CHAPTER I: INTRODUCTION

Forensic science is the application of scientific knowledge and methodology to criminal investigations and legal problems. Forensic scientists are who help to collect, preserve, and examining physical evidence during the course of an investigation.[1] Forensic Scientist must be methodical, accurate and unbiased. There are so many fields in Forensic science which may help in solving the crimes. In that field Forensic Toxicology is an important field that help to solving the crimes that related the determination of drug use, poisoning, or exposure to toxic substances as part a legal investigation. In forensic toxicology I studied the poisonous cases which related to plants. In plant poisoning cases I studied the poisoning and analysis of Cerbera Odollam using IR spectroscopy.

Cerbera odollam is a dicotyledonous angiosperm, a plant species in the family Apocynaceae and commonly known as the Suicide Tree, Pong_Pong, Mintolla, and Othalam. It bears a fruit known as Othalanga that yields a potent poison that has been used for suicide and murder. It is a species native to India and other parts of southern Asia, growing preferentially in coastal salt swamps and in marshy areas but also grown as a hedge plant between home compound. Its fruit, when still green, looks like a small mango, with a green fibrous shell enclosing an ovoid kernel measuring approximately 2 cm × 1.5 cm and consisting of two cross-matching white fleshy halves. On exposure to air, the white kernel turns violet, then dark grey, and ultimately brown, or black. Its killing effect is due to the excessively toxic chemical called *cerberin* in its seeds. Cerberin is a cardiac glycoside, which is a class of organic compounds that slows your heart rate.[22] There's enough cerebin in one Cerbera odollam seed to kill an adult human. The way it works to stop your heart is similar to that of lethal injection.[17][18]

The kernels of *Cerbera odollam* contain *cerberin*, a digoxin-type cardenolide and cardiac glycoside toxin that blocks the calcium ion channels in heart muscle, causing disruption of the heartbeat, most often fatally.[12] The most common

symptom of toxicity in humans was noted to be vomiting.[5] Electrocardiographic abnormalities were noted to be common, the most common being sinus bradycardia.

Around half of the patients develops thrombocytopenia.[4][14] Temporary cardiac pacing has been used in the management, apart from other supportive measures. The difficulty in detecting cerberin in autopsies and the ability of strong spices to mask its taste makes it an agent of homicide and suicide in India;[19] there were more than 500 cases of fatal Cerbera poisoning between 1989 and 1999 in the southwest Indian state of Kerala. In kerala, this plant is responsible for an average of 50 deaths a year; and approximately 70% victims are women.[7] Although the plant is a well-known poison in many regions of Madagascar and Southeast Asia, the prevalence of Cerbera odollam poisonings has not been well documented in these regions.[10] This plant is responsible for more suicide deaths than any other plant in Kerala. The toxicity of cerbera odollam is more danger than the other poisonous plants.[8][9]

A fatal dose of the poison is contained in one kernel, leading to death within 1–2 days. Common symptoms include: burning sensation in mouth, violent vomiting, irregular respiration, headache, irregular heartbeat, coma and eventual death.

The kernels found at the core of the fruit are toxin rich, and this is the part used for suicidal and homicidal purposes.[5] The fatal amount is probably dependent on the body mass index of the consumer. Animal studies have demonstrated that ingestion of extremely small amounts of the kernel can result in death with a lethal dose of 1.8mg/kg and 3.8mg/kg in dogs and cats respectively. Hence, ingesting a half or whole kernel could be sufficient to cause death. The green husk of the seed is removed to extract the fleshy kernel.[16] The kernel is then mixed with sweet or spicy food in an effort to mask the bitter taste of the poison and then consumed. Death usually occurs within 3 to 6 hours after ingestion.

