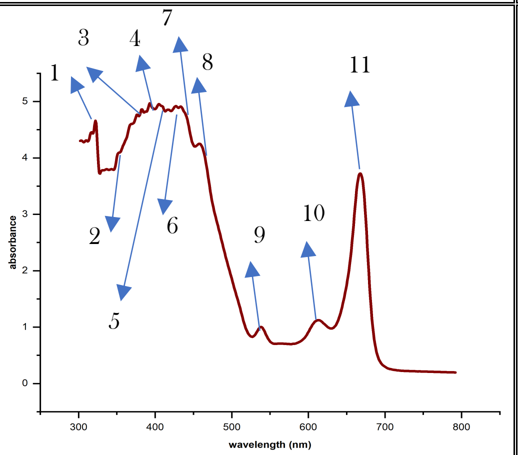


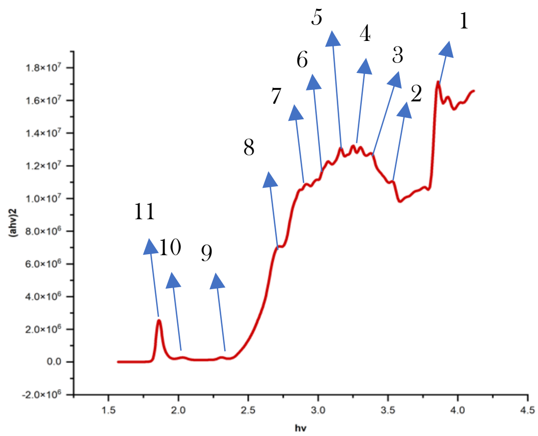
Figure 17

Energy levels

Plant Specie	
Colocasia esculenta	
Arbi Leaves	



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
1	315.84
2	322.40
3	352.63
4	367.23
5	375.00
6	381.92
7	392.80
8	405.64
9	428.02
10	459.72
11	670.95

Peak wavelengths

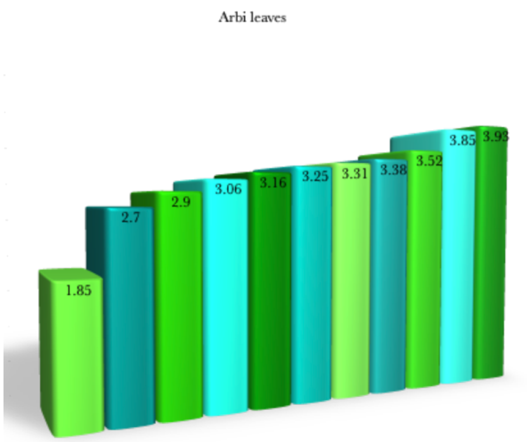


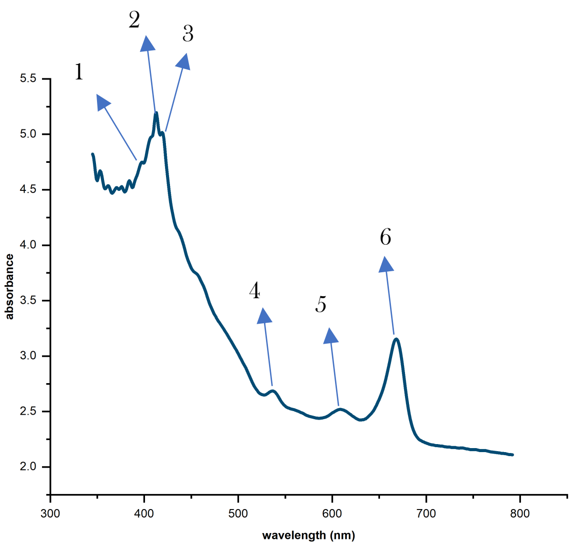
Figure 18

Energy levels

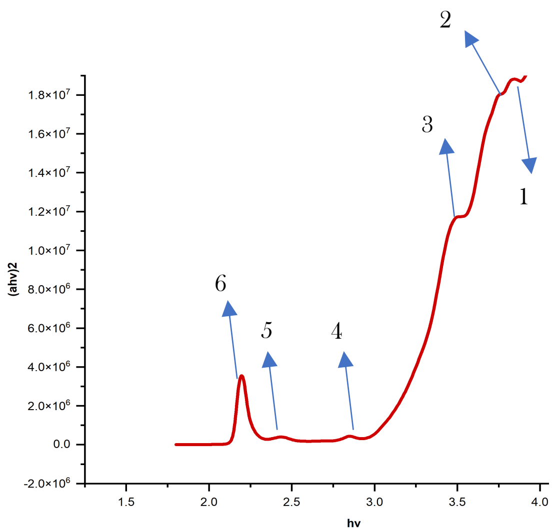
Plant Specie

Polyalthia Longifolia

Asopalav



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
1	322.40
2	331.00
3	356.68
4	437.06
5	510.80
6	566.78

Peak wavelengths

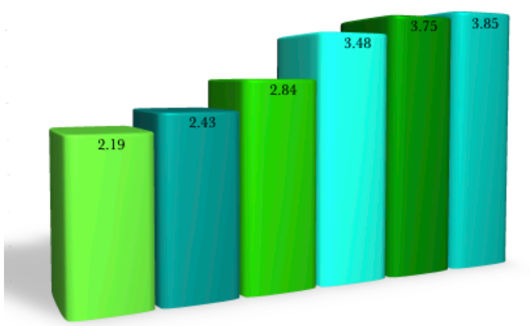


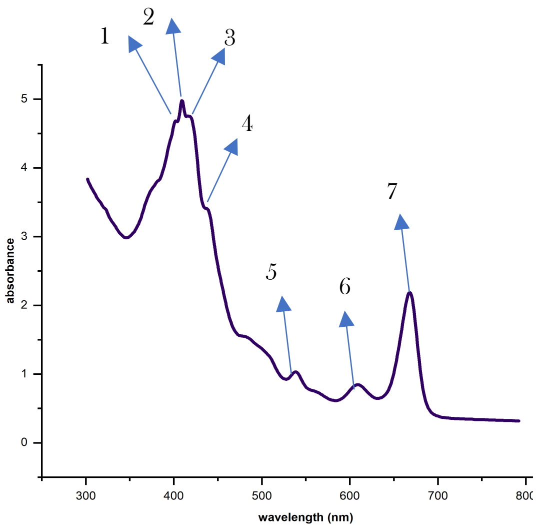
Figure 19

Energy levels

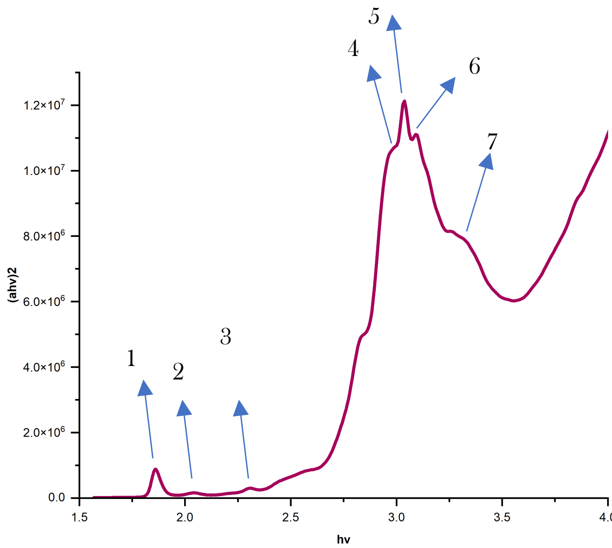
Plant Specie

Ficus Benghalesis

Banyan



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength
1	380.75
2	401.70
3	408.31
4	437.06
5	537.34
6	614.48
7	670.95

