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Uv-Vis Spectroscopy in Analysis of Phytochemicals

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ABSTRACT :
Plants are recognized in the pharmaceutical industry for their broad structural diversity as well as their wide range of pharmacological activities. The biologically active compounds present in plants are called phytochemicals.Phytochemicals have two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contain terpenoids and alkaloids These phytochemicals are derived from various parts of plants such as leaves, flowers, seeds, barks, roots and pulps. They are used as sources of direct medicinal agents and serve as a raw material base for elaboration of more complex semi-synthetic chemical compounds. UV-visible spectroscopy can be performed for qualitative analysis and for identification of certain classes of chemical compounds in both pure and biological mixtures. This review covers the brief description of extraction of active compounds from the various parts of plants and the qualitative and quantitative analysis of the phytochemicals of chemical composition of plant extractsusing a UV spectrophotometer which is a scientific instrument to separate and measure spectral components of physical phenomenon.

KEYWORDS : Phytochemicals, medicinal agents, UV-Visible spectroscopy, qualitative and quantitative analysis, UV-Spectrophotometer.

I. INTRODUCTION :

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg,1999)[1].Phytochemicals are also well known for their tendency of zero side effects unlike pharmaceutical chemicals, and hence given a name "MAN FRIENDLY MEDICINES". Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc.They also protect plants from disease and damage and contribute to the plant's color, aroma andflavor. In general, the plant chemicals that protect plant cells fromenvironmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are known as phytochemicals (Mathai, 2000)[2]. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds[3].More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher and Thomson,1999). Generally,phytochemicals have been classified into six major categories based on their chemical structures and characteristics. These categories include carbohydrate, lipids, phenolics, terpenoids and alkaloids, and other nitrogen-containing compounds(Figure 1; Harborne and Baxter, 1993; Campos-Vega and Oomah, 2013)[4]. Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional system , mordern system, nutraceuticals food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

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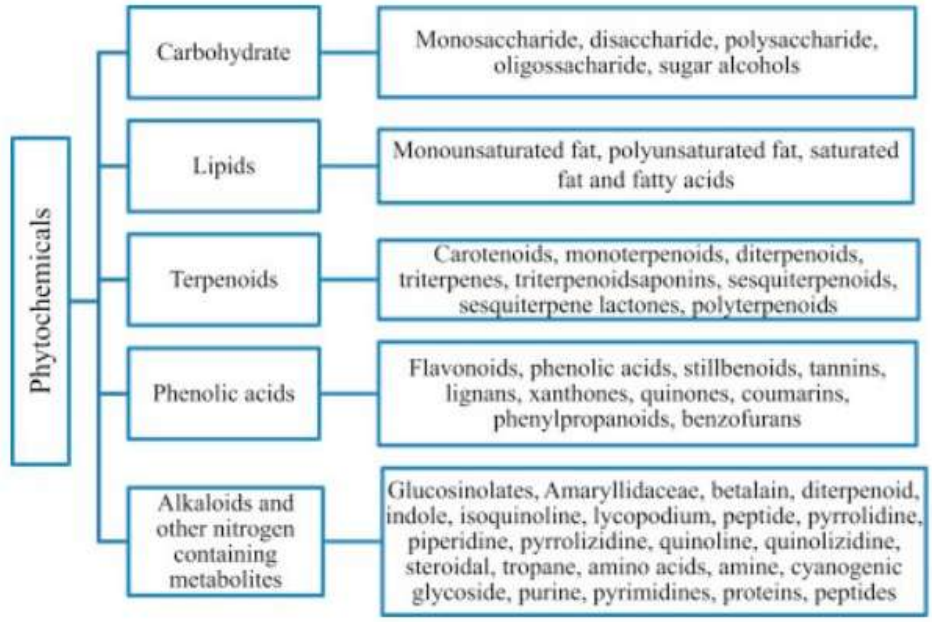

Phytochemical analysis :

Phytochemical analysis refers to the extraction, screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, carotenoids, tannin, antioxidants and phenolic compounds.

Phytochemicals analysis of a plant includes several aspects:

- Extraction of the compounds to be analyzed from a sample or specimen.
- Separation and isolation of them
- Identification or characterisation of the isolated compound.
- Investigation of the biosynthetic routes of a certain molecule.
- Determination or quantitaive assessment[5].

Ultraviolet-visible spectroscopy :

Spectroscopy is the branch of science dealing with the study of interaction between Electromagnetic radiation and matter. UV-Visible spectrophotometry is one of the most frequently employed technique in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measure the ratio, or function of ratio, of the intensity of two beams of light in the UV-Visible region are called Ultraviolet-Visible spectrophotometers. In quantitative analysis, spectrophotometre is used to ascertain the quantity of molecular species absorbing the radiation. The fundamental law that governs the quantitative spectrophotometric analysis is the Beer - Lambert law. Beer-Lambert law states that when beam of parallel monochromatic light is passed through a transparent cell containing a solution of an absorbing substance, intensity of light may decreases. Mathematically, Beer- Lambert law is indicated as:

$$A=a.b.c. \quad \ldots \text{Eq. (1)}$$

where, 'A' is absorbance, 'a' is absorptivity or extinction coefficient, 'b' is path length of radiation through sample (cm), 'c' is concentration of solute in solution (Davidson, 2002)[6-8].

UV/Vis spectroscopy is in its simplest form, a sample is placed between a light source and a photo detector, and the intensity of a beam of light is measured before and after passing through the sample. These measurements are compared at each wavelength to quantify the sample's wavelength dependent extinction spectrum. The data is typically plotted as extinction as a function of wavelength. Each spectrum is background corrected using a "blank" - a cuvette filled with only the dispersing medium to guarantee that spectral features from the solvent are not included in the sample extinction spectrum.(Markham, 1982)[9].

II. METHODS AND MATERIALS :

The systemic investigations of plant material for its phytochemical behaviour involve four different stages[10] :

1. The procurement of raw material and quality control.

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2. Extraction, purification and characterization of the constituents of pharmaceutical interest and in process quality control.
3. Investigation of biosynthetic pathways to particular compound
4. Quantitative evaluation.

1. Collection of plant material :

1.1.Collection of Plants

Plants under consideration may be collected either from wild forests or fromherbariums. After the plants are collected from wild or from herbarium they have to be processed for cleaning in order to prevent the deterioration of phytochemicals present in plants.

1.2.Cleaning of Plants

After plants collection they have to be cleaned properly. The process may involve the following steps. Cleaning, washing, peeling or stripping leaves from stems. Cleaning has to be done by hands in order to get better results.

1.3.Drying

The main purpose of drying is to remove the water content from plants so that the plants can be stored. Plants have to be dried immediately as soon as the plants collection or this will lead to spoilage of plant materials. The drying consists of two methods. Drying can be done either by natural process or by artificial process[11].

2. Extraction and Isolation :

Extraction is the separation of medicinally active portions of plant tissues using selective solvents through standard procedures. The dried plant material generally used for extraction. The fresh plant part when used are homogenized or macerated with a solvent.The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstrum.

