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CHAPTER I: INTRODUCTION

Forensic toxicology is the use of toxicology and disciplines such as analytical chemistry, pharmacology and clinical chemistry to aid medical or legal investigation of death, poisoning, and drug use. The primary concern for forensic toxicology is not legal outcome of the toxicological investigation or technology utilized but rather the obtainment and interpretation of results. A toxicological analysis can be done to various kinds of samples. A forensic toxicologist must consider the context of an investigation, in particular any physical symptoms recorded and any evidence collected at the crime scene that may narrow the search, such as pill bottles, powders, trace residues, and any available chemicals. Provided with the information and samples with which to work, the forensic toxicologist must determine which toxic substances are present in what concentrations, and the portable effect of those chemicals on person.

Poisons are any substance which is solids, liquids, or gas when administered in living organisms through any means like inhalation, ingestion, injection, local or surface absorption it will produce ill health or even death due to its physical properties. This is classified into many types of which plant poisons comes under irritants in the mode of action of poisons. Examples of some poisonous plants are *Datura stramonium, Caladium bicolor, Asparagus officianalis, Laportea interrupta* etc.

Laportea interrupta a genus of plants in the family Utricaceae. They are herbaceous, either annual or perennial. Like many plants of Utricaceae, they have stinging hairs and non-stinging hairs on the same plant. It is commonly known as stinging nettle.

Fig 1: *Laportea interrupta* plant with flower, fruit, leaves and stem

Fig 2: *L. interrupta* from the Western Ghats of Kerala

Fig 3: L. *interrupta* leaves and hairy stem

Fig 4: Flower and fruit of L. *interrupta*

Classification:

Kingdom : Plantae

Phylum :Tracheophyta

Class : Mangnoliopsida

Order : Rosales

Family : Utricaceae

Genus : *Laportea*

Species : *Laportea interrupt*

Synonyms:

*Boehmeria javanica (*BI.)Hassk., *Boehmeria interrupta* (L.)Wild.*Utrica interrupta* L.

L. interrupta is a small, hardly branched herb bearing hairs that irritate the skin. The stem grows up to 20-80 cm tall. Leaves are ovate, narrow tipped, 6-9 cm long, 5-6 cm wide. Leaves have internally grooved petioles, and are broader towards the base and very near to the tip with a short furrow, cuspidate towards the anterior part with a longer tip, are also hairy and burning. The whole marginshas incised with thick teeth, and has riblets standing out on the lower side and with the mid-rib proceeding from the petiole, one on both sides.

Flowers appear in short and branched peduncle, in long tender stalk-lets or petioles which arise from the origin of the leaves and in one head many collectively, and are small, light green buds and consisting of minute, white and less conspicuous leaves.

Occasional side effects includes mild stomach upset, fluid retention, sweating, diarrhoea, and hives or rashes (mainly on topical use). It is important to be careful when handling the nettle plant because touching it can cause an allergic rash.

In case of poisoning cases these plants compositions can be found out in trace quantities and these are rarely used in cases like suicidal, accidental, homicidal cases etc. Also it connects the culprit, suspect, or a victim to a crime scene; these are the known significances of this plant in forensic science.

But the plant has claimed to have the biological properties like foetal-maternal health, anti-inflammatory, antimicrobial, antipyretic activity in the experimental animal model system. However, during the literature search, there are no such detailed reports were found in the aspects of various chemical compounds of *L. interrupta*. So the present study has been taken up to evaluate the methanolic and aqueous extract of *L. interrupta* Leaf and the entire plant for its spectroscopic characteristics.

Infrared spectroscopy involves the interaction of infrared radiations with matter. It covers a range of techniques, mostly based on absorption spectroscopy. As with all spectroscopic techniques, it can be used to identify and study chemical substances. Samples may be solid, liquid, or gas.

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.

Mainly in the analysis of plants Fourier Transform Infrared (FTIR) spectroscopy is used. This relies upon various frequencies of light to produce a spectrum. The basic theory at work is that the bonds between different elements absorb light at different frequencies. The light is measured using an infrared spectrometer which produces the output of an infrared spectrum. It has a source, sample, two mirrors, a laser reference and a detector, but the assembly of components also includes a beam splitter and the two strategic mirrors that function as an interferometer.