Peak wavelengths

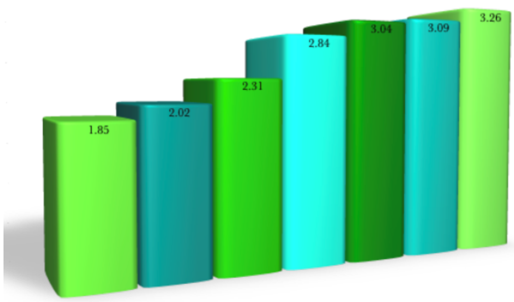


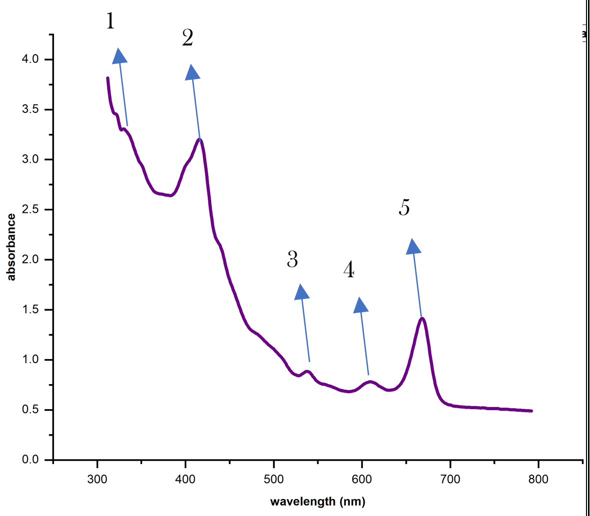
Figure 20

Energy levels

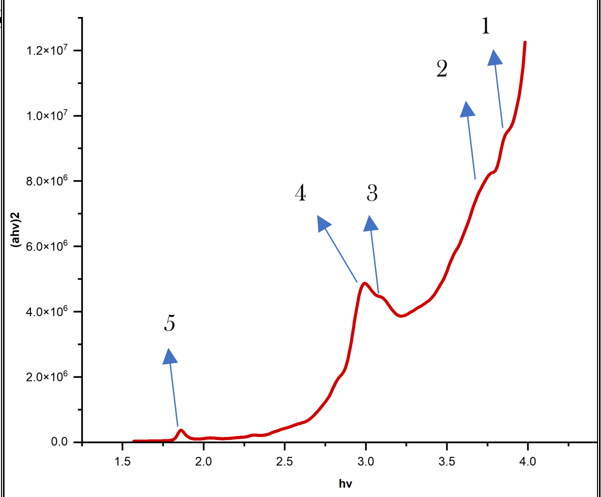
Plant Specie

Aegle Marmelos

Bilva



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
1	319.91
2	328.37
3	400.40
4	416.53
5	670.95

Peak wavelengths

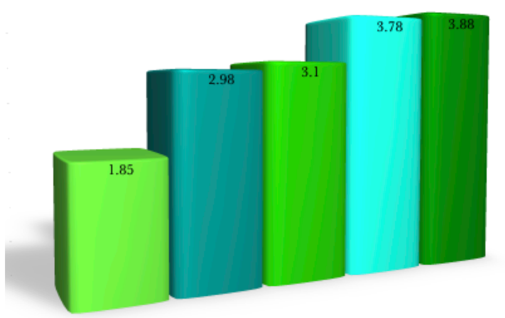
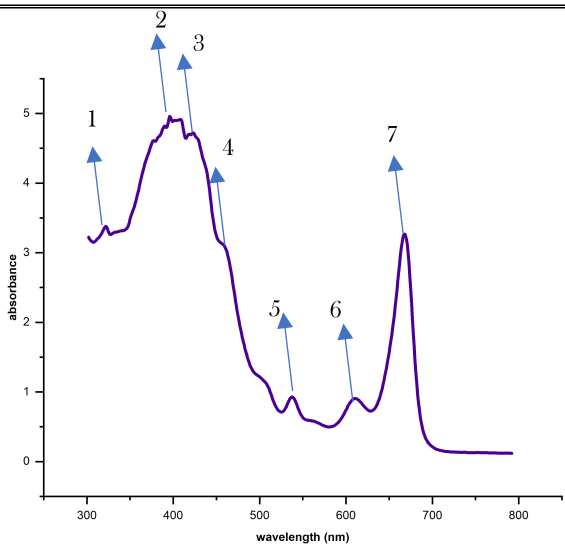


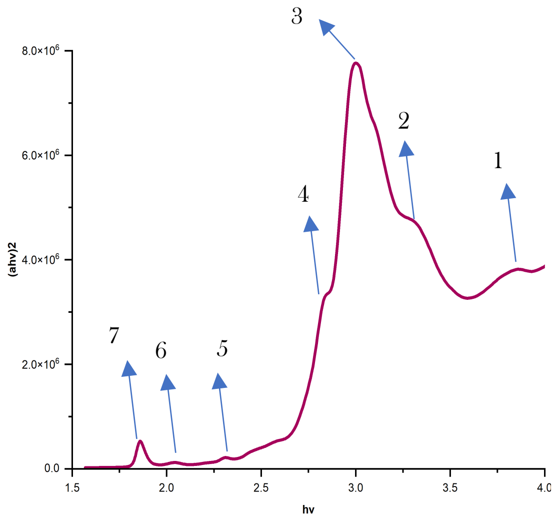
Figure 21

Energy levels

Plant Specie	
Coriandrum sativum	
Coriander	



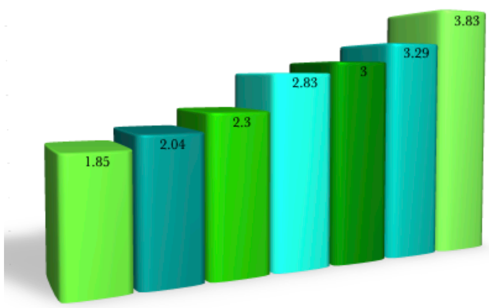
UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
1	324.09
2	377.28
3	413.75
4	438.60
5	539.67
6	608.46
7	670.95