General method of extraction :

The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, Soxhlet extraction, aqueous-alcoholic extraction by fermentation, countercurrent extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydrofluorocarbon solvents).

For aromatic plants :- hydrodistillation techniques (water distillation, steam distillation, water and steam distillation), hydrolytic maceration followed by distillation, expression and enfleurage (cold fat extraction) may be employed.

Some of the latest extraction methods for aromatic plants include headspace trapping, solid phase microextraction, protoplast extraction, microdistillation, thermomicrodistillation and molecular distillation[10].

A general procedure forextraction of different constituents from fresh plant and fractionating into different class described in the following chart:[10]

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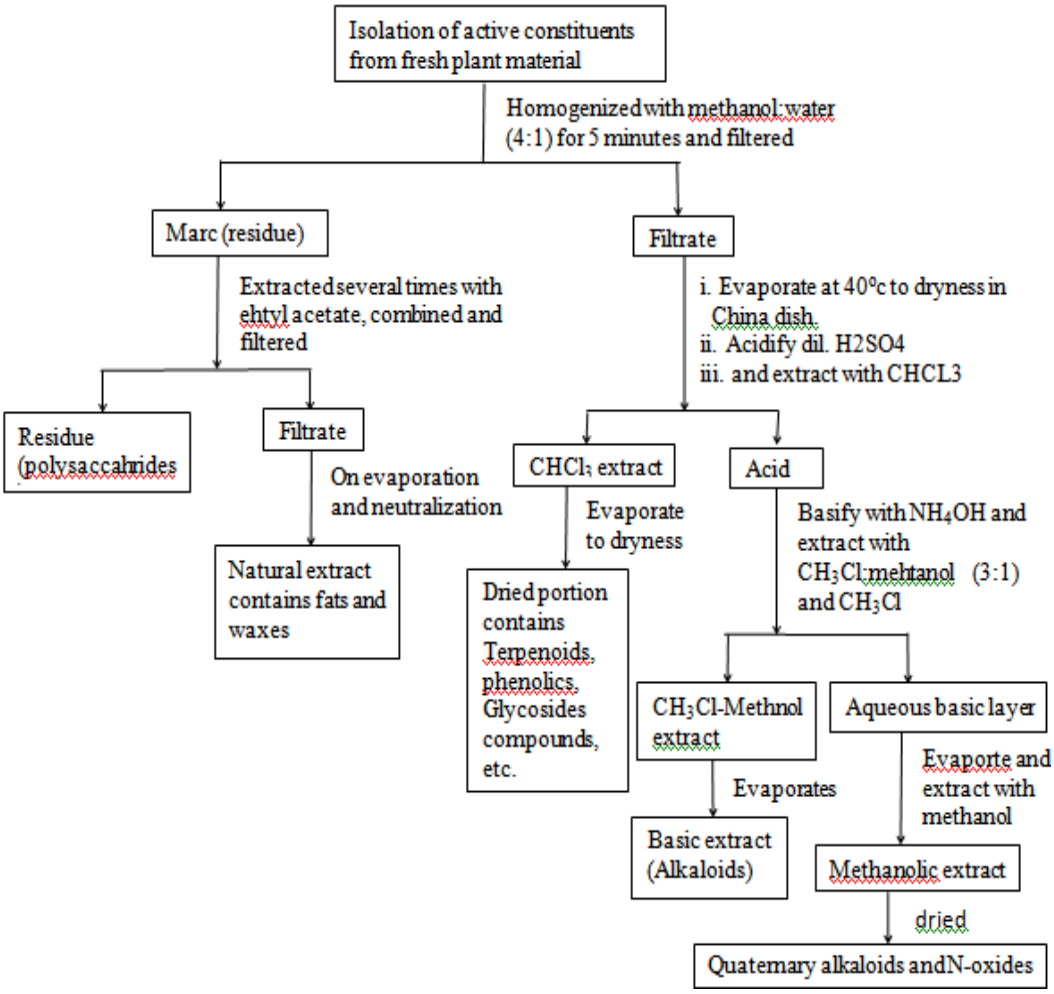

3. Methods of separation and purofocation of compoun:[10]

1. Paper chromatography
2. Thin layer Chromatography (TLC)
3. Gas layer Chromatography (GLC)
4. High performance Liquid Chromatography (HPTLC)
5. Column Chromatography
6. Gel Permeation Chromatography (Gel Filtration, ,Molecular Sieves)
7. AffinityChromatography

4. Methods of detection of phytochemicals in quantitative analysis :[10]

1. UV-VIS spectroscopy
2. Infra-red spectroscopy
3. Fluorescence Analysis
4. Nuclear Magnetic Resonance Spectroscopy (NMR)
5. Mass spectrometry
6. X-ray Difraction
7. Radioimmune Assays (RIA)

Principle of UV-Vis Spectroscopy :

1. Basically, spectroscopy is related to the interaction of light with matter.
2. As light is absorbed by matter, the result is an increase in the energy content of the atoms or molecules.
3. When ultraviolet radiations are absorbed, this results in the excitation of the electrons from the ground state towards a higher energy state.
4. Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb energy in the form of ultraviolet light to excite these electrons to higher anti-bonding molecular orbitals.
5. The more easily excited the electrons, the longer the wavelength of light it can absorb. There are four possible types of transitions (π–π*, n–π*, σ–σ*, and

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n–σ*), and they can be ordered as follows: σ–σ* > n–σ* > π–π* > n–π*[12]

i. σ–σ* transition :- An electron in a bonding σ orbital of a molecule is excited to the corresponding anti-bonding orbitalby the absortption of a radiation. To induce this, it requires large energy so takes place with highly energetic (shorter wavelength) radiation (λ< 185/100-200nm).

ii. n–σ* transition :- In this type, saturated compounds containing atoms with unshared pair of electrons undergo n–σ* transition. It occurs at less energetic (longer wavelength) radiation (λ> 200/150-250nm)

iii. π–π*&n–π* transiiton :- Most organic compounds undergo this transitions.They occurs at small energy (longer wavelengths) radiation (200-400 nm).[13] n → π* and π → π* are associated with both UV and larger wavelength VIS radiation.

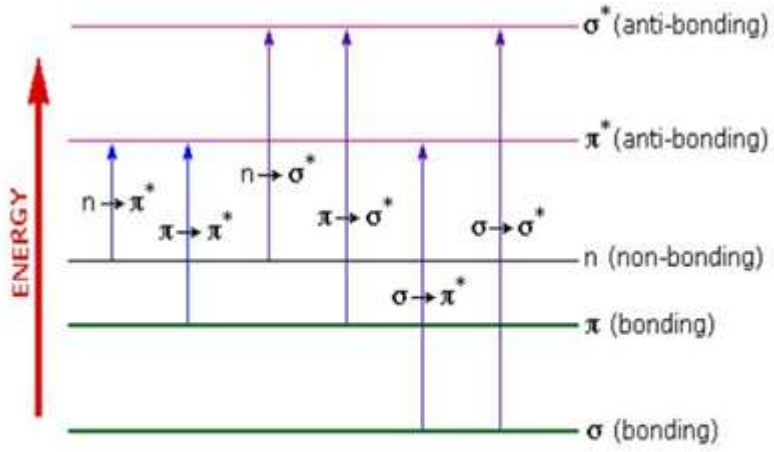

Fig. 02. Energy Scheme for molecular orbits

6. The absorption of ultraviolet light by a chemical compound will produce a distinct spectrum which aids in the identification of the compound[12].

a. Chromophores :-
Any isolated compounds covalently bonded group that are responsible for imparting a color to the compound and shows a characteristic absorption of electromagnetic radiation in the UV-vis range. Eg. C=C, C=O.

