A STUDY OF INTERACTION OF ANJANAKALLU WITH ECLIPTA ALBA

A Project report submitted to
Mahatma Gandhi University, Kottayam
in partial fulfillment of requirement for the award of the degree of

B.Sc CHEMISTRY

BY
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Under the supervision of

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DEPARTMENT OF CHEMISTRY

BHARATA MATA COLLEGE THRIKKAKARA

(Affiliated to Mahatma Gandhi University, Kottayam)

2017-2020

DEPARTMENT OF CHEMISTRY

BHARATA MATA COLLEGE, THRIKKAKARA

(Affiliated to Mahatma Gandhi University, Kottayam)

CERTIFICATE

This is to certify that the Project titled **"A Study of interaction of anjanakallu with eclipta alba "** is a bonafide work carried out by **Amala Seby, Reg No: 170021025594 ,** B.Sc Chemistry student, under my supervision and guidance and that no part of this has been presented earlier for the award of any other degree, diploma or other similar titles of recognition.

Forwarded by

Guide

Project

Dr. Litty Sebastian
Head of the Department
Department of Chemistry
Bharata Mata College,

Place:

Date:

DECLARATION

I, Amala Seby , declare that the project report entitled "A study of interaction of anjanakallu with eclipta alba", submitted to Mahatma Gandhi University, Kottayam, in partial fulfillment for the award of the degree of BSc Chemistry, is an authentic record of original work done by me, under the supervision of Dr. Litty Sebastian, Department of Chemistry, Bharata Mata College, Thrikkakara and no part of this has been previously formed on the basis for the award of any degree or assistantship of any other institution.

Place: Amala Seby.

Date:

ACKNOWLEDGMENT

First and foremost, I praise God almighty, being the unfailing source of support, comfort and strength throughout the successful completion of my work.

With great appreciation, I would like to express my sincere gratitude to Dr. Litty Sebastian, Head of the Department, for her valuable suggestions, guidance and support throughout the accomplishment of my project.

Words are inadequate to express my sincere gratitude to my guide, Dr. Litty Sebastian, for her sustained guidance and encouragement throughout the course of this project. Without her solicitude, I would not have been able to make such a study. I am also thankful to all my teachers in the department for their kind cooperation and help.

I extend my sincere thanks to the lab assistants Mr. Shinto and Ms. Siji for their help and kind cooperation.

On my personal note, I would like to thank my parents who gave me support and encouragement and motivated me on all my endeavors.

Finally, I would also like to thank my friends who have helped me, directly or indirectly, at the various stages of this product.

Amala Seby

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CHAPTER 1.INTRODUCTION

The project that has been done during the academic year 2017 - 2020 under the surveillance of Dr. Litty Sebastian, Head Of Department of chemistry in Bharata Mata College. This project aimed at knowing the scavenging property of ayurvedic medicinal plant Eclipta alba on Anjana kallu. Anjana kallu or Kohl stone consists of a poisonous element of lead, which has been scavenged by the action of eclipta alba by the formation of a complex. By unrevealing that element in eclipta alba that forms a complex with lead in anjanakallu and turns into a non - poisonous substance to human body, along the medicinal values of eclipta alba, may this action of scavenging of poison can also be cited.

ANJANA KALLU (KOHL STONE)

Anjana is a thick eyeliner made with herbs. It is an ayurvedic herbal product. Women and girls have been using collyrium or anjana for the beautification, facial lift and maintenance of eye health since ages. Ayurveda has used and advocated the use of anjana not only for eye beautification and maintenance of eye but also to prevent and treat many disorders of eye. Thus anjana has a therapeutic importance and has been an integral part and prescription in ayurvedic ophthalmology. Anjana, surma or kohl is widely used in traditional practices as well as medicines. Kohl may be defined as an ultra fine powder of kohl stone that is used for various ailments and it is used in middle east, India, Pakistan and parts of Africa that often contains high levels of lead.