Peak wavelengths



Relative Energy Levels

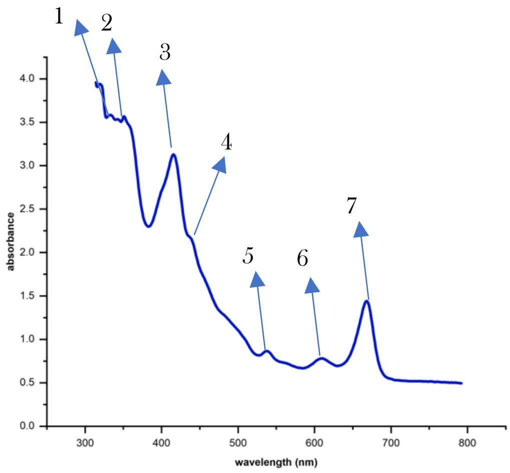
Figure 22

Energy levels

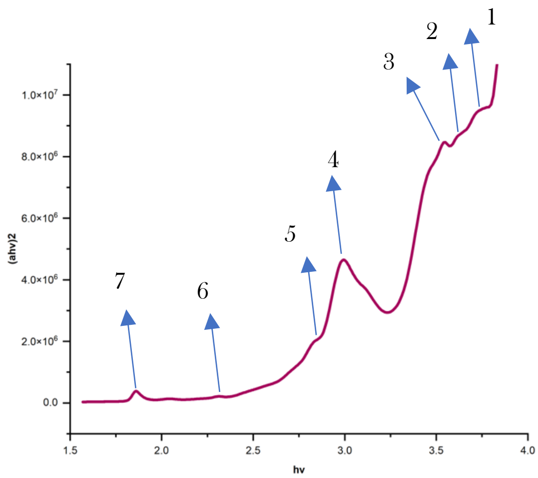
Plant Specie

Murraya Koenigii

Curry leaves



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
1	670.95
2	537.34
3	438.60
4	416.53
5	358.74
6	351.63
7	334.57

Peak wavelengths

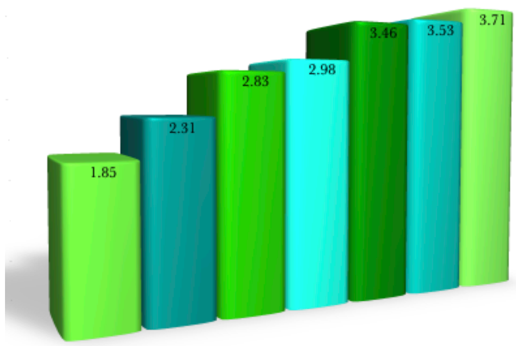


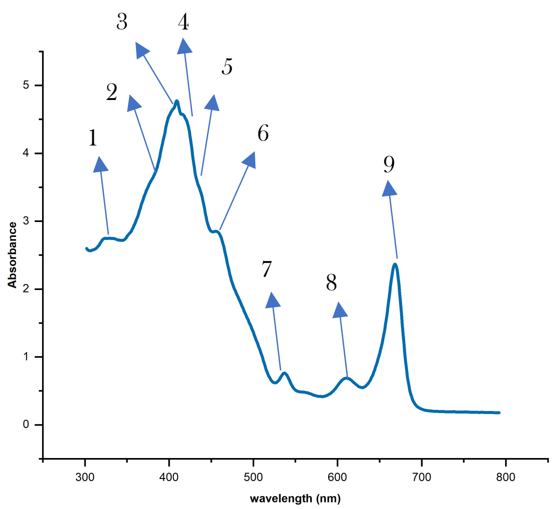
Figure 23

Energy levels

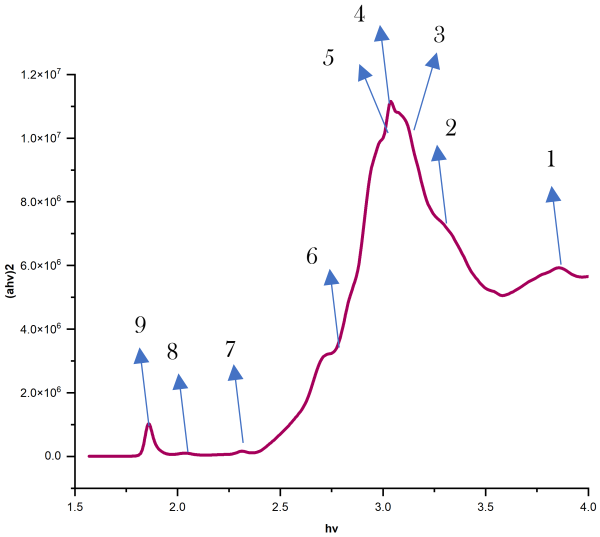
Plant Specie

Ocimum basillicum

Sweet Basil



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
1	670.95
2	614.48
3	537.34
4	458.03
5	417.93
6	409.65
7	377.28
8	352.63
9	322.40

Peak wavelengths

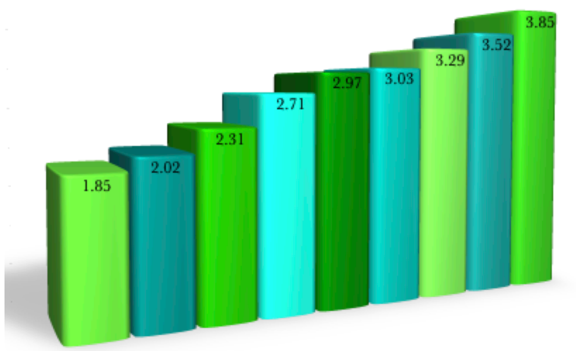
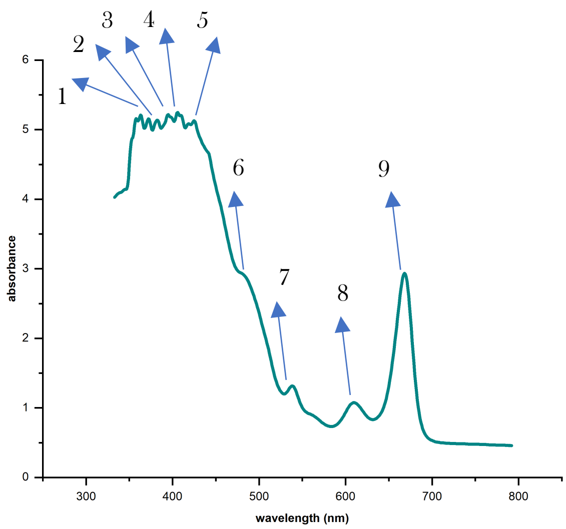


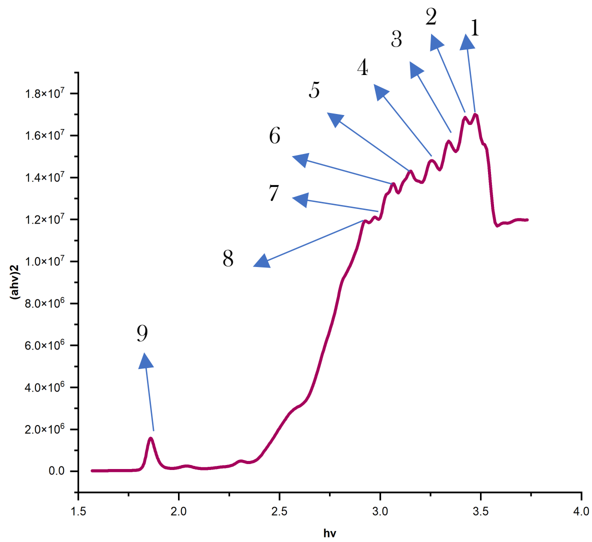
Figure 24

Energy levels

Plant Specie	
Durento	
Golden Duedrop	



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
1	322.40
2	329.24
3	367.23
4	408.31
5	416.53
6	438.60
7	535.02
8	608.46
9	670.95