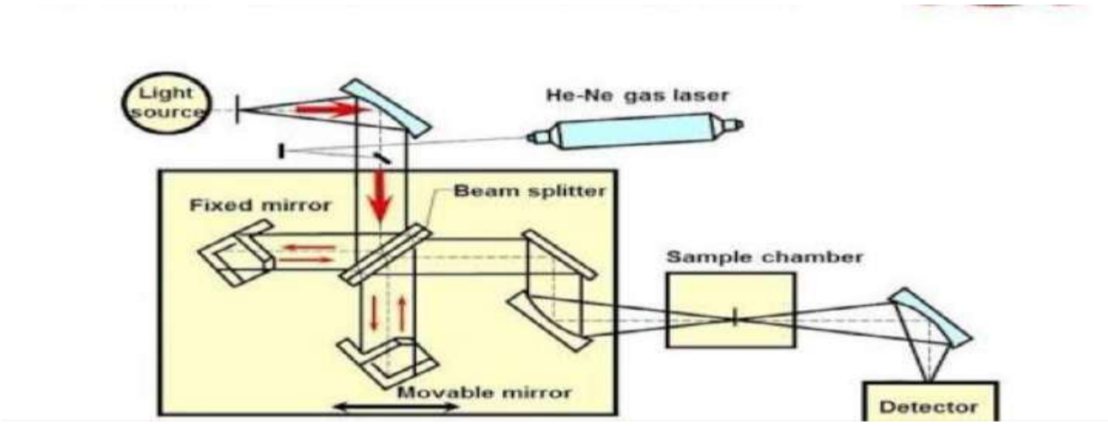

Fig 5: FTIR instrumentation

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

FTIR can be used in all applications where a dispersive spectrometer was used in the past. In addition, the improved sensitivity and speed have opened up new areas of application. Spectra can be measured in situations where very little energy reaches the detector and scan rates can exceed 50 spectra a second. FTIR is used in geology, chemistry, materials and biology research fields.

Physiochemical analysis were conducted in this plant before so in this the phytochemical analysis using IR spectroscopy will be conducted in order to identify the chemical compounds present in this.

CHAPTER II: LITERATURE REVIEW

M N Bobby et.al, during the year 2012 has made an attempt to establish FT-IR profile and identify the functional components of *Albizia lebbeck Benth*. This was done on a Thermo scientific spectrometer system which was used to detect the characteristics peak values and their functional values. The result was confirmed the presence of amide, alkynes, alkanes, carboxylic acid, alkenes, aromatic, aliphatic, amines and alkyl halides compounds which showed major peaks. This study produced the FTIR spectrum profile for this plant.

Dales R Gardner et.al, during the year 1997 has studied about the quantitative analysis of Norditerpenoid alkaloids in Larkspur (*Delphinium spp.*) by Fourier Transform Infrared spectroscopy. Alkaloids were isolated from dry plant materials using a simplified extraction procedure and quantitated using FT-IR. Calibration of the method was made from selected Larkspur samples with known alkaloid levels determined by high pressure liquid chromatography and gravimetric methods. Thirty nine samples from three different Larkspur species (***Delphinium barbeyi, D. glaucescens and D. occidentale***) were analysed to demonstrate the ability of the FT-IR method to measure toxic and total alkaloid concentration in these plants.

Dale R Gardner et.al, during the year 1999 conducted an analysis of toxic Norditerpenoid alkaloid in *Delphinium* species by electrospray, atmospheric pressure chemical ionization, and sequential tandem mass spectrometry. The electrospray mass spectrometry was calibrated using two standard alkaloids methyllycaconitine and deltaline. The electrospray ionization combined with sequential tandem mass spectrometry was done. This resulted in the identification of toxic Norditerpenoid alkaloid in this plant.

TK Schoch et.al, during 1998 conducted a study Fourier transform infrared (FT-IR) for rapid analysis toxic compounds in larkspurs and milkvetches. A number of techniques were used in the quantitative estimation but the accurate, effective, fast, sensitive, and simple method was FT-IR which was used for the quantitative analysis of specific compounds in complex mixtures. The amount of toxic substances was found out through the FR-IR spectroscopy.

Dale R Gardner et.al during the year 2009 conducted HPLC-MS analysis of toxic Norditerpenoid alkaloids in refinement of low larkspurs (*Delphinium spp.*). This was conducted using HPLC-MS spectrometer. The low larkspur contains a number of toxic alkaloids in addition to the alkaloid methyllycaconitine that need to be assessed when considering the toxicity of plants.