On the basis of its properties and therapeutic action, anjana is classified as Lekhana anjana (scraping), Ropana (healing), Prasadhana (cosmetic). On the basis of form of medicines used it is classified as Gutika anjana, Rasa anjana, Churna anjana. On the basis of the taste of herbs choosen to prepare the collyrium, it is classified as Madhura, Amla, Lavana, Katu, Tikta and Kashaya Anjana.

Uses of Anjana/Kohl

Kohl has certain uses. Applying kohl to the eyes will protect the eyes by forming a thin film on the eye lens thus avoiding the direct contact of harmful UV radiations and glare of sun with lenses. Black and shining particles of galena (PbS) in kohl shields the eyes from those glares and reflections of sun and thus protect the eyes. It also helps in stopping internal eye bleeding, helps to strengthen eye sight and clearing eyes by protecting them from infections.

Composition of Anjana Kallu

The main composition of Anjana Kallu is Galena or Lead Sulphide (PbS). Compositionally, Galena contains 85-87 % lead and 11-13% sulphur.

A study of kohl manufactured in Egypt and India found that one third samples studied contains lead while the remaining two-third contained amorphous carbon, zincite, cuprite, elemental silicon, aluminum and organic compounds.

In Northern India, the Galena derived preparations is called surma and it contains lead but the eye cosmetic kajal is prepared from carbon soot and it is free from lead. Many studies have reported the chemical contents of kohl or surma is particular lead contents. Analysis of kohl or surma composition by X-ray powder diffraction and Scanning Electron Microscopy provides information on speciation of the metals and on organic compounds. From these analysis and

studies, majority of Omani, Saudi - Arabia , Egyptian and Indian kohls sample contains 70 % Lead Sulphide (PbS).

Chemical composition of Kohl stone and surma.

Element		Kohl stone	Surma from sold on the street under brand name
Lead	(Pb)	85.51%	5.974%
Sulphur	(S)	11.43	0.054
Antimony	(Sb)	2.06	ND
Carbon	(C)	0.689	0.152
Iron	(Fe)	0.02	0.094
Chromium	(Cr)	0.002	ND
Copper	(Cu)	ND	ND
Nickel	(Ni)	ND	ND
Zinc	(Zn)	ND	ND
Magnesium	(Mg)	ND	0.01
Calcium	(Ca)	ND	0.02
Sodium	(Na)	ND	ND
Potassium	(K)	ND	ND

ND = Not detected
Chemical analysis: Performed at Mineral Processing Research Centre, Pakistan Council of Scientific & Industrial Research, Lahore, Pakistan.

Results of chemical analysis of kohl stone and surma are given above in the table. The main constituents estimated are Lead (85.51%), Sulphur (11.43%), and Antimony (2.06%). Carbon is also detected in small quantity (0.689%). Since lead and antimony are found in nature in combination with sulphur , therefore, lead detected in test sample in the form of Galena or PbS as major and antimony as SbS as minor component. Similarly, a sample of surma powder sold on the streets was also analysed by the same method. As per the chemical analysis it contains very little amount of lead (5.974%) as compared to amount of lead in kohl stone sample. S, Fe and C are also found in little amount as compared to that present in the kohl stone sample.

Toxicity of Galena

Lead is ubiquitous environment contaminant to which human are exposed throughout life from dust, air and water. Extensive investigation of lead - induced toxicity for past 40 years have established that lead is toxic to nervous and neuroendocrine system of fetus, infants, pre -

adolescents and aging adults and that lead causes renal and cardiovascular toxicity. Potential sources of exposure of lead are traditional medicine and cosmetics like kohl.

Environmental exposure to lead can be hazardous to human health. Lead poisoning is a global problem, considered to be the most important environment disease in children. High lead levels are detected during the sample study of surma or kohl. Use of surma is associated with high blood lead concentration with significant reduction of haemoglobin levels. Lead contaminated kohl or surma use has been linked to increased levels of lead in the blood stream, putting its users at risk of lead poisoning and lead intoxication. Complications of lead poisoning includes anemia , growth retardation , low IQ , convulsions and in severe cases, death.