Peak wavelengths

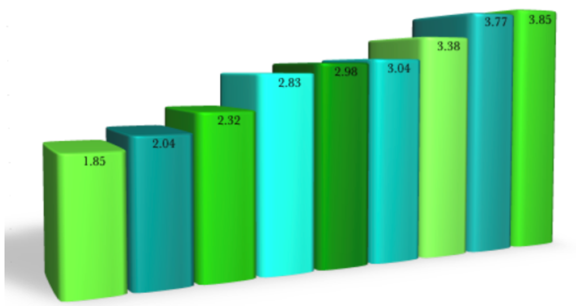


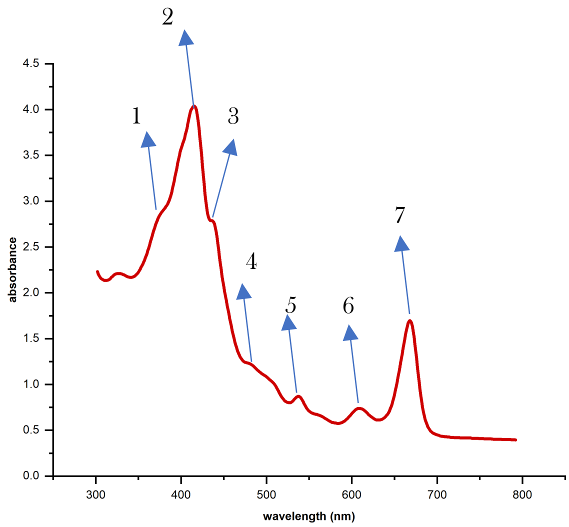
Figure 25

Energy levels

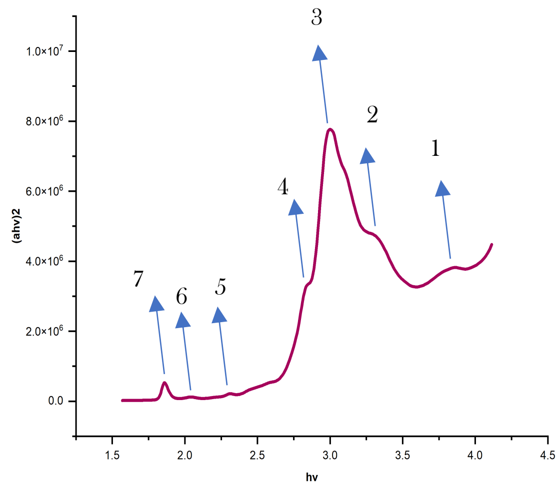
Plant Specie

Anethum Graveolens

Dill leaves



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
1	323.24
2	373.87
3	413.75
4	437.06
5	537.34
6	614.48
7	667.34

Peak wavelengths

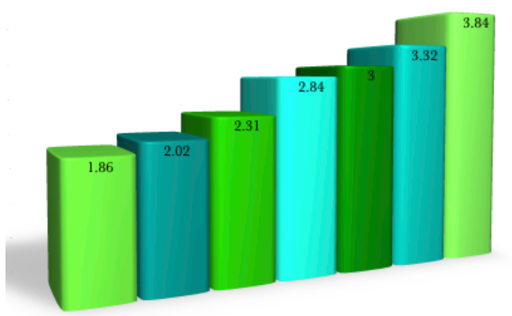


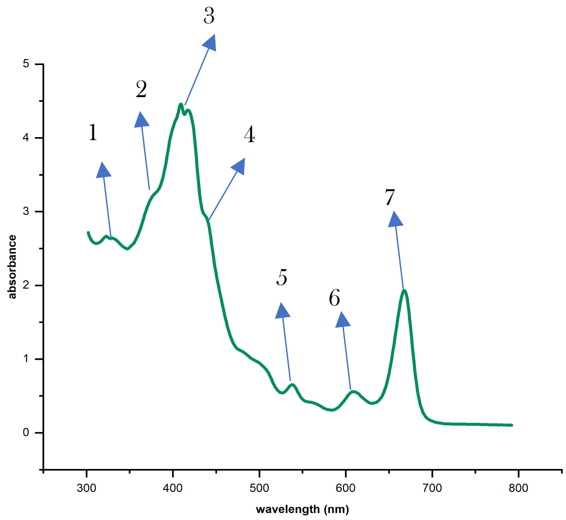
Figure 26

Energy levels

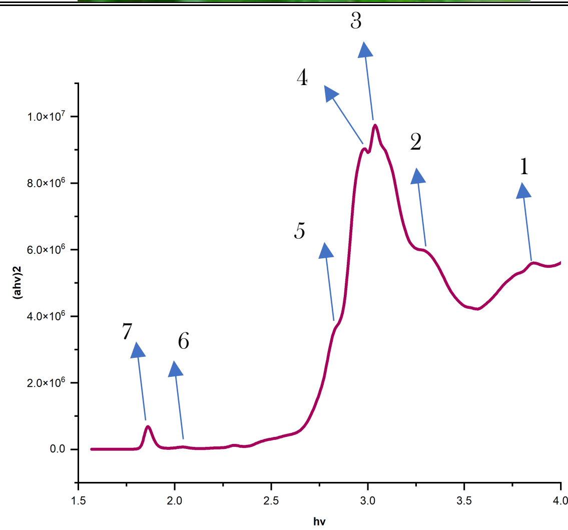
Plant Specie

Lawsonia Inermis

Heena



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
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2	380.75
3	409.65
4	458.03
5	535.02
6	614.48
7	670.95