Priya Banerjee et.al, during the year 2014, conducted a research on the leaf extract mediated green synthesis of silver nano particles from widely available Indian plants, along with their synthesis, characterization, antimicrobial property and toxicity analysis. In this the sample is mixed with 1 mM silver nano particles (AgNP's), $AgNO_3$ and using leaf extract. These were characterized by UV visible spectrometer, particle size analyser (DLS), scanning electron microscopy (SEM), and transmission electron microscopy (TEM), energy dispersive spectroscopy (EDS). Results of which showed that seeds treated with AgNP solution exhibited better rates of germination and oxidative stress enzyme activity nearing control levels, though detailed mechanism of uptake and translocation are yet to be analysed.

John C Craig et.al, during 1978 presented a new GLC analysis of urushiol congeners in different plant parts of poisonous ivy, Toxicondendron radicans. Methods are presented for the direct GLC analysis of catechol C_{15} alkenyl side chain congeners contained in the urushiol fraction of poison ivy and the C_{17} homologous of poison oak. A number of liquid phases were investigated and demonstrated varying degrees of separation. The methods developed were applied to the analysis of urushiol fractions obtained from different plant parts of poison ivy. The effects of extraction before and after drying demonstrated that a larger percentage of urushiol was obtained when the fresh plant material was extracted with ethanol.

C Mungenge et.al, during the year 2014, studied the phytochemical screening, toxicity and insecticidal activity of fish poison plant *Synaptolepis alternifolia* Oliv. (Thymelaeaceae). This was achieved by the Brine shrimp lethality (BSL) bioassay. The result shows a huge potential of *Synaptolepis alternifolia* as an insecticide.

CHAPTER III: AIMS AND OBJECTIVES

Aim

To analyse*Laportea interrupta* using Infrared spectroscopy.

Objectives

To find the unknown chemical compounds present in it.

CHAPTER IV: MATERIALS AND METHODOLOGY

Materials Required

- **Apparatus**

1. Dried samples of leaves and entire plant
2. Electronic blender
3. Weighing machine
4. Weighing glass
5. Measuring cylinder
6. Cuvette
7. Beaker
8. Filter paper
9. Funnel
10. Conical flask
11. Stopwatch

- **Chemicals**

Methanol

Instruments required

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

Fig 6: Shimadzu-8400 Fourier transform infrared system

Method

The plant materials were collected from Western Ghats of Kerala. The plant was authenticated by the Taxonomist, Centre for Medicinal Plants Research, Malappuram. The leaves and plant samples were collected and shade dried. These were grinded separately by using electronic blendertill the entire sample became finelypowder like texture. These powdered samples were collected in a glass container and labelled. Samples were weighed to 1gm each by using electronic weighing machine and are separated. One gram each of dried and powdered samples of leaves and entire plant was extracted with 10 ml of methanol for 10 minutes filtered and concentrated to dryness. . The dried extracts were analysed by Fourier transform infrared spectroscopy (FTIR). The analysis was conducted by Shimadzu-8400 FTIR system with potassium bromide (KBr) optics. The pellets were prepared in FTIR grade potassium bromide after back ground scan with KBr. The transmittance was measured between 400 and 4000 cm^{-1}.

Fig 7: Grinding and extracting the sample

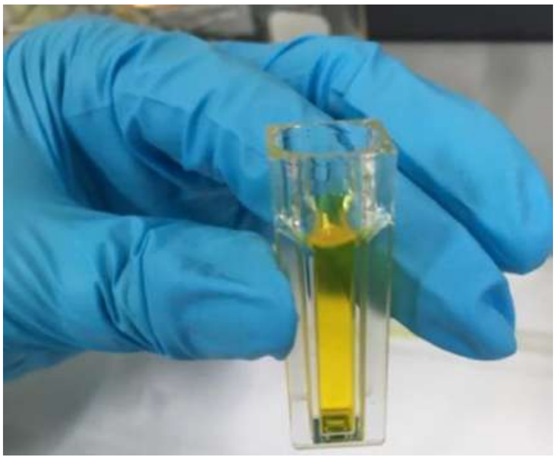

Fig 8: Concentrating the sample using methanol

CHAPTER V: OBSERVATION

The following graphs were obtained when wavelength is plotted against absorbance for both samples when it was subjected to the IR radiations ranging from 400-4000 cm^{-1}:

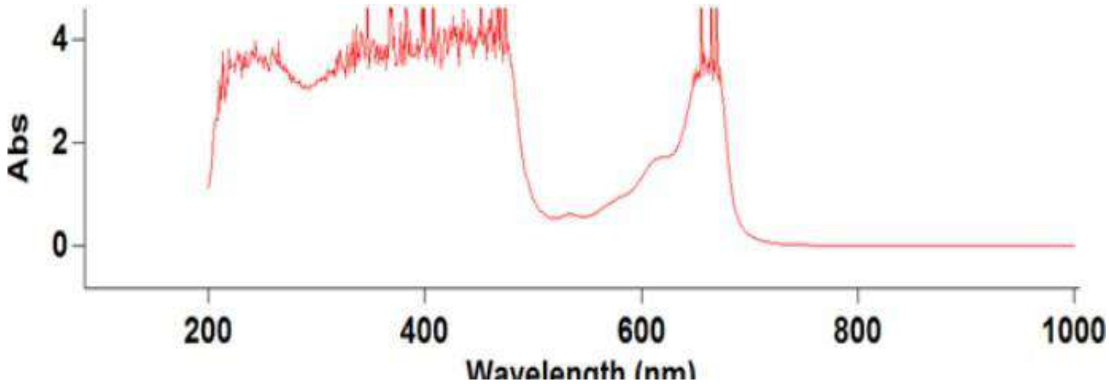

Fig 9: Spectroscopic analysis of methanolic extract of *L. interrupta* Leaf

Wavelength	Absorbance
673.0	3.463
658.0	3.735
619.0	1.729
534.0	0.613
477.0	3.923
435.0	4.584
416.0	3.631
319.0	3.695

Table 1: major peaks and their corresponding wavelengths of methanolic extract of L. *interrupta* Leaf

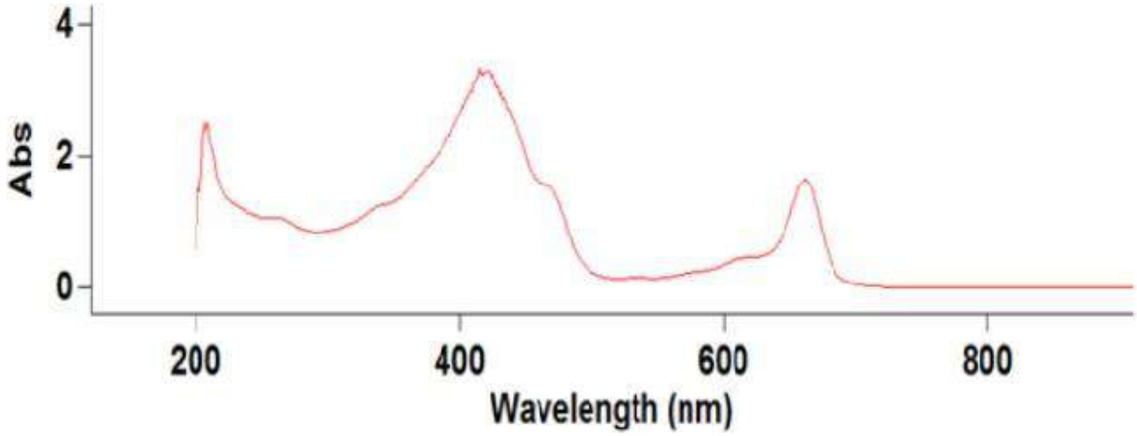

Fig 10: Spectroscopic analysis of methanolic extract of *L. interrupta* fruit

Wavelength	Absorbance
662.0	1.639
534.0	0.158
432.0	2.955
425.0	3.246
209.0	2.527

Table 2: Major peaks and their corresponding wavelengths of *L. interrupta* leaf at the concentration of 12.5 mg%.