Two types of chromophores are known which are as follows ,
1. Chromophores in which the groups have π electrons undergo π-π* transitions. For examples:-ethylenes, acetylenes etc.
2. Chromophores having both π- electrons and n (non-bonding) electrons undergo two types of transitions. i.e., π-π* and n-π*, for examples:- carbonyls, nitriles, azo compounds and nitro compounds etc[14].

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Group	Structure	nm
Carbonyl	> C = O	280
Azo	-N = N-	262
Nitro	-N=O	270
Thioketone	-C =S	330
Nitrite	-NO2	230
Conjugated Diene	-C=C-C=C-	233
Conjugated Triene	-C=C-C=C-C=C-	268
Conjugated Tetraene	-C=C-C=C-C=C-C=C-	315
Benzene		261

Fig.03. Chromophoric Group

b. Auxochromes :-

It is a group which itself does not act as a chromophore but when attached to a chromophore, it shifts the adsorption towards longer wavelength along with an increase in the intensity of absorption. Some commonly known auxochromic groups are: -OH, -NH2, -OR, -NHR, and –NR2. For example:- When the auxochrome –NH2 group is attached to benzene ring. Its absorption change from λ max 225 (εmax 203) to λmax 280 (ε_{max}1430)[14].

Auxochrome is colour enhancing group. Chromophore + Auxochrome = Newer Chromophore. The effect is due to its ability to extend the conjugation of a chromophore by sharing the non-bonding electrons. The new chromophore formed have a different value of absorption maximum as well as extinction coefficient.

c. Absorption shift and intensity shifts caused by Auxochrome :

Substituenst may have any four effects on a chromophore which are as followed:

i. Bathochromic Shift (red shift) : Absorption shifts towards longer wavelength. Those group which deepens the colour of chromogen are called as bathochromic groups. n-π* for carbonyl compound experiences bathochromic shift when polarity of solvent is decreased.

ii. Hypsochromic Shift (blue shift) : Absorption shifts towards shorter wavelength.

Those compound which diminish or lighten the colour of chromogen are called as hypsochromic groups. It occurs due to change of solvent towards higher polarity or removal conjugation.

iii. Hyperchromic Shift : Shift occurs towards higher absorbance due to increase in intensity -ε_{max} increases

Eg. Pyridine = 257nm and ε_{max} is 2750, 2-methyl pyridine = 262 nm and ε_{max} is 3560.

iv. Hypochromic Shift : Shift occurs towards lower absorbance due to decrease in intensity - ε_{max} decreases.

Eg. Biphenyl absorption is at 250 nm and 19000 ε_{max}[15]

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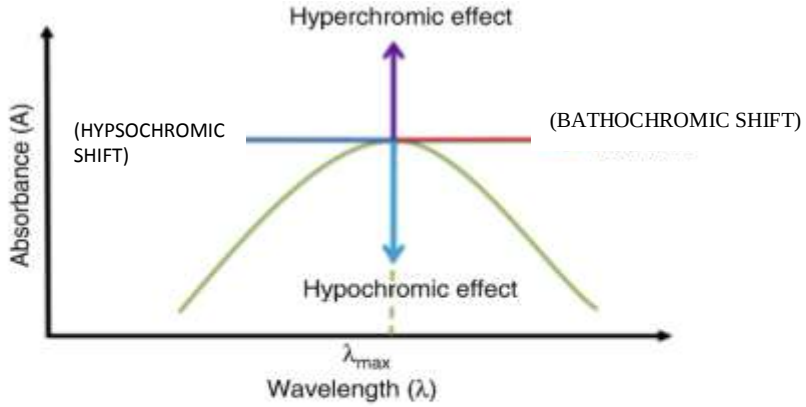

Descriptive term	Nature of the shift
Bathochromic shift (Red shift)	Towards longer wavelength
Hypsochromic shift (Blue shift)	Towards shorter wavelength
Hyperchromic effect	Towards higher absorbance
Hypochromic effect	Towards lower absorbance

Fig. 04. Absorption and intensity shift by auxochrome

Instrmentation of UV-vis Spectroscopy:

a. Light Source

Tungsten filament lamps and Hydrogen-Deuterium lamps are most widely used and suitable light source as they cover the whole UV region.

Tungsten filament lamps are rich in red radiations; more specifically they emit the radiations of 375 nm, while the intensity of Hydrogen-Deuterium lamps falls below 375 nm.

b. Monochromator

Monochromators generally is composed of prisms and slits.Most of the spectrophotometers are double beam spectrophotometers.The radiation emitted from the primary source is dispersed with the help of rotating prisms.The various wavelengths of the light source which are separated by the prism are then selected by the slits such the rotation of the prism results in a series of continuously increasing wavelength to pass through the slits for recording purpose.The beam selected by the slit is monochromatic and further divided into two beams with the help of another prism.

c. Sample and reference cells

One of the two divided beams is passed through the sample solution and second beam is passé through the reference solution.Both sample and reference solution are contained in the cells.These cells are made of either silica or quartz. Glass can't be used for the cells as it also absorbs light in the UV region.

d. Detector

Generally two photocells serve the purpose of detector in UV spectroscopy.One of the photocell receives the beam from sample cell and second detector receives the beam from the reference.The intensity of the radiation from the reference cell is stronger than the beam of sample cell. This results in the generation of pulsating or alternating currents in the photocells.

e. Amplifier

The alternating current generated in the photocells is transferred to the amplifier.The amplifier is coupled to a small servometer.Generally current generated in the photocells is of very low intensity, the main purpose of amplifier is to amplify the signals many times so we can get clear and recordable signals.Most of the time amplifier is coupled to a pen recorder which is connected to the computer.Computer stores all the data generated and produces the spectrum of the desired compound[12].