Studies have been revealed that most commercially produced kajal or surma contains high levels of lead , Galena (PbS), minimum (Pb_3O_4), amorphous carbon, magnetite (Fe_3O_4) and zincite (ZnO). Prolonged applications of surma or kohl may cause excessing lead storage in the body affecting the brain and bone marro. In addition to the kohl stone, it may also contains herbs, minerals and marine products of therapeutic values. Thus the use of kohl or surma is both beneficial and dangerous too. So reduce the use of kohl products and build up a healthy body.

ECLIPTA ALBA

Eclipta alba (or synonym Eclipta prostrate) is an annual herbaceous plant which is also known as false daisy. It belongs to the family Asteraceae. There are four main varieties of the herb *Eclipta alba* based on the colour of their blossom, that is, red, yellow, white, and blue. The white and yellow ones assume an essential part in traditional medicine, but it is the white species (*Eclipta alba*) that is most commonly harvested for its therapeutic advantages as it grows wildly in moist places, as a weed, and it can be easily propagated. The extracts from the leaves and flowers of this medicinal herb can be applied in numerous ways, both topically and internally, to soothe many ailments. The latin name for the yellow variety is is Wedelia chinensis (also called as Wedelia calendulacea).

The white variety is commonly found in Kerala. In Malayalam, it is known as Kayyonni. Other names include, Kariyalanganni, Kaiyenna, Kaiyanayam, Kanjuanyam, Jalabringa etc. In Hindi, it is known as Bringha and in Punjabi, Bhangra and in Sanskrit, Kesharaja.

The taxonomic hierarchy of the plant is given below:

Kingdom	Plantae
Subkingdom	Viridaeplantae
Infrakingdom	Streptophyta
Division	Tracheophyta
Subdivision	Spermatophytina

Infradivision	Angiospermae
Class	Magnoliopsida
Superorder	Asteranae
Order	Asterales
Family	Asteraceae
Genus	Eclipta L.
Species	Eclipta alba (L.) Hassk.

Fig 1. Picture of Eclipta Alba.

It is also commonly known as Bhringaraj and Karisilakanni which is a common weed found throughout India ascending up to 6000ft.The genus name comes from the Greek word meaning "Deficient,", with reference to the absence of the bristles and awns on the fruits. It is one of the well known and valuable medicinal plant in India. It is popularly known as "King of hair", used in indigenous system of medicine. It also has an important role in the traditional Ayurvedic, Siddha and Unani system of medicine.

Eclipta alba has been mentioned in the ancient Ayurveda textbooks like Paraskara Grihyasutram, Keshava Yoga, Shonakya Atharvam and Kaushika Sutra. They describe the medicinal powers of the plant. It is known as Bhringaraj in Ayurveda and has been generally utilized for a very long time as a part of conventional prescription for ailments especially related to the liver and hair. In Ayurveda, it is used to prepare nilabhringaditailam, Narasinharasayanam, kunnunyaditailam, bhringarajasavam.

In Ayurvedic science, its juice is used for headaches and hair loss, and is mentioned in the famous Charaka Samhita and Ashtanga hridaya. After squeezing the juice, the leaves are grinded

with kalchakam and squeezed into sesame oil. It is mentioned in various texts that applying this oil on the head helps hair growth.

COMPONENTS OF ECLIPTA ALBA

Phytochemical studies on Eclipta alba discovered the presence of alkaloids like nicotine and ecliptine and bio-active steroidal alkaloids like verazine, dehydroverazine, ecliptalbine and many hydrocarbons like ecliptal, α-formylterthienyl. Whole part is said to have many triterpenene like saponin, eclalbatin, along with α-amyrin and β-amyrin, ursolic acid, oleanolic acid and six new oleanane triterpene glycosides, eclalbasaponins I-VI were also reported to be present in the whole plant.