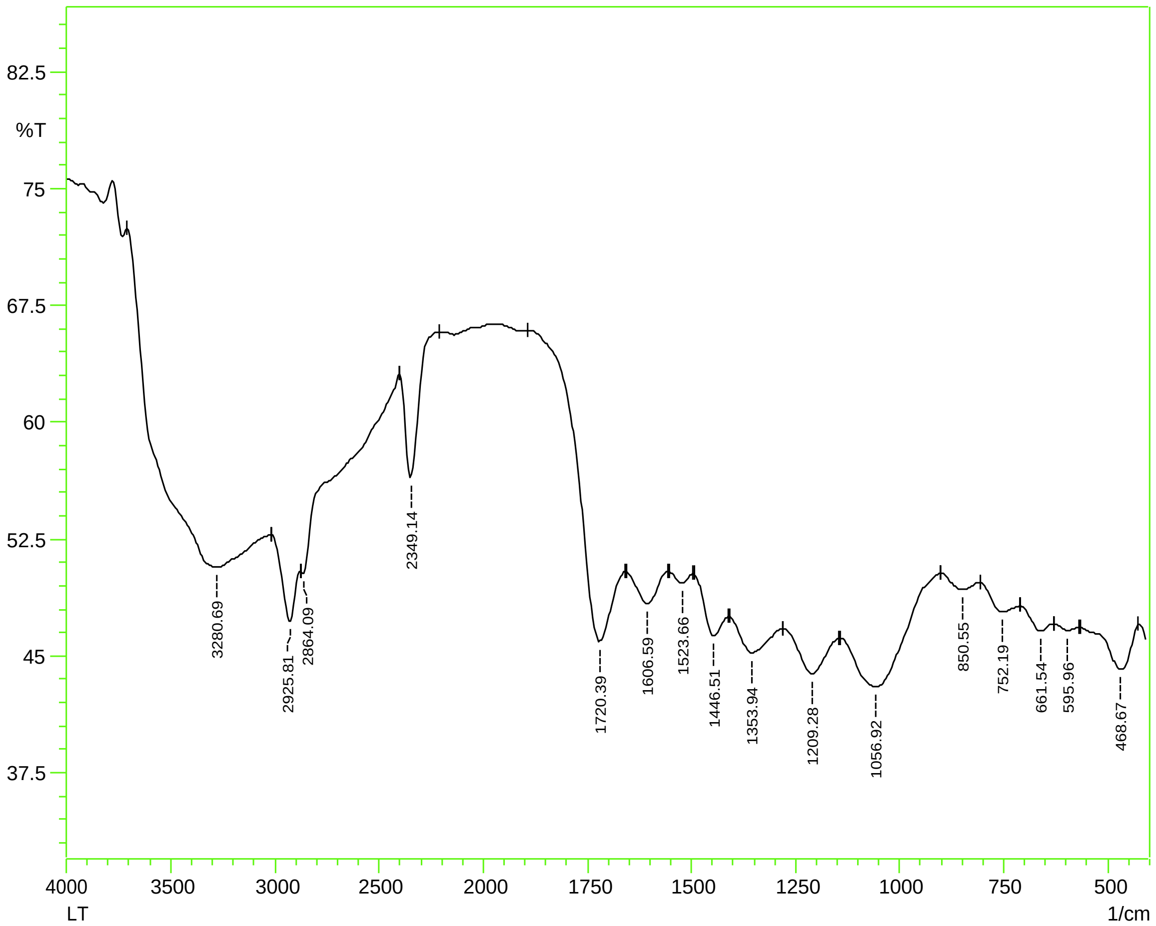

Fig 11: spectroscopic analysis of methanolic extract of *L.interrupta* stem.

CHAPTER VI: RESULTS AND CONCLUSION

Result

Spectroscopic analysis of methanolic extract of *L. interrupta* leaf showed the major peaks at the wavelengths of 673, 619, 477, 435, 319 nm representing the presence of group of various compounds in the category of chlorophylls, phycobilins, xanthophylls, cytochromes and flavonoids respectively. The presence of coumarins (209 nm) and chlorophylls (662 nm) are observed in the methanolic extract of L. interrupta leaf studied at the concentration level of 12.5 mg%.

Conclusion

The spectroscopic analysis showed the presence of various group of compounds like xanthophylls, flavonoids, anthocyanins and carotenoids. The presence of such group of compounds is expected to be responsible for its various biological activities.

However, the detailed scientific studies are essential for the confirmation of the compounds present in them using HPLC, GC, GCMS and other instrumentations.

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