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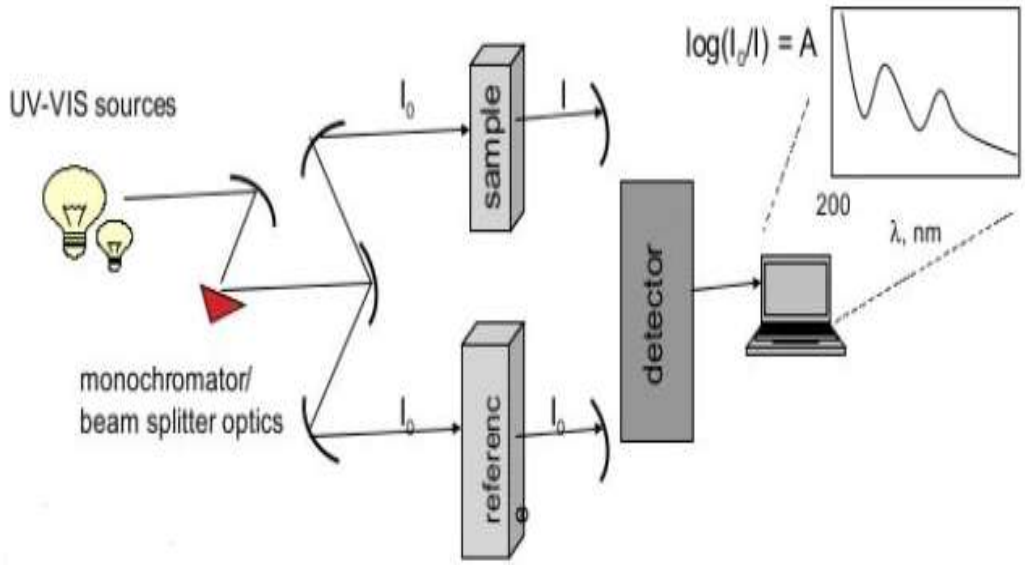

Fig. 05. Instrumentation of dual beam UV-VIS Spectrophotometer

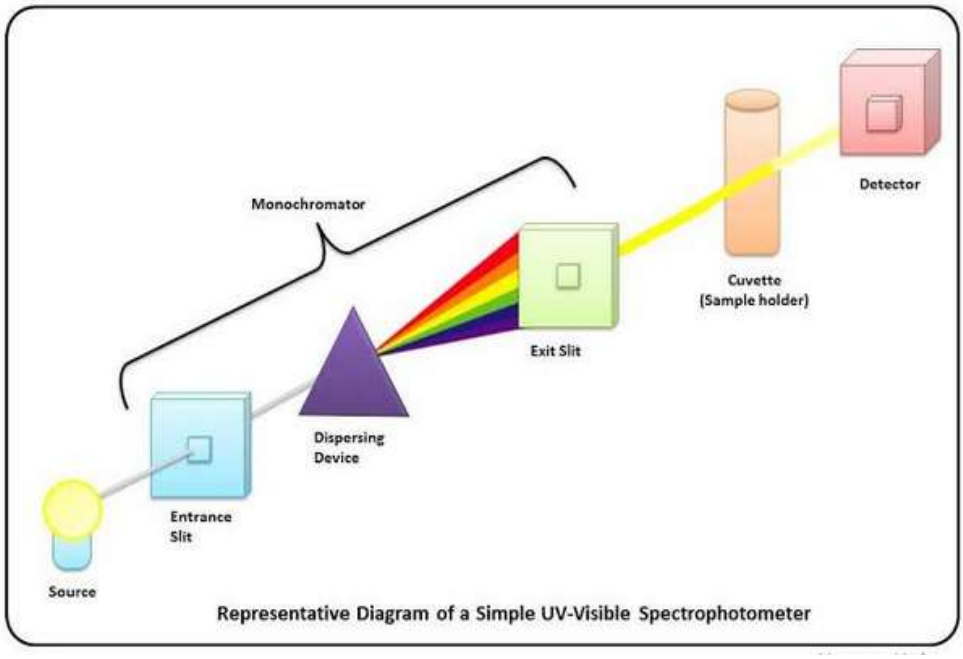

Fig. 06. Representative diagram of a simple UV-vis Spectrophotometer

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All of these instruments have a light source (usually a deuterium or tungsten lamp), a sample holder and a detector, but somehave a filter for selecting one wavelength at a time. The single beam instrument (fig.07) has a filter or a monochromator between the source and the sample to analyze one wavelength at a time. The double beaminstrument (fig.08) has a single source and a monochromator and then there is a splitter and a series of mirrors to get the beam to a reference sample and the sample to be analyzed, this allows for more accurate readings. In contrast, the simultaneousinstrument (fig.09) does not have a monochromator between the sample and thesource; instead, it has a diode array detector that allows the instrument tosimultaneously detect the absorbance at all wavelengths. The simultaneous instrumentis usually much faster and more efficient, but all of these types of spectrometers work well.[16]

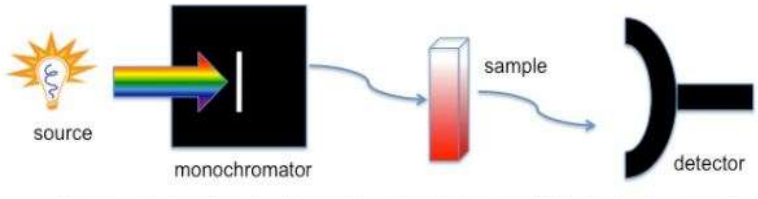

Fig. 07. Illustration of a single beam UV-vis instrument

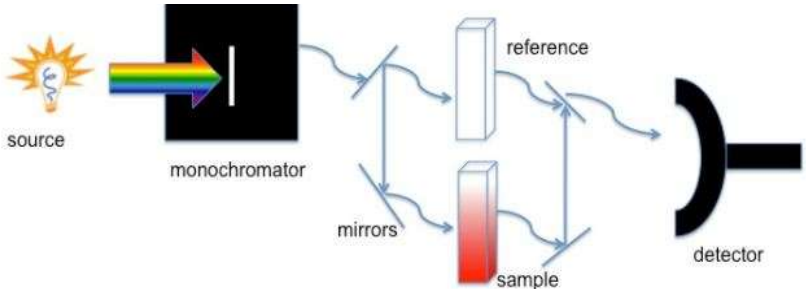

Fig. 08. Illustration of a double beam UV-vis instrument

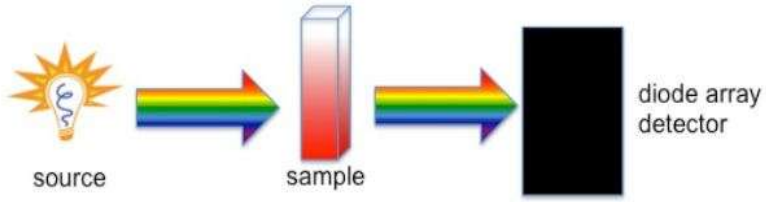

Fig. 09. Illustration of a simultaneous UV-vis instrument

Procedure :

- **Calibrate the Spectrophotometer:**

1) **Turn on the spectrophotometer.**
Most spectrophotometers need to warm up before they can give an accurate reading. Turn on the machine and let it stabilize for at least 15 minutes before running any samples.

2) **Prepare a blank solution.**
Fill a cuvette with the solvent for the sample. It has only the chemical solvent in which the solute to be analyzed is dissolved in.

3) **Wipe the outside of the cuvette.**
Before placing the cuvette into the spectrophotometer you want to make sure it is as clean as possible to avoid interference from dirt or dust particles. Using a lint free cloth, remove any water droplets or dust that may be on the outside of the cuvette.

4) **Place the cuvette in the spectrometer.**
Make sure to align the cuvette properly, as often the cuvette has two sides, which are meant for handling (may be grooved) and are not meant to shine light through.