Peak wavelengths

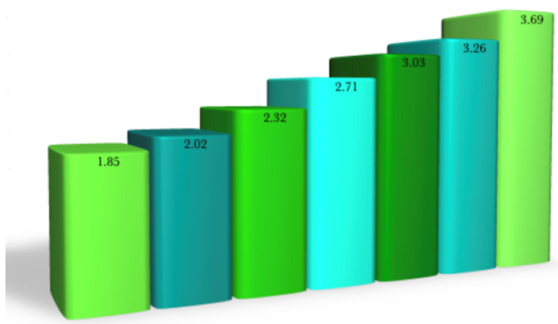


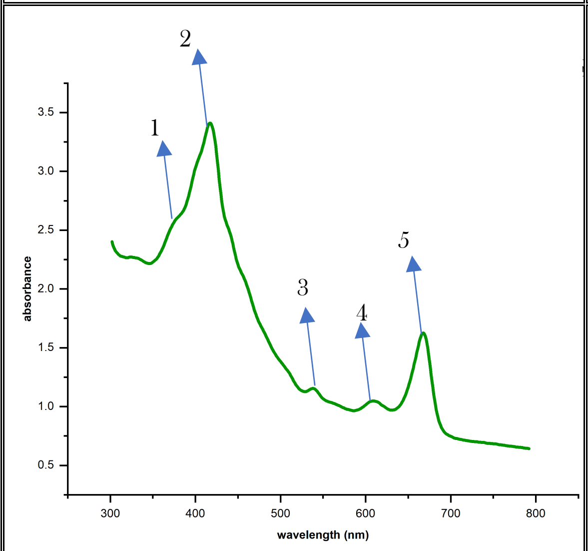
Figure 27

Energy levels

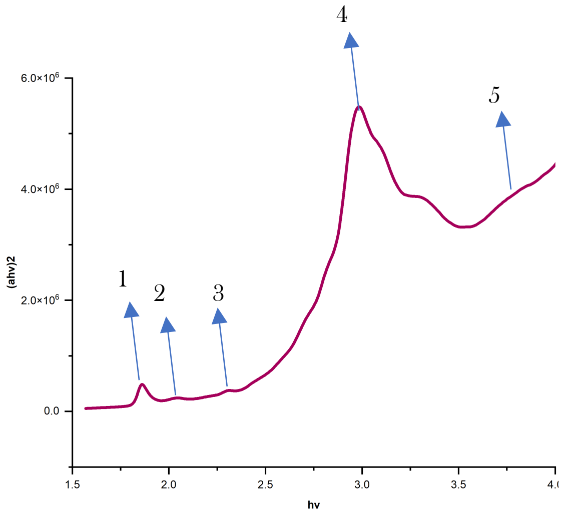
Plant Specie

Trigonella foenumgraecum

fenugreek



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
1	373.87
2	416.53
3	535.02
4	608.46
5	670.95

Peak wavelengths

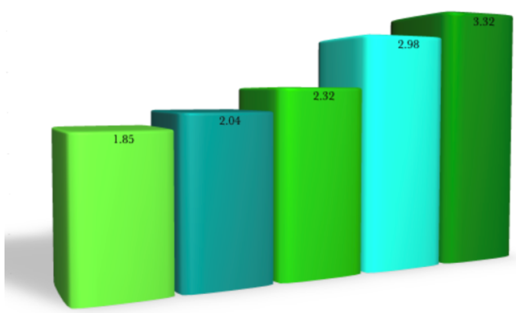
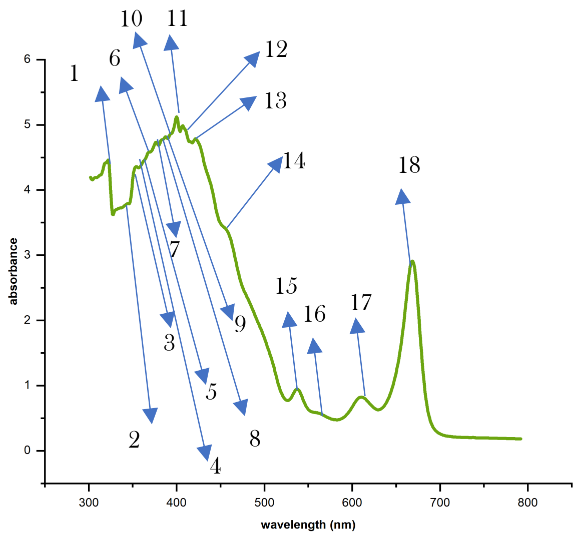


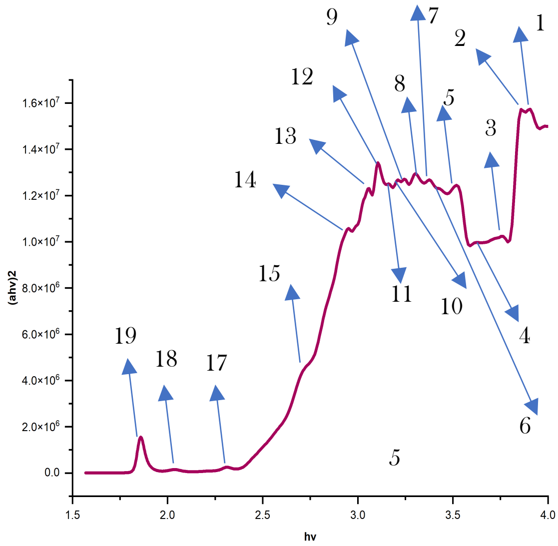
Figure 28

Energy levels

Plant Specie	
Camellia sinensis	
Green tea	



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
1	318.27
2	322.40
3	330.12
4	342.89
5	353.63
6	361.88
7	368.32
8	375.00
9	381.92
10	386.68
11	392.80
12	400.40
13	406.97
14	420.76
15	458.03
16	535.02
17	614.48
18	670.95

Peak wavelengths

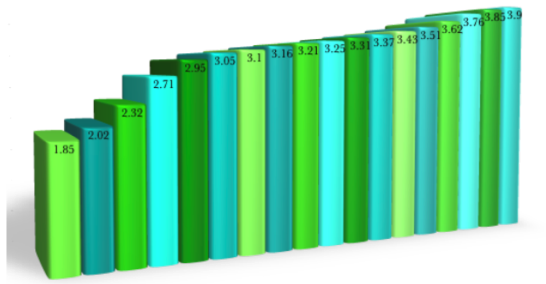


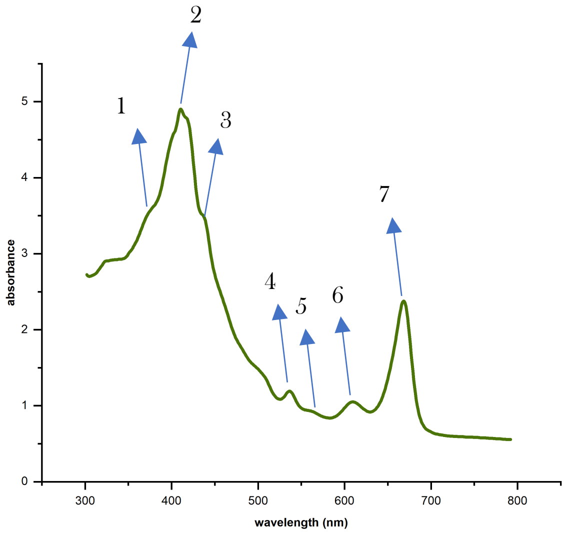
Figure 29

Energy levels

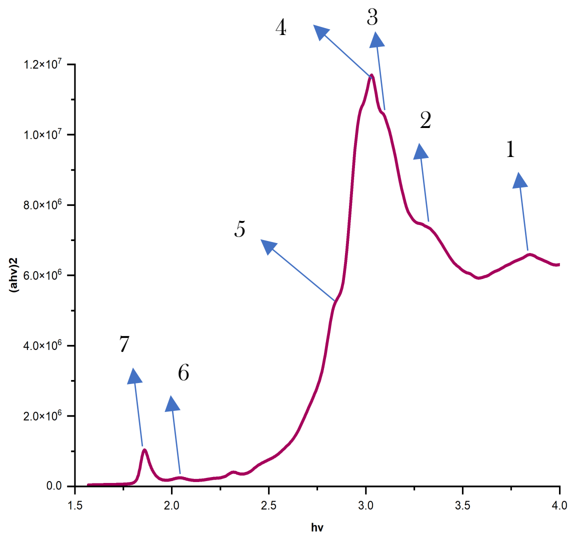
Plant Specie

Ocimum sanctum

Holy basil



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
1	322.40
2	377.28
3	409.65
4	437.06
5	537.34
6	614.48
7	670.95