Figure 1 – Cerbera odollam tree

Figure 2- Cerbera odollam fruit

Figure 3- Cerbera odollam dried fruit

Figure 4- Cerbera odollam leaf

IR spectroscopy deals with the infrared region of the electromagnetic spectrum, i.e. light having a longer wavelength and a lower frequency than visible light. Infrared Spectroscopy generally refers to the analysis of the interaction of a molecule with infrared light. The IR spectroscopy concept can generally be analyzed in three ways: by measuring reflection, emission, and absorption. The major use of infrared spectroscopy is to determine the functional groups of molecules, relevant to both organic and inorganic chemistry, using the FTIR for getting more accurate result.

It is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid, or gas.[1] An FTIR spectrometer simultaneously collects high spectral range. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelengths at a time

IR Spectroscopy detects frequencies of infrared light that are absorbed by a molecule. Molecules tend to absorb these specific frequencies of light since they correspond to the frequency of the vibration of bonds in the molecule. An IR spectrum is essentially a graph plotted with the infrared light absorbed on the Y-axis against. frequency or wavelength on the X-axis. The energy required to excite the bonds belonging to a molecule, and to make them vibrate with more amplitude, occurs in the Infrared region. A bond will only interact with the electromagnetic infrared radiation, however, if it is polar. The presence of separate areas of partial positive and negative charge in a molecule allows the electric field component of the electromagnetic wave to excite the vibrational energy of the molecule.[6]

Most of the bands that indicate what functional group is present are found in the region from 4000 cm-1 to 1300 cm-1. Their bands can be identified and used to determine the functional group of an unknown compound.[2]

Bands that are unique to each molecule, similar to a fingerprint, are found in the fingerprint region, from 1300 cm-1 to 400 cm-1. These bands are only used to compare the spectra of one compound to another.

The samples used in IR spectroscopy can be either in the solid, liquid, or gaseous state. Solid samples can be prepared by crushing the sample with a mulling agent which has an oily texture. A thin layer of this mull can now be applied on a salt plate to be measured.

Liquid samples are generally kept between two salt plates and measured since the plates are transparent to IR light. Salt plates can be made up of sodium chloride, calcium fluoride, or even potassium bromide.

Since the concentration of gaseous samples can be in parts per million, the sample cell must have a relatively long pathlength, i.e. light must travel for a relatively long distance in the sample cell.

Thus, samples of multiple physical states can be used in Infrared Spectroscopy. The IR spectroscopy theory utilizes the concept that molecules tend to absorb specific frequencies of light that are characteristic of the corresponding structure of the molecules. The energies are reliant on the shape of the molecular surfaces, the associate a vibronic coupling, and the mass corresponding to the atoms.[15]

For instance, the molecule can absorb the energy contained in the incident light and the result is a faster rotation or a more pronounced vibration.

First, a beam of IR light from the source is split into two and passed through the reference ant the sample respectively.

Now, both of these beams are reflected to pass through a splitter and then through a detector. Finally, the required reading is printed out after the processor deciphers the data passed through the detector.

Infrared spectroscopy is widely used in industry as well as in research. It is a simple and reliable technique for measurement, quality control and dynamic measurement. It is also employed in forensic analysis in civil and criminal analysis.

Some of the major applications of IR spectroscopy are as follows:

- Identification of functional group and structure elucidation
- Identification of substances
- Studying the progress of the reaction
- Detection of impurities
- Quantitative analysis

The term Fourier transform infrared spectroscopy originates from the fact that a Fourier transform is required to convert the raw data into the actual spectrum.

A common FTIR spectrometer consists of a source, interferometer, sample compartment, detector, amplifier, A/D convertor, and a computer.

The source generates radiation which passes the sample through the interferometer and reaches the detector.