PHYTOCHEMISTRY

Eclipta alba contains wide range of active principles which includes coumestans, alkaloids, flavonoids, glycosides, triterpenoids, volatile oils, saponins, sterols, alcohols and polyacetylene substituted thiophene. The leaves contain stigmasterol, β terthienylmethanol, wedelolactone, demethylwedelolactone and demethylwedelolactone-7-glucoside. The roots give hentriacontanol and heptacosanol. The roots contain polyacetylene substituted thiophene. The aerial part contains phytosterol, β-amyrin in the n-hexane extract and luteolin-7-glucoside, β-glucoside of phytosterol, a glucoside of a triterpenic acid and wedelolactone. The polypeptides isolated from the plant yield cystine, glutamic acid, phenyl alanine, tyrosine and methionine on hydrolysis. Nicotine and nicotinic acid also occur in this plant.

CHEMICAL CONSTITUENTS PRESENT IN PLANT PARTS

LEAVES	Wedelolactone(1.6%), Demethylwedelolactone, Demethylwedelolactone-7-glucoside, Stigmasterol
ROOTS	Hentriacontanol, Heptacosanol, Stigmasterol, Ecliptal, Eclalbatin
AERIAL PARTS	β-amyrin and Luteolin-7-glucoside, Apigenin, Cinnaroside, Sulphur compounds, Eclalbasaponins I-VI
STEMS	Wedelolactone
SEEDS	Sterols, Ecliptalbine (alkaloid)
WHOLE PLANT	Resin, Ecliptine, Reducing sugar, Nicotine, Stigmasterol, Triterpene,

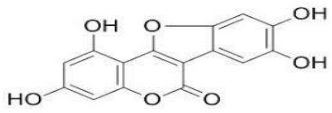
Saponin, Eclalbatin, Ursolic acid, Oleanolic acid

COUMESTAN

Coumestan is an organic compound that is a derivative of coumarin. Coumestan forms the central core of a variety of natural compounds known collectively as coumestans. The major coumestan isolated from Eclipta alba includes wedelolactone(0.5-0.55%) and demethylwedelolactone. Demethylwedelolactone, isodemethylwedelolactone, and strychnolactone can be obtained by percolation and hot extraction of Eclipta alba whole plant. Dried leaves of Eclipta alba gives wedelolactone and its derivatives, demethylwedelolactone, isodemethylwedelol actone demethylwedelolactone-7-glucoside and strychnolactone.

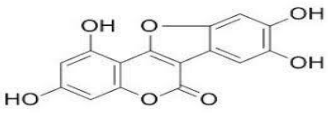
The coumestan wedelolactone and demethylwedelolactone posses potent anti-hepatotoxic activity and is utilized for the treatment of hepatitis and cirrhosis. Wedelolactone has more potent activity than other coumestans. It has a protective effect on liver as well as against liver disorders. The coumestan derivative, wedelolactone, has been found to be a potent and selective inhibitor of 5-Lox which inhibits 5-Lox activity by an oxygen radical scavenging mechanism. Thus wedelolactone has emerged as a candidate drug for prevention as well as treatment of inflammatory diseases and cancer.

a) Wedelolactone



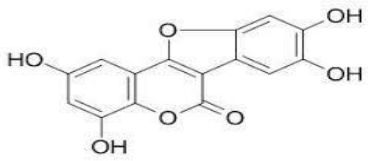
$C_{16}H_{10}O_7$

b) Dimethyl wedelolactone



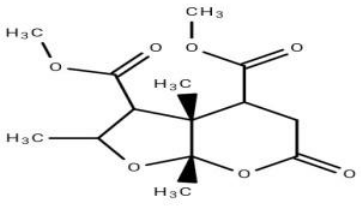
$C_{15}H_8O_7$

c) Isodimethylwedeloctone



$C_{15}H_8O_7$

d) Strychnolactone



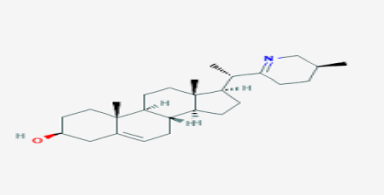
$C_{12}H_{16}O_7$

ALKALOIDS

Phytochemical studies on Eclipta alba revealed the presence of alkaloids like ecliptine and nicotine, bio-active steroidal alkaloids verazine, dehyroverazine, ecliptalbine.