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5) **Take a reading for the blank.**
The absorbance should be minimal, but any absorbance should be subtracted out from future samples. Some instruments might store the blank data and perform the subtraction automatically.A blank reference is needed at the very beginning of the analysis of the solvent and if concentration analysis needs to be performed, calibration solutions need to be made accurately[17].

- **Perform an Absorbance Spectrum:**

1. Fill the cuvette with the sample. To make sure the transfer is quantitative, rinse the cuvette twice with the sample and then fill it about ¾ full. Make sure the outside is clean of any fingerprints, etc.
2. Place the cuvette in the spectrometer in the correct direction.
3. Cover the cuvette to prevent any ambient light.
4. Collect an absorbance spectrum by allowing the instrument to scan through different wavelengths and collect the absorbance. The wavelength range can be set with information about the specific sample, but a range of 200–800 nm is standard. A diode-array instrument is able to collect an entire absorbance spectrum in one run.
5. From the collected absorbance spectrum, determine the absorbance maximum (λmax). Repeat the collection of spectra to get an estimate of error in λmax.
6.Calculate the transmittance and absorbance of the sample. Transmittance is how much of the light that passed through the sample reached the spectrophotometer. Absorbance is how much of the light has been absorbed by one of the chemicals in the solute.
8. Plot the absorbance values versus the wavelengths on a graph. The absorbance value is plotted on the vertical y-axis against the wavelength of light used for a given test plotted on the horizontal x-axis. Plotting the maximum absorbance values for each wavelength of light tested, produces the sample's absorbance spectrum and identifies the compounds making up the test substance and their proportions[17].

Choice of solvents & cuvettes :
Every solvent has a UV-vis absorbance cutoff wavelength. The solvent cutoff is the wavelength below which the solvent itself absorbs all of the light. So when choosing a solvent be aware of its absorbance cutoff and where the compound under investigation is thought to absorb. Table 03 provides an example of solvent cutoffs.

Water	Ethanol	Methanol	Choloroform	Ether	Acetone
Anthocyanins	Tannins	Anthocyanins	Terpenoids	Alkaloids	Phenol
Starches	Polyphenol	Terpenoids	Flavonoids	Terpenoids	Flavonols
Tannins	Polyacetylenes	Saponins		Coumarins	
Saponins	Flavonol	Tannins		Fatty acids	
Terpenoids	Terpenoids	Xanthoxylline s			
Polypeptides	Sterols	Totarol			
Lectins	Alkaloids	Quassinoids			
		Lactones Flavones Phenones			
		Polyphenols			

Table 01. Example of solvents along with the phytochemicals

The material the cuvette (the sample holder) is made from will also have a UV-visabsorbance cutoff. Glass will absorb all of the light higher in energy starting at about 300 nm, so if the sample absorbs in the UV, a quartz cuvette will be more practical as the absorbance cutoff is around 160 nm for quartz (Table 02)[16]

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Material	Wavelength Range (nm)
Glass	380-780
Plastic	380-780
Fused Quartz	< 380

Table 02. Three different types of cuvettes commonly used, with different usable wavelengths.

Solvent	UV Absorbance Cutoff (nm)
Acetone	329
Benzene	278
Dimethylformamide	267
Ethanol	205
Toluene	285
Water	180

Table 03. UV absorbance cutoff of various common solvents

Working :

The intensity of the sample beam is defined as **I.** Over a short period of time, the spectrometer automatically scans all the component wavelengths. The ultraviolet (UV) region scanned is normally from 200 to 400 nm, and the visible portion is from 400 to 800 nm.In UV-Vis, a beam with a wavelength varying passes through a solution in a cuvette. The sample in the cuvette absorbs this UV or visible radiation.

I_0 is the radiation coming in,I the radiation coming out

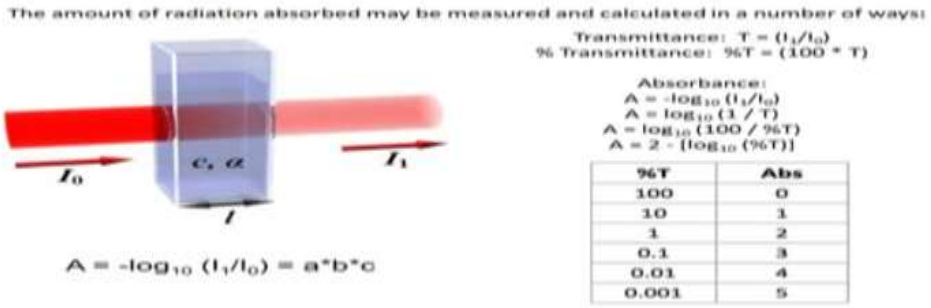

Fig. 10. Tramission of light beam through cuvette

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The amount of light that is absorbed by the solution dependson the concentration, the path length of the light through the cuvette and how well the analyte the light absorbs at a certain wavelength. The transmittance I/I0 is an indication of the concentration of the analyte in the sample. I/I0 is defined as the transmittance (or transmission) T. If there is no absorption of the light passing through the solution, the transmittance is 100%.The most-used term in UV-Vis spectrometry to indicate the amount of absorbed light is the absorbance, defined as: $A = - \log 10\ T = -\log 10\ (I/I_0).1/T = 10(A)$ The relation of absorbance to concentration is given by **Lambert-Beer's law (Beer's law)**: $A = \varepsilon lc$

(where A is absorbance (unitless), ε (epsilon) is molar absorption coefficient (or molar absorption constant) of the analyte for a certain wavelength ($l \cdot mol^{-1} \cdot cm^{-1}$), l is path length (cm) through your cuvette and c is the concentration of the analyte ($mol \cdot l^{-1}$)**.**From the Beer–Lambert law it is clear that the greater the number of molecules capable of absorbing light of a given wavelength, the greater the extent of light absorption. This is the basic principle of UV spectroscopy[18].

A diagram of the components of a typical spectrophotometer are shown in the following diagram

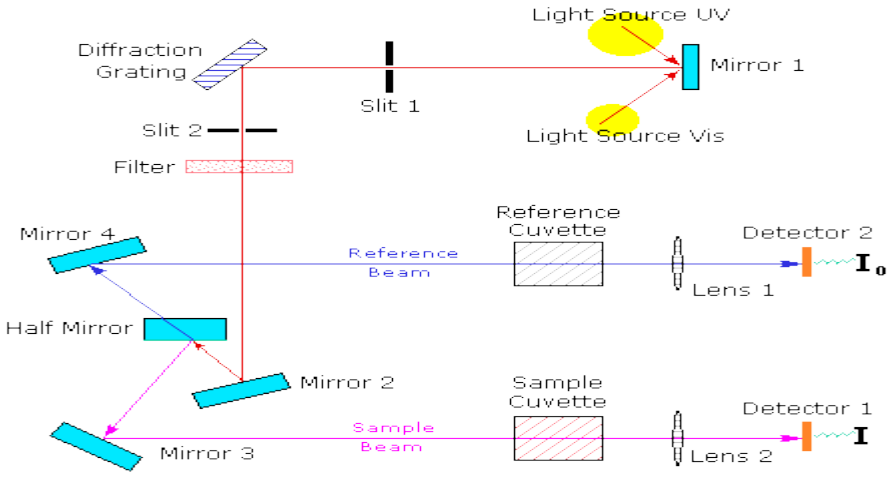

Fig. 11. Optical arrangement in a dual beam spectrophotometer

The functioning of this instrument is relatively straightforward.