Then the signal is amplified and converted to digital signal by the amplifier and analog-to-digital converter, respectively. Eventually, the signal is transferred to a computer in which Fourier transform is carried out.[11]

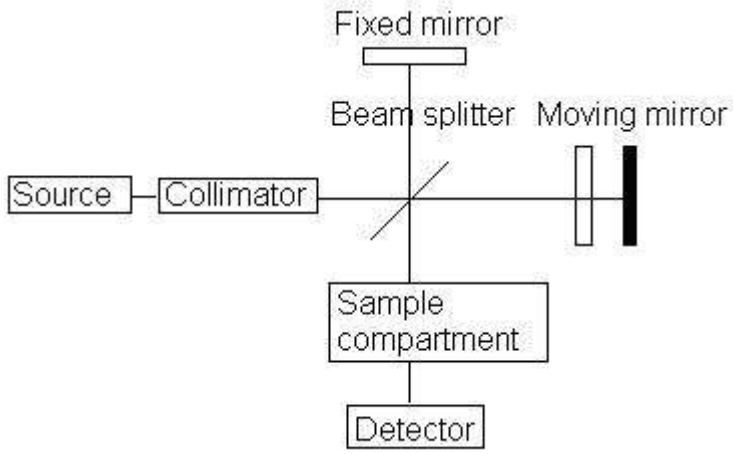


Figure 5- FTIR instrumentation

FTIR spectrometers are mostly used for measurements in the mid and near IR regions. For the mid-IR region, 2−25 μm (5000–400 cm−1), the most common source is a silicon carbide element heated to about 1200 K (Globar). The output is similar to a blackbody.[3] Shorter wavelengths of the near-IR, 1−2.5 μm (10000–4000 cm−1), require a higher temperature source, typically a tungsten-halogen lamp. The long wavelength output of these is limited to about 5 μm (2000 cm−1) by the absorption of the quartz envelope.

For the far-IR, especially at wavelengths beyond 50 μm (200 cm−1) a mercury discharge lamp gives higher output than a thermal source.

An ideal beam-splitter transmits and reflects 50% of the incident radiation. However, as any material has a limited range of optical transmittance, several beam-splitters may be used interchangeably to cover a wide spectral range.

For the mid-IR region, the beamsplitter is usually made of KBr with a germanium-based coating that makes it semi-reflective. KBr absorbs strongly at wavelengths beyond 25 μm (400 cm−1) so CsI is sometimes used to extend the range to about 50 μm (200 cm−1).

ZnSe is an alternative where moisture vapor can be a problem but is limited to about 20μm (500 cm−1). CaF2 is the usual material for the near-IR, being both harder and less sensitive to moisture than KBr but cannot be used beyond about 8 μm (1200 cm−1). In a simple Michelson interferometer one beam passes twice through the beamsplitter but the other passes through only once. To correct for this an additional compensator plate of equal thickness is incorporated.

Far-IR beamsplitters are mostly based on polymer films and cover a limited wavelength range.[20] It is one accessory of FTIR spectrophotometer to measure surface properties of solid or thin film samples rather than their bulk properties. Generally, ATR has a penetration depth of around 1 or 2 micrometres depending on your sample conditions.

The interferogram in practice consists of a set of intensities measured for discrete values of retardation. The difference between successive retardation values is constant. Thus, a discrete Fourier transform is needed. The fast Fourier transform (FFT) algorithm is used.[23]

Cerbera odollam is a powerful toxic plant that is responsible both for suicide and homicide. The seeds are excessively toxic, containing cerberin as the main active cardenolide. Cerberin is difficult to detect in autopsies and its taste can be masked with strong spices.

Therefore, it is often used in homicide and suicide especially in India. Pathologists would not be able to identifty Cerbera poisoning unless there is evidence the victim had eaten the plant. In this research I want to highlight the recent incidence of poisoning, present analytical techniques used as well the recent advances in identification of the *cerberin toxin.*[21]

In the past studies around 20 researches are done that related to Cerbera odollam, methods like TLC, HPLC, UV, chemical analysis, etc. In the present study that to identify the composition of odollam by using IR spectroscopy. And which also help to the identification of glycosides present in the plant. In the present study to collect the Cerebera odollam leaf and fruit for the analysis.