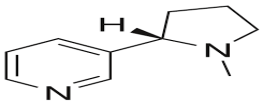
Due to the presence of these alkaloids Eclipta alba has anti-inflammatory and analgesic properties. The alkaloids ecliptalbine and verazine are lipid lowering agents. The active compound 25-β-hydroxyverazine showed good activity against Candida albicans. The in vitro antifungal activity of Eclipta alba extract was investigated against Candida tropicalis, Rhodotorula glutinis and Candida albicans.

a) Verazine



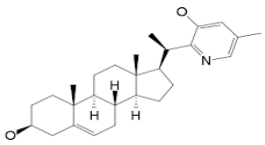
$C_{27}H_{43}NO$

b) Nicotine



$C_{10}H_{14}N_2$

c) Ecliptalbine



$C_{27}\ H_{39}O_2N$

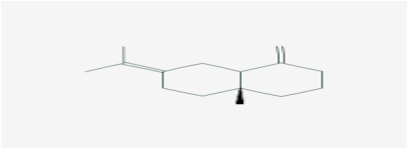
VOLATILE OILS

The volatile components were isolated from the aerial parts of eclipta alba by hydrodistillation and analysed by GC-MS. A total of 55 compounds, which were the major part of the volatiles, were identified by matching mass spectra with a mass spectrum library. The different types of volatile components can be isolated from the aerial parts of the plant by hydro distillation and can be analyzed by GC–MS technique. The main components include heptadecane, 6,10,14-trimethyl-2-pentadecanone, n-hexadecanoic acid, pentadecane, eudesma-4(14), 11-diene, phytol, octadec9-enoic acid, 1,2-benzenedicarboxylic acid diisooctyl ester, (Z,Z)-9,12- octadecadienoic acid, (Z)-7,11-dimethyl3- methylene-1,6,10-dodecatriene and (Z,Z,Z)-1,5,9,9- tetramethyl-1,4,7-

cycloundecatriene. D-dithienyl acetylene ester, ecliptal or α-terthienyl aldehyde, α-terthienyl-methanol and α-formylterthienyl.

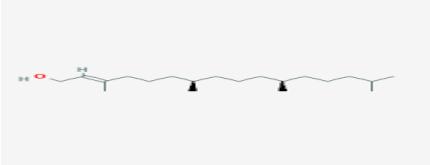
Structures of some of the components are given below:

a)Eudesma-4(14),7(11)-diene



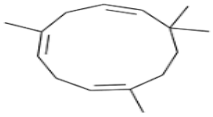
$C_{15}H_{24}$

b) Phytol



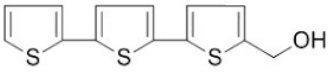
$C_{2O}H_{40}O$

c) 1,5,9,9-tetramethyl-1,4,7-cycloundecatrien



$C_{15}H_{24}$

d) Alpha terthienyl-methanol

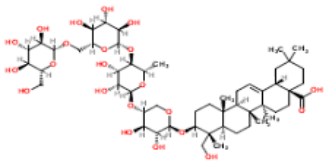


$C_{13}H_{10}OS_3$

SAPONINS

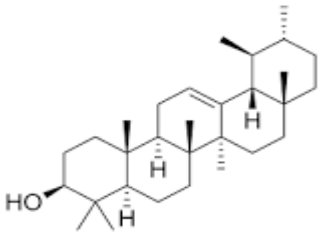
From the whole plant of Eclipta alba, a triterpene saponin, named eclalbatin, together with alpha-amyrin, ursolic acid and oleanolic acid were isolated.