Peak wavelengths

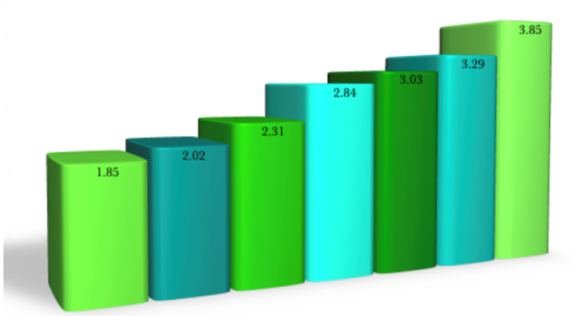


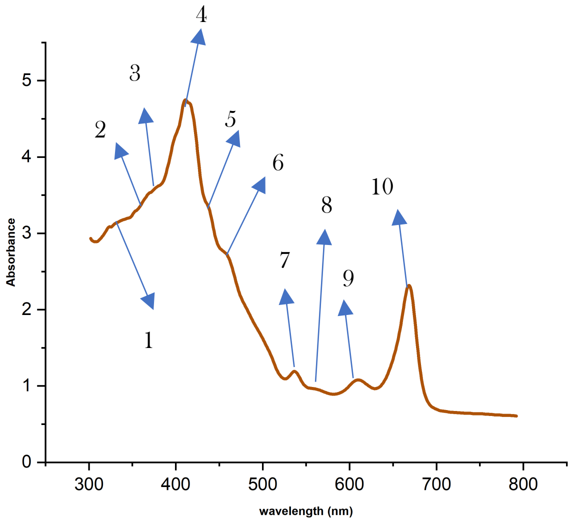
Figure 30

Energy levels

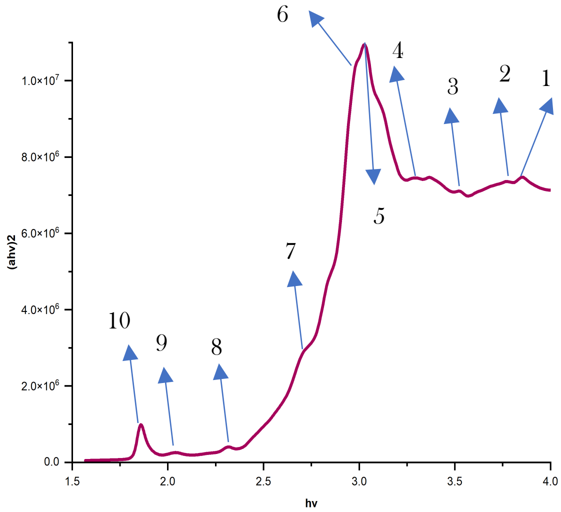
Plant Specie

Mentha Spicata

Mint



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
1	323.24
2	330.12
3	353.63
4	368.32
5	409.65
6	437.06
7	458.03
8	537.34
9	614.48
10	670.95

Peak wavelengths

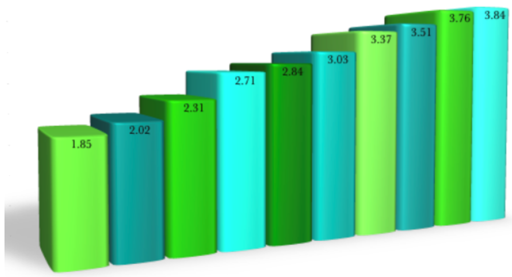
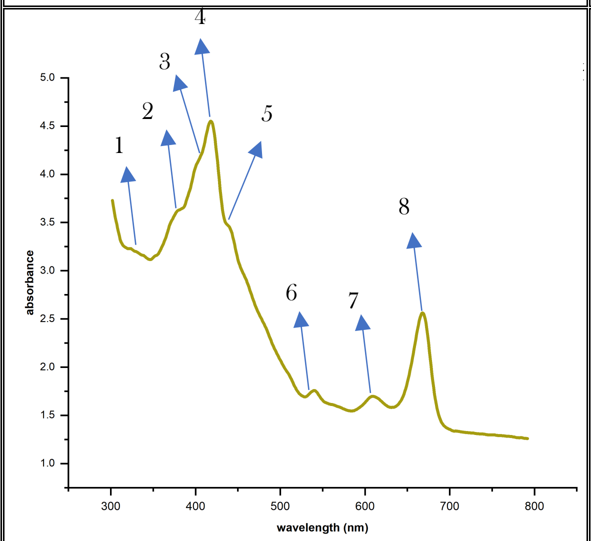


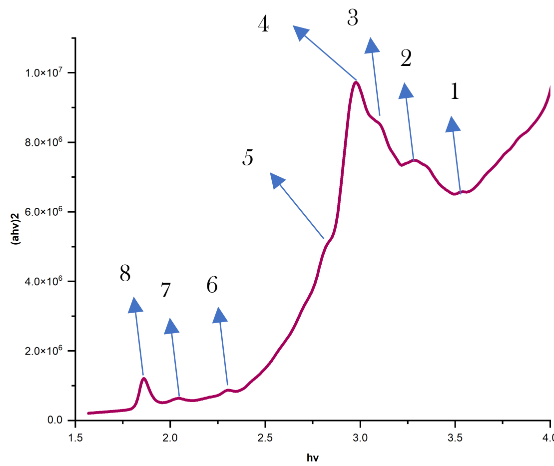
Figure 31

Energy levels

Plant Specie	
Azadirachta indica	
Neem	



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
1	351.63
2	378.43
3	401.70
4	417.93
5	441.73
6	535.02
7	614.48
8	670.95