CHAPTER II: LITERATURE REVIEW

Ritesh G. Menzes et.al (2018) studied Cerbera Odollam ToxicityThey use TLC for detect the Cerbera Odollam poisoning and the biologically active compound cerberin appears negative on digoxin assays. The chromatograms from the plant extract and autopsy tissue are compared to identify the presence of the toxin in the tissue. Another technique is High Performance Liquid Chromatography coupled with tandem Mass Spectrometry has been shown to be highly effective detecting a variety of plant poisons. Cerbera Odollam contains a glycoside very much like digitalis that is potentially cardiotoxic and even fatal. While the plant is endemic in South India. Currently there are no laboratory techniques to quickly detect the toxin of cerbera odollam in emergency cases. Administration of digoxin immune fab could also be considered in severe Cerbera Odollam poisoning despite limited data.

Tran Thi Minh Hien, Ch. Navarro-Delmasure and Tran Vy (2002) studied Toxicity and Effect on the Central Nervous System of a Cerbera Odollam leaf extract. Necropsy was performed on all animal dying after administration of the extract and on all 30 days survivors. They use macroscopic examination externally and the heart, aorta, spleen, kidneys, liver, lungs. Anthiconvsulant potential of the leaf extract was studied by investigating its capacity to a tagonize pentylenetetrazole included convulsions. The toxicity of C odollam total leaf extract by the given as a single dose to mice. The largest dose never fatal was 14.5 g/kg and the dose consistently killing all the animals was equivalent to 30g/kg of dried leaves. Most of the animals are dying due to the eating of this plant leaf.

S. S Prasanth and Rajasekaran Aiyula (2015) Quantitative Determination of Cerberin in seed extract of *Cerbera odollam* and Rat Serum by HPLC. Preparation of standard solution by taking 2 mg of marker standard cerberin was dissolved in 2ml of methanol to get cerberin and solution was used for hplc analysis.

Preparation of plant seed extract that is ripe seed of *Cerbera odollam* were collected and about 14.2 g was extracted with ethyl acetate – ethanol mixture [1:1]. Chromatography technique by using HPTLC. The developed HPTLC technique is precise, specific and accurate for the determination of cerberin in plant seed extract and rat serum. The cerberin in plasma and other biological fluids of human in case of accidental or intentional poisoning. This method can used for detection of *Cerbera Odollam* poisoning by clinical toxicology.

K. Malathy and A. Krishnamoorthy (2002) Detection of *Cerbero Odollam* by Thin layer chromatography the sample of viscera was extracted with ethanol ethyl acetate (1: 1). Then they do the TLC technique for the detection of *Cerbera odollam.* The proposed technique of extraction and detection was successfully applied in several cases of poisoning by C odollam. The components of C.odollam were extracted from different autopsy tissue by the proposed method. We find out the Rf value of *Cerbera odollam* by using TLC.

Mary E. Wermuth et.al (2018) Cardiac toxicity from intentional ingestion of pong pong seeds, they use various case study for the detection of the cardiac toxicity and also study the symptoms of the poisoning persons. To the determination and the study of case study the treatment approach for patients in their case series was variable. Most patients received conventional treatment for bradycardia and hyperkalaemia.

UK Ramakrishnan, VV Pillay, SL Arathy Forensic implication of viscera analysis in death due to *cerbera odollam* poisoning the main aim of this study was to perform thin layer chromatography on plant extract of *cerbera odolam* and Manihot esculenta and ascertain. And also used the HPLC to find out whether it can be differentiated with two compounds they get the Rf value of the particular *cerbera odollam* plant.

Charan K Shetty (2016) Forensic Relevance of the Suicide Tree' *Cerbera Odollam'* using TLC, HPLC they are mainly used and then they used the method for the determination of cerberin by UPLC – MS method.

By using the analytical method that help the detection of *cerbera odollam* poisoning it also help the law enforcement to solve cases.