- A beam of light from a visible and/or UV light source (colored red) is separated into its component wavelengths by a prism or diffraction grating.
- Each monochromatic (single wavelength) beam in turn is split into two equal intensity beams by a half-mirrored device. One beam, the sample beam (colored magenta), passes through a small transparent container (cuvette) containing a solution of the compound being studied in a transparent solvent.
- The other beam, the reference (colored blue), passes through an identical cuvette containing only the solvent.
- The intensities of these light beams are then measured by electronic detectors and compared.

The intensity of the reference beam, which should have suffered little or no light absorption, is defined as $\mathbf{I_0}$. The intensity of the sample beam is defined as **I**. Over a short period of time, the spectrometer automatically scans all the componentwavelengths in the manner described. The ultraviolet (UV) region scanned is normally from 200 to 400 nm, and the visible portion is from 400 to 800 nm.If the sample compound does not absorb light of of a

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given wavelength, $I = I_0$. However, if the sample compound absorbs light then I is less than I_0, and this difference may be plotted on a graph versus wavelength. Absorption may bepresented as transmittance ($T = I/I_0$) or absorbance ($A = \log I_0/I$). If no absorption has occurred, T = 1.0 and A= 0. Most spectrometers display absorbance on the vertical axis, and the commonly observed range is from 0 (100% transmittance) to 2 (1% transmittance). The wavelength of maximum absorbance is a characteristic value, designated as λ_{max}.Different compounds may have very different absorption maxima and absorbances. Intenselyabsorbing compounds must be examined in dilute solution, so that significant light energy is received by the detector, and this requires the use of completely transparent (non-absorbing) solvents.

The absorbance of a sample is proportional to its molar concentration in the sample cuvette, a corrected absorption value known as the molar absorptivity is used when comparing the spectra of different compounds. This is defined as:

Molar Absorptivity,ε = A/ cl (where A= absorbance, **c** = sample concentration in moles/liter & **l** = length of light path through the cuvette in cm.)[19]

The UV-Vis spectrum shows the absorbance of one or more sample component in the cuvette when we scan through various wavelengths in the UV/Vis region of the electromagnetic spectrum.An optical spectrometer records the wavelengths at which absorption occurs, together with the degree of absorption at each wavelength known as absorption spectrum. The resulting spectrum is presented as a graph of absorbance (A) versus wavelength.

- The x-axis (horizontal) shows the wavelength.
- The y-axis (vertical) shows the dependent variable; the absorbance[18].

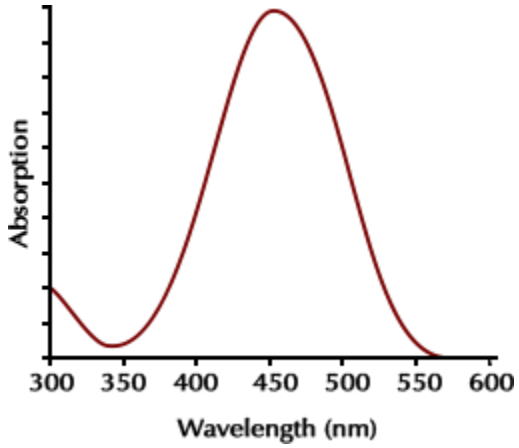

Fig. 12. Absorption Spectrum

Example :

Let's do a phytochemical analysis of Senna Alexandrina by UV- VISSpectrophotometer to investigates the characterization of the bioactive constituents present in leaf extract.

1. Plant Material

The leaf of Senna alexandrina was purchased from the local market, it was cleaned and dried under shade and made them into powder and the selected solvents Methanol, Acetone and ethyl acetate were procured from the National scientific products, Guntur and were used for the preparation of plant extracts by Soxhlet method, the extract were dried and stored in cool conditions for further use. The dried leaves thencrushed in mechanical grinder till it becomes a fine powder and then it was stored in air tight container at room temperature.

2. Phytochemical Screening

The methanolic extract of Senna Alexandrina was subjected to phytochemicalscreening for the presence of various compounds like alkaloids and flavonoids.

- **Preparation of reagents :**

Phosphate buffer solution (pH 4.7):

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The buffer solution was prepared by adjusting the pH of 2M sodium phosphate (71.6 g Na2HPO4 in 1 L distilled water) to 4.7 with 0.2 M Citric acid (42.02 g citric acid in 1L distilled water).

Sample Preparation:

10 mg of the plant extract was taken and is dissolved in the 10ml of Ethanol which is sonicated for 10minutes. From this 1ml of the extract was taken and was employed for further analysis.

A. Estimation of Alkaloids

- **Standard Preparation:**

Atropine was taken as a standard for the estimation of the Alkaloid content in the plant extract. 10mg of Atropine was taken which is dissolved in 10ml with the suitable solvent i.e., methanol and it is sonicated for 5minutes. This is the stock solution of concentration of 1000 µg/ml which was kept aside for further use. From the stock solution, 1ml was taken and 9ml of same solvent is added to obtain the concentration of 100µg/ml solution and then again 1ml was taken from this which is diluted to 10ml with the same solvent to get 10 µg/ml solutions. This process was repeated till the concentration of 1 µg/ml was obtained.

- **Preparation of Standard Calibration curve:**

Calibration curve was prepared by taking 2ml, 4ml, 6ml, 8ml, 10ml and 12ml solutions from the concentration of 10 µg/ml and applying the procedure for the estimation of Alkaloids and the standard curve was plotted by taking the sample concentration on X-axis and absorbance values on Y-axis.

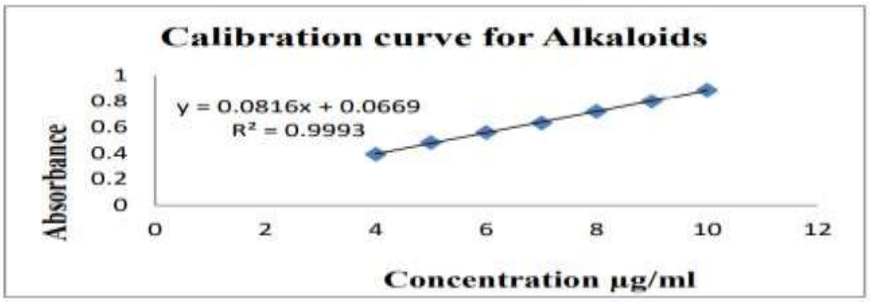

Fig. 13. Standard calibration curve for the estimation of Alkaloids in Senna

- **Procedure for the estimation of Alkaloid content:**

1ml of the extract was added with 5 ml of phosphate buffer (pH 4.7) and 5 ml BCG solution and the mixture was shaken with 4 ml of Chloroform. The extract was collected in a 10-ml volumetric flask and is diluted to make the final volume with Chloroform. The blank was prepared as above but without the extract and the absorbance of the complex in chloroform was measured at 470 nm against the blank. And the results of the assay were compared with Atropine equivalents.