Peak wavelengths

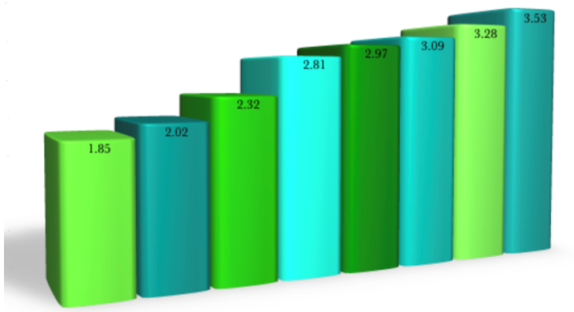


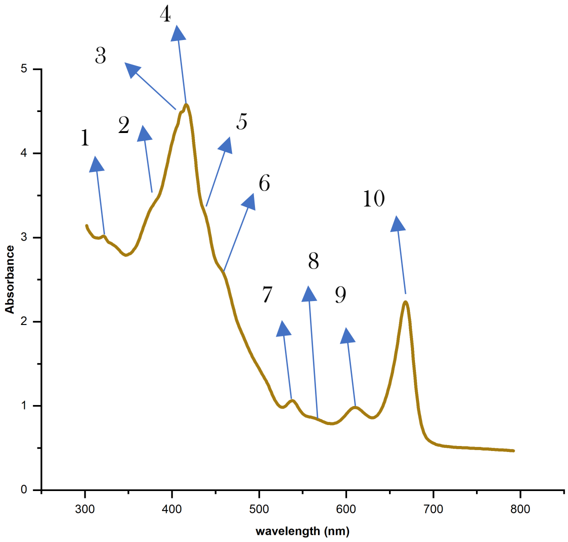
Figure 32

Energy levels

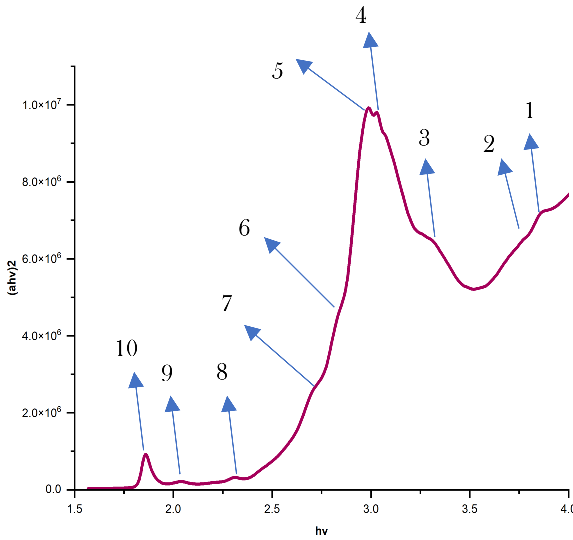
Plant Specie

Ficus religiosa

Peepal



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
1	323.24
2	330.12
3	353.63
4	368.32
5	409.65
6	437.06
7	458.03
8	537.34
9	614.48
10	670.95

Peak wavelengths

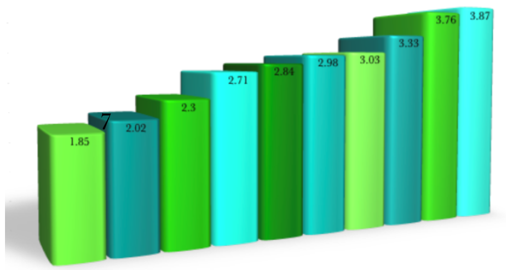


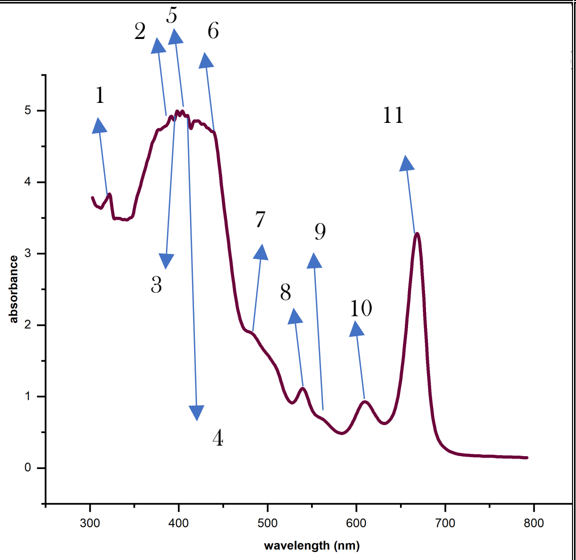
Figure 33

Energy levels

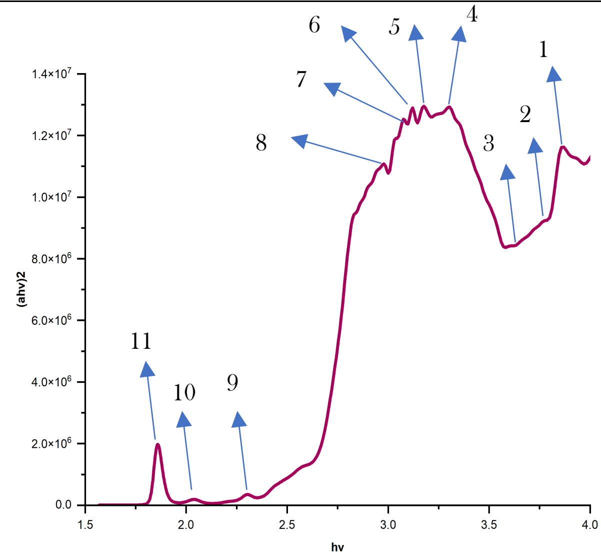
Plant Specie

Raphanus sativus

Radish



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
1	320.74
2	376.14
3	391.56
4	397.84
5	403.00
6	409.65
7	417.93
8	481.10
9	537.34
10	611.45
11	670.95

Peak wavelengths

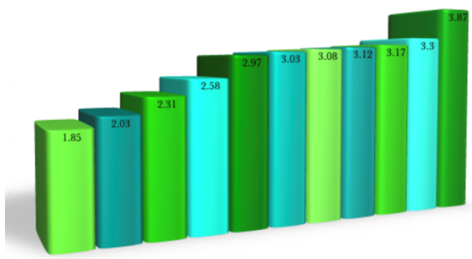
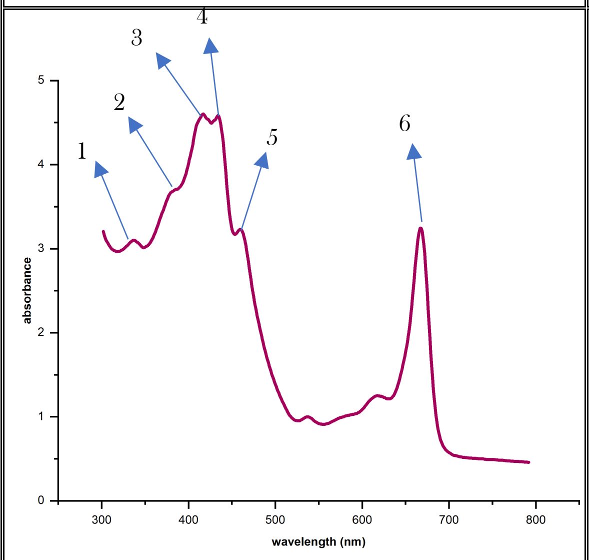