F Ahmed et.al (2008) Antibacterial, cytotoxic and Neuropharmacological activities of *Cerbera Odollam* seeds. Plant material collection and extraction, test for different chemical groups in animals, microorganisms, drugs, and antibacterial activity, cytotoxic activity and neuropharmacological activity. The MeOH extracted from the cerbera seed that possesses antibacterial, cytotoxic, and CN depressant effects which correlate the traditional use of plant.

Firoj Ahmed et.al. (2006) Antinociceptive and sedative effects of the bark of *Cerbera Odollam* plant material collection and extraction, pharmacological actives the crude extract of *cerbera odollam* may possess anti – nociceptive and CNS depressant effect. However further researches are necessary to find out the active principle responsible for these activities.

Yvan Gaillard et.al (2004) *Cerbera Odollam*: a suicide tree and cause of death in the state of Kerala. Pharmacological action of crude alcoholic extract of odollam seeds, using TLC and HPLC to determine the Rf values of solvent. This retrospective study has made it possible to bring to light an extremely toxic plant that is relatively unknown to physicians and forensic toxicologist. To the best knowledge no plant in the world is responsible for many deaths by suicide as the odollam tree.

CHAPTER III: AIMS AND OBJECTIVES

Aim:

To analyse the *Cerbera Odollam* by using Fourier-Transform Infrared Spectroscopy.

Objectives:

- To identify the glycosides in the fruit and leaf of *Cerbera odollam.*

CHAPTER IV: MATERIALS AND METHODOLOGY

Materials Required:

1. Beaker
2. Measuring cylinder
3. Electronic computer
4. Sample chamber
5. Knife
6. Electronic Blender
7. Watch glass
8. Dropper
9. Conical Flask

Chemical Reagent:

- Methanol

Instrument Required:

- Shimadzu 8400 FTIR system with potassium bromide (KBr) optics
- Electronic weighing machine

Figure 6- Shimadzu 8400

Figure 7- Electronic weighing machine

Methods:

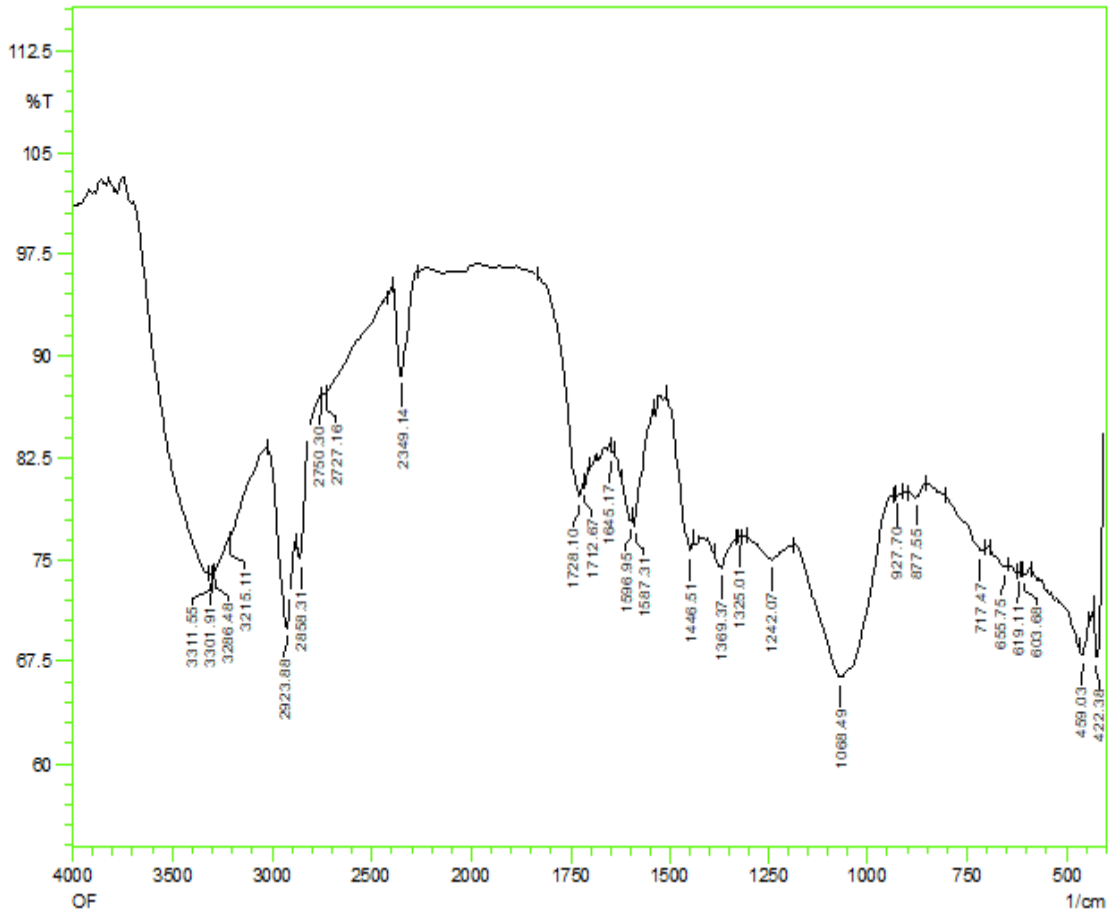
Fresh odollam leaves and fruits were collected and shade dried it, these were grinded by using electronic blender till the entire sample became finely powdered like texture. These powdered samples were collected in a glass container and labelled. Samples were weighed to 1 gm for leaves and 0.5 gm for fruits by using electronic weighing machine and are separated Then 1 gram of Cerbera Odollam leaf was extracted with 10 ml of methanol for 10 minutes. Then filtered the sample and concentrated to dryness. Then 0.5 gram of Cerbera odollam fruit was extracted with 10 ml of methanol for 10 minutes.