Sr. No	Name of Phytochemical compound	Senna alexandrina	
		Methanol extract Absorbance(nm)	Amount
1.	Alkaloids	0.457	0.483

Table 04. Results of Total Alkaloids Estimation of Senna Alexandrina

B. Estimation of Flavonoids :

- **Standard Preparation**

Quercetin was taken as a standard for the estimation of the Flavonoid content in the plant extract. 10mg of Quercetin was taken and dissolved in 10ml of the Methanol and it is sonicated for 5minutes. This is the stock solution of 1000 µg/mlconcentration which was kept aside for further use. 1ml of this

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solution was taken and 9ml of solvent is added to obtain thesolution with a final concentration of 100µg/ml from which 1ml was taken and is further diluted with the solvent to get the solution with 10 µg/ml concentration. The same process was repeated till the concentration of 1 µg/ml was obtained.

- **Preparation of Standard Calibration curve**

Calibration curve was prepared by taking 1ml, 2ml, 3ml, 4ml, 5ml and 6ml solutions from the concentration of 1 µg/ml and applying estimation procedure of Flavonoids. A graph was plotted by taking absorbance on Y-axis andconcentration on X-axis. The values obtained are substituted in the regression equation i.e., Y=mx+c2 . Here Y is average absorbance of the unknown sample, term m shows slope and c indicates intercept.

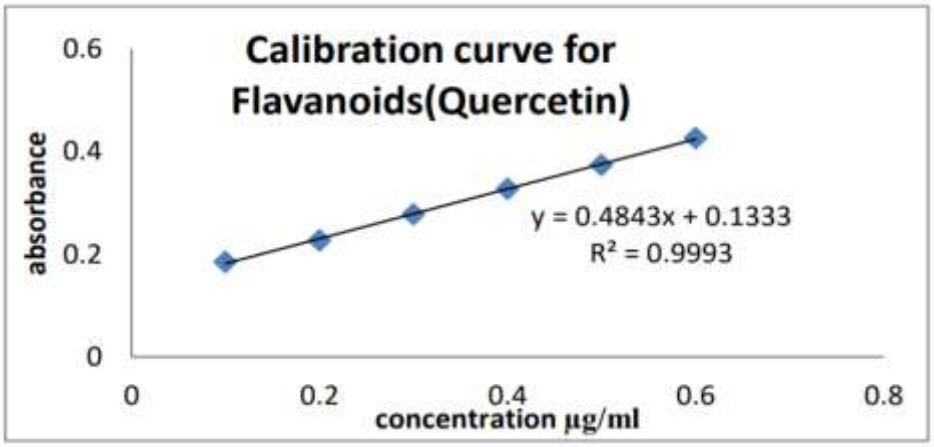

Fig. 14. Standard calibration curve for the estimation of Flavonoids in Senna Alexandrina

- **Procedure for the estimation of total Flavonoids:**

Total flavonoid content was estimated using Aluminium chloride (AlCl3) according to a known method, using Quercetin as a standard. 1ml of the plant extract was taken in a test tube which is added with 2ml of 5% NaNO2and 3 ml of AlCl3 (10%) was added to this after 5 min. Then the reaction mixture was treated with 2 ml of 1 M NaOH for another 5 min and finally the reaction mixture was made up to 10 ml with water and the absorbance was measured at 510 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was taken. For the blank, the extracts were replaced with an equal volume of distilled water. The Flavonoids content in extracts was expressed in terms of Quercetin equivalents.

Sr. No	Name of Phytochemical compound	Senna alexandrina Methanol extract	
		Absorbance(nm)	Amount
1.	Flavonoids	0.162	0.616

Table 05. Results of Total Flavonoids Estimation in Senna Alexandrina.

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- **Results of Quantitative analysis of the selected Phytochemical compounds from Senna alexandrina plant extract:**

The quantitative analysis of the same plant extract by UV Visible spectrophotometric method with Bromocresol green and Atropine As the standard for alkaloids and Aluminium chloride method with quercetin as standard for Flavonoids were carried out. The standard calibration curves obtained for the estimation of Alkaloids and Flavonoids were given in Figure 13 and 14 respectively. Based on the results of quantitative analysis it was confirmed that leaf extract of Senna alexandrina contains 0.483mg/g of alkaloids and0.616mg/g of Flavonoids respectively as it was shown in Tables 04 and 05.

3.Identification of Bioactive Compounds by UV-VIS Spectroscopic analysis:

UV-VIS Spectra:

The extract was examined under UV-visible light for proximate analysis. These spectral measurements are important for screening the crude plant extract for the presence of particular classes of compounds in their identification. The extract was centrifuged at 3000 rpm for 10 min and filtered through Whatman No.1 filter paper by using a high pressure vacuum pump and was subjected to UV-VIS spectrophotometric analysis. The sample was diluted to 10 times with the same solvent and the extract was analysed in the UV wavelength ranging from 200-800 nm by employing Perkin Elmer Spectrophotometer and the characteristic peaks were detected.

Sr. No.	Senna alexandrina	
	Wavelength (nm)	Absorption value
1.	449.01	2.727
2.	532.00	0.837
3.	606.0	0.589
4.	665.0	1.276

Table 06. Peak values in UV-VIS Spectrum of Senna alexandrina extract.

4. Result of UV-Vis Spectroscopic analysis of Senna Alexandrina :

Results of the Bioactive compound identification by UV-Visible Spectrophotometric Analysis are as follows :-

The UV-VIS profile of plant extract was analysed at 200 to800 nm wavelength to identify the compounds containing σ- bonds, π-bonds, andlone pair of electrons, chromophores and aromatic rings. The profile showed the peaks at 449.01nm, 532.00 nm, 606.0 nm and 665.0 nm with the absorbance values of 2.727, 0.837, 0.589 and 1.276 Senna alexandrina.The various phytochemicals including flavonoids, alkaloids, carbohydrates and protein were found to be present in the extract of Senna Alexandrinaby the spectroscopic studies showing the characteristic peaks obtained in Ultraviolet - Visible region.