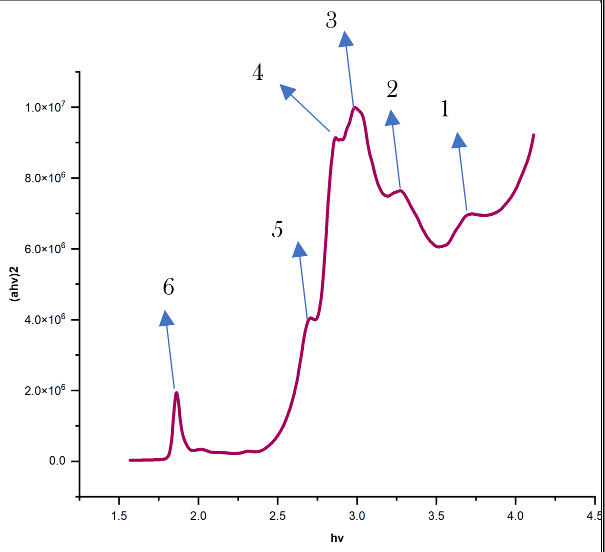
Figure 34

Energy levels

Plant Specie	
Spinacia Oleracea	
Spinach	



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
1	337.30
2	380.75
3	416.53
4	434.00
5	459.72
6	670.95

Peak wavelengths

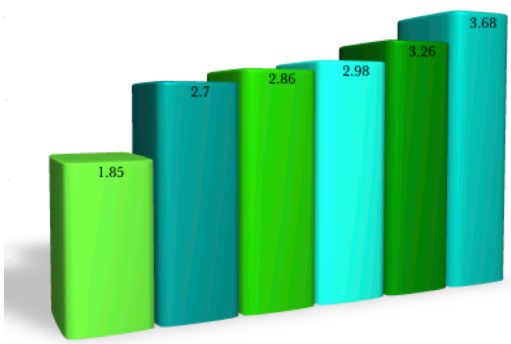


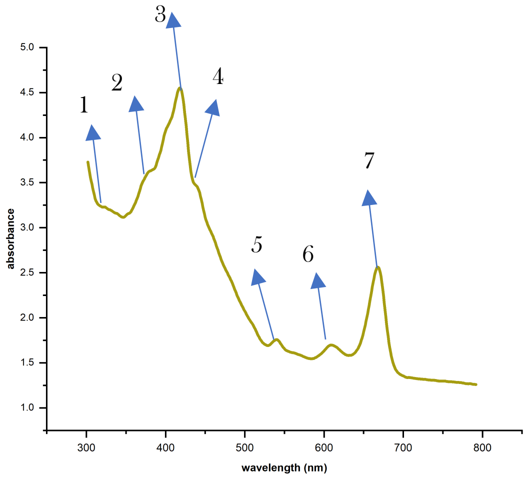
Figure 35

Energy levels

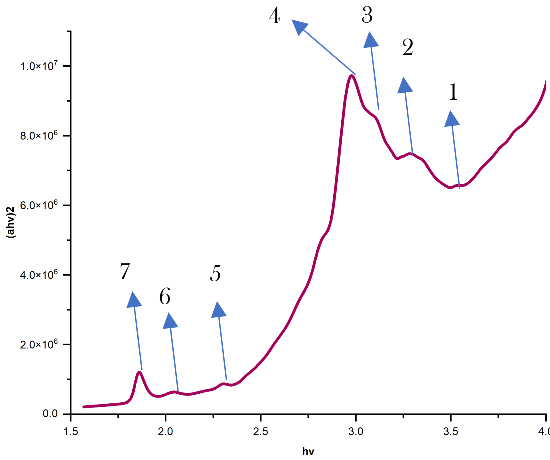
Plant Specie

Amaranthus

Tandaja



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
1	336.38
2	380.75
3	409.65
4	458.03
5	535.02
6	614.48
7	670.95

Peak wavelengths

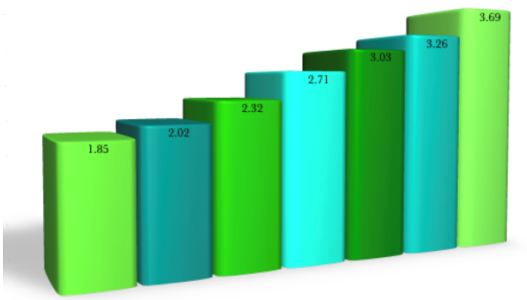
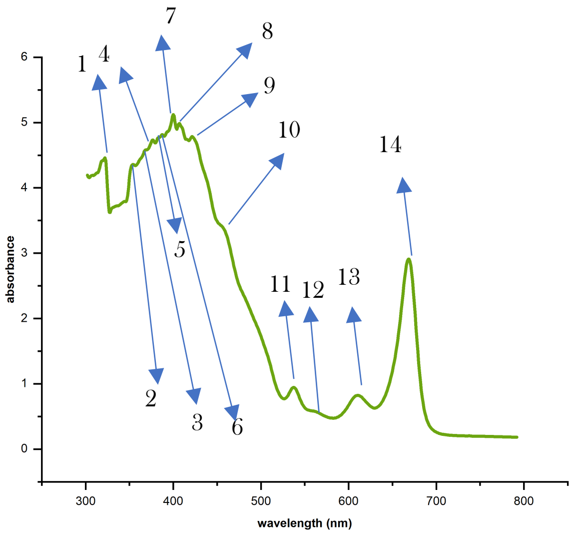


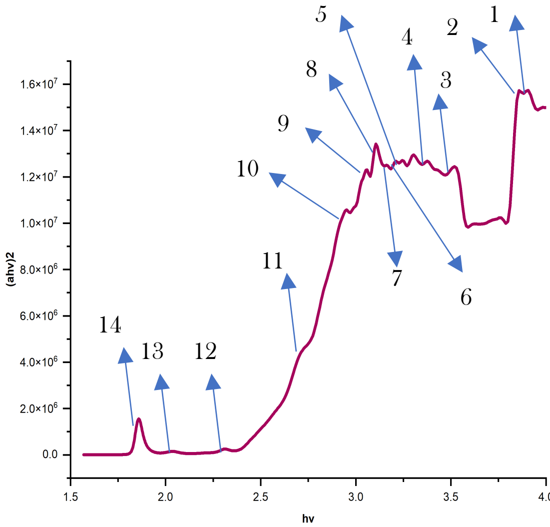
Figure 36

Energy levels

Plant Specie	
Datura Stramonium	
Thorn Apple	



UV- Vis Analysis



Tauc's plot

Peak No.	Wavelength (nm)
1	353.63
2	356.68
3	362.94
4	372.75
5	380.75
6	394.05
7	405.64
8	417.93
9	423.63
10	440.16
11	482.98
12	537.34
13	608.46
14	670.95

Peak wavelengths

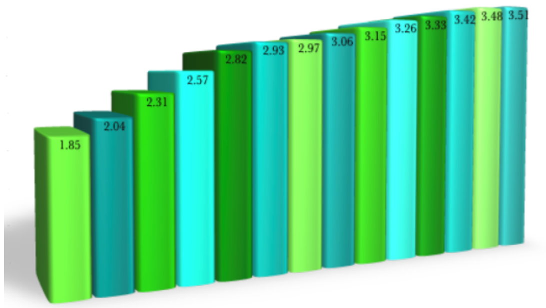


Figure 37

Energy levels