Filtered the sample and concentrated to dryness These samples were used for the FTIR test. The dried powdered samples of different extract were analysed by Fourier-transform infrared spectroscopy.

The analysis was conducted by Shimadzu-8400 FTIR system with Potassium bromide (KBr) optics. The pellets were prepared in FTIR grade potassium after back ground scan with KBr. The standard method to prepare solid sample for FTIR is to use KBr. Sample and about 200 mg KBr were grinded together. The practical size should be unified and less than two micrometres, so the mixture was squeezed to form transparent pellets which can be measured directly. The second step was getting a background spectrum by collecting an interferogram and its subsequent conversion to frequency data by inverse Fourier-transform. The background spectrum will contain information about the species of solvent molecule. The back ground spectrum also takes into account several other factors related to the instrument performance. Which also includes information about the source, interferometer, detector and the ambient water and carbon dioxide present in the optical bench. Next, we have collected the single-beam spectrum of the sample, which will contain absorption band from the sample as well as the back ground. The ratio of the single-beam sample spectrum and the single-beam background spectrum gave the spectrum of the sample. Data analysis was done by assigning the observed absorption frequency bands in the sample spectrum to appropriate normal modes of vibrations in the molecules.

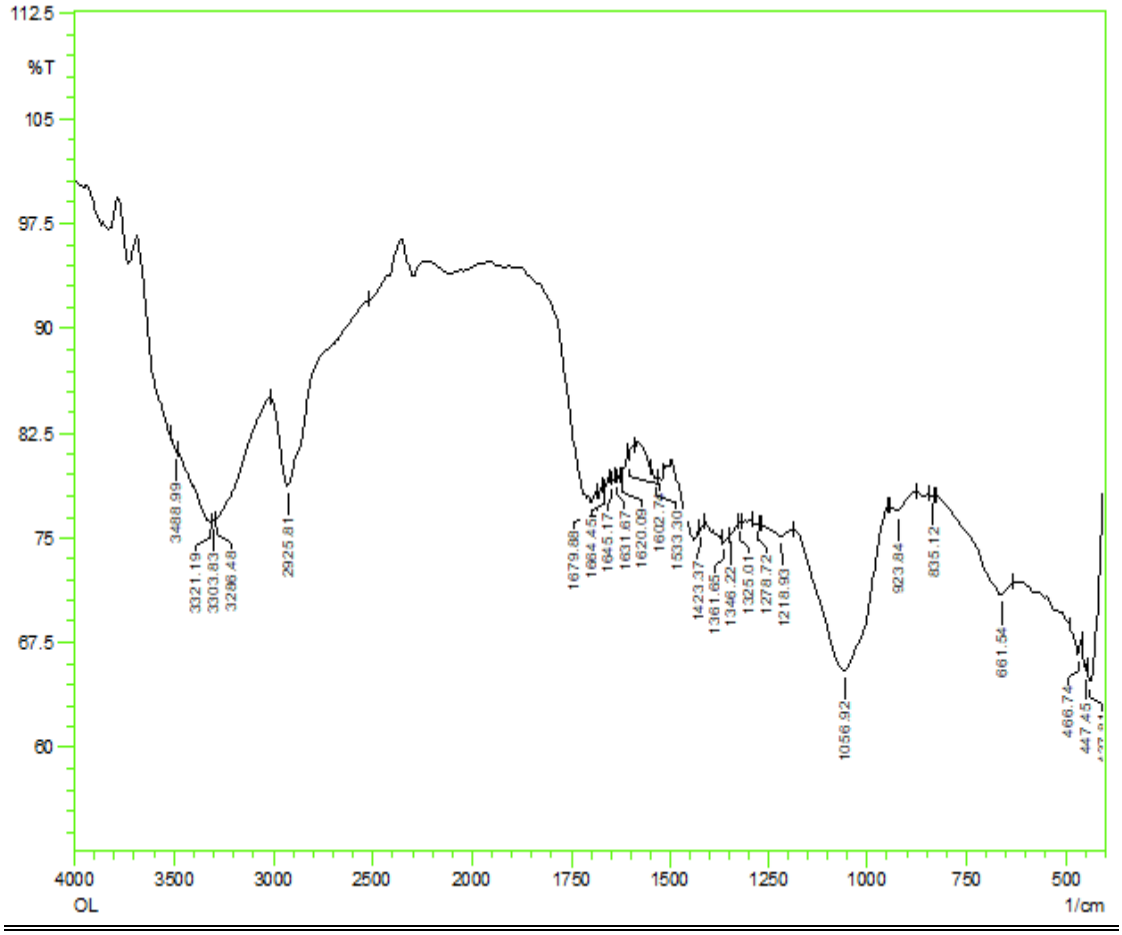
CHAPTER V: OBSERVATION

IR Spectrum of *Cerbera odollam* Fruit:



Graph 1- FTIR spectrum of Cerbera odollam fruit

IR Spectrum of *Cerbera Odollam* Leaf:



Graph 2- FTIR spectrum of Cerbera odollam leaf

CHAPTER VI: RESULT AND CONCLUSION

Result:

Using the analysis of *Cerbera odollam* leaves and fruit by IR spectroscopy, the chemical composition was identified. Cerberin, which is a cardiac glycoside was identified from both the samples. Through the analysis the transmittance was measured between 400 to 4000 cm^{-1}. The major peaks of *Cerbera odollam* fruit sample are shown at the wavelengths - 3432 cm^{-1},3311cm^{-1},3301cm^{-1}. The major peaks of *Cerbera odollam* leaf sample are shown at the wavelength –3488 cm^{-1}, 3410cm^{-1}, 3321 cm^{-1}.

Conclusion:

In this the cerberin and cardiac glycoside were identified by using the IR spectroscopy in Cerbera odollam leaf and fruit. The presence of 2-acetyl derivative of neriifolin (which is the cardiac glycoside) was also confirmed by the above method.

Other chemical components which are responsible for the various biological activities can be found out by using other techniques like Gas chromatography, mass spectroscopy, high performance liquid chromatography and other various techniques.

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