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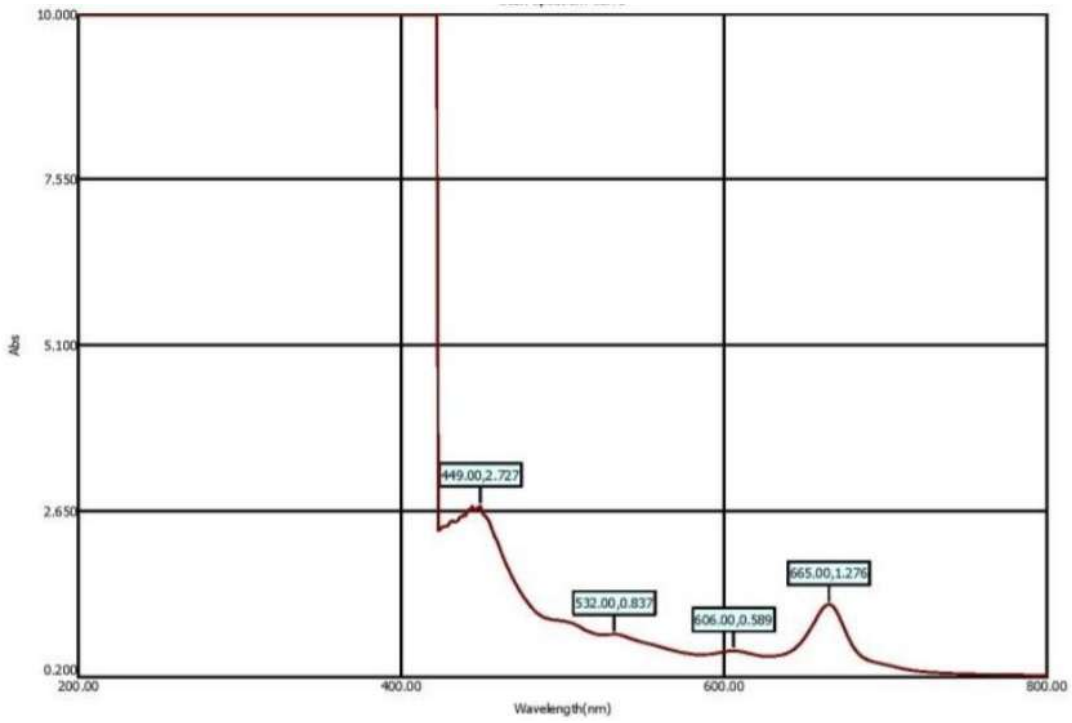

Fig. 15.UV-Vis Spectra of methanolic extract of Senna Alexandrina.

Thus the phytochemical profile showed the occurrence of peaks with in the 234-800 nm range of UV, which confirms the presence of phenolics, flavonoids and alkaloids in the leaf extract of Senna alexandrina. The results of the preliminary phytochemical studies, basing on biochemical protocols have confirmed that the extracts of the plants how positive results for more number of phytochemical components as shown in Table 3.1 and it can be concluded that both the plant contain Alkaloids and Flavonoids predominantly in methanolic and ethyl acetate extracts. These phytochemicals could contribute to the various medicinal applications of Senna alexandrina plant. The current results are in line with that the secondary metabolites (phytochemicals) and other chemical constituents of the medicinal plant are responsible for their medicinal values[20, 21, 22, 23, 24].

III. CONCLUSION :

From the above study, it is concluded that the UV-VIS spectroscopy can be performed for quantitative analysis of phytochemicals and for identification of certain classes of chemical compound in both pure and biological mixtures. The bioactive substances such as phenolic compounds, flavonoids, alkaloids, carotenoids, tannins and antioxidants can be derived from plants using UV-vis Spectroscopy.The basis for the identification is that the absorption spectrum shows a number of absorption bands associated with structural units within the molecule.Phytochemical analysis deals with the extraction, screening and identification of the medicinally active substances found in plants. The phytochemicals derived from various parts of plants are used as sources of direct medicinal agents and serve as a raw material base for elaboration of more complex semi-synthetic chemical compounds. Further, these compound can be isolated further screened for different kind of biological activities depending on their therapeutic uses.

REFERENCE :

[1]. Hasler CM and Blumberg JB (1999) Phytochemicals: Biochemistry and physiology. Introduction. Journal of Nutrition 129: 756S–757S.

[2]. Mathai K(2000). Nutrition in the Adult Years. In Krause‟s Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K

[3]. Introduction to phytochemicals:Secondary Metabolites from plants with active principles

for pharmacological importance- By Nadia Mendoza and Eleazer M. Escamilla Silva
[4]. Handbook of Plant Food Phytochemicals: Sources, Stability and Extraction - By Rocio Campos-Vega and B. Dave Oomah
[5]. Krishnaiah D, Sarbatly R, Bono A (2007) Phytochemical antioxidants for health and medicine: A move towards nature. Biotechnol Mol Biol Rev 1: 97-104.
[6]. Willard-Hobart H, Merritt, JLL, Dean, JA. Instrumental Methods of Analysis, fifth ed. Von Nostrand, University of Michigan, 1974.
[7]. Chatwal GR, Anand S. Instrumental Methods of Chemical Analysis, fifth ed. Himalaya Publishing House, New Delhi, 2002.
[8]. Davidson AG. Ultraviolet-visible absorption spectrophotometry, In: Beckett AH, Stenlake JB. Practical Pharmaceutical chemistry, fourth ed. CBS Publishers and distributors, New Delhi, 2002, 275-278.
[9]. Markham KR. Techniques of flavonoid identification. London:Academic Press; 1982
[10]. Pharmacognosy book by Dr. C.K. Kokate, A.P. Purohit and S.B. Gokhale
[11]. General Techniques Involved in Phytochemical Analysis International Journal of Advanced Research in Chemical Science (IJARCS) Volume 2, Issue 4, April 2015, PP 25-32
[12]. https://microbenotes.com/uv-spectroscopy-principle-instrumentation-applications/
[13]. https://www.slideshare.net/mariomS7/uvvis-spectroscopy
[14]. https://www.pharmatutor.org/pharma-analysis/define-and-exemplify-chromophores
[15]. https://www.slideshare.net/krakeshguptha/chromophore
[16]. https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Book%3A_Physical_Methods_in_Chemistry_and_Nano_Science_(Barron)/04%3A_Chemical_Speciation/4.04%3A_UV-Visible_Spectroscopy
[17]. https://www.jove.com/v/10204/ultraviolet-visible-uv-vis-spectroscopy
[18]. https://www.chromedia.org/chromedia?waxtrapp=fotjtbEsHiemBpdmBlIEcCFpB&subNav=lnijabEsHiemBpdmBlIEcCFpBN
[19]. https://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/spectrpy/uv-vis/uvspec.htm
[20]. Jasper C, Maruzzella JC, Henry PA. (1958): The antimicrobial activity of perfume oils. J Am Pharm Asso 47:471-476.
[21]. Sofowora A. (1993): Medicinal Plants and Traditional Medicine in Afric. John Wiley and son Ltd 150-153.
[22]. Amir Muhammad Khan, Rizwana Aleem Qureshi, Faizan Ullah, Syed AneelGilani,AsiaNosheen, SumairaSahreen, Muhammad Khan Laghari, Muhammad Yousif Laghari, Shafiq-Ur-Rehman1, Ishtiaq Hussain and Waheed Murad.(2011):"Phytochemical analysis of selected medicinal plants of Margalla Hills and surroundings Journal of Medicinal Plants Research" 5(25):6017- 6023
[23]. Thite SV., Chavan YR., Aparadh VT and Kore BA. (2013): "Preliminary Phytochemical Screening of Some Medicinal Plants". IJPCBS 3(1):87-90.
[24]. Minakshi B, Jharna D, Chanbi Devi E, Nayan T, Partha PK, Kundal N and Manash(2016): Phytochemical Analysis of Traditional Medicinal Plants and their Antimicrobial Activity: An Experience from North East India Open Access Journal of Pharmaceutical Research 1(1):1-7.