Eclalbatin



$C_{53}H_{50}O_{22}$

b) α- amyrin

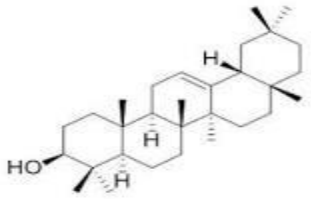


$C_{30}H_{50}O$

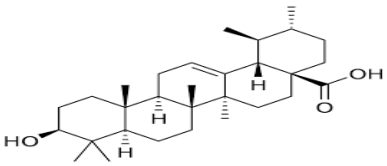
c) β- amyrin

d)Ursolic acid

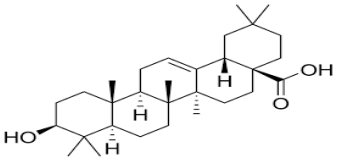
e) Oleanoic acid



$C_{30}H_{50}O$



$C_{30}H_{48}O_3$



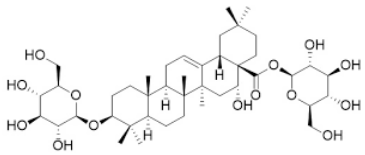
$C_{30}H_{48}O_3$

TERPENOIDS AND THEIR GLYCOSIDES

From the whole part of the Eclipta alba Hassk, six triterpene glycosides, named eclalbasaponins I-VI, were isolated. These structures were characterized as echinocystic acid glycosides and those of V-VI were revealed to be sulphated saponins.

Taraxastane triterpene glycosides , named eclalbasaponins VII-X were also isolated. Two oleanane type glycosides eclalbasaponins I and eclalbasaponins II along with the ubiquitous steroid stigmasterol were isolated from an n- hexane extract of the stem bark of Eclipta alba.

Eclalbasaponin 1

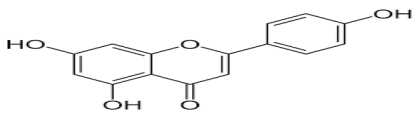


$C_{42}H_{68}O_{14}$

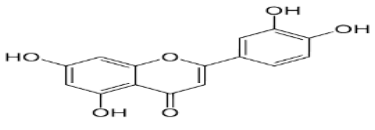
FLAVONOIDS

Flavonoids seen in Eclipta alba are apigenin, luteolin and luteolin-7-glucoside.The flavonoids present in Eclipta alba are secondary metabolites which act as antioxidants and help in free radical scavenging,metal ions chelation and protects against human disease like cardiac-disorder, thrombosis, hepatotoxicity, anticarcinogenic, anti-mutagenic etc. Total flavonoid content (TFC) was determined by $AlCl_3$ colorimetric method.

a)Apigenin

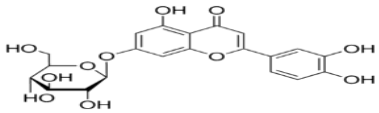


b) Luteolin



$C_{15}H_{10}O_5$ $C_{15}H_{10}O_6$

Luteolin-7-glucoside



$C_{21}H_{20}O_{11}$

Medicinal Uses

All the parts of Eclipta alba and its chemical constituents are used as anticancer, antileprotic, analgesic, antioxidant, antimyotoxic , antihaemorrhagic, antihepatotoxic, antiviral, antibacterial, spasmogenic, hypotensive, ovicidal etc. It also promotes the blackening and growth of hair. It has immunomodulatory properties and can restore the youthful vigor and appearance. It shows an antivenom property and corrosion pickling inhibition action on mild steel in hydrochloric acid.

It is an active ingredient of many herbal formulations prescribed for liver ailments and shows effect on liver cell regeneration. It is used as a tonic and diuretic in hepatic and spleen enlargement. It is also used in catarrhal jaundice and for skin diseases. The alcoholic extract shows antiviral activity against Ranikhet disease virus. The fresh juice of the leaves is used for increasing appetite, improving digestion and as a mild bowel regulator. The plant has a reputation as an antiageing agent in Ayurveda. It is used as a general tonic for debility. It has a profound antihepatotoxic activity. A complete relief in epigastria pain, nausea and vomiting in ulcer patients has been observed. In China, it has been used as a food additive and herbal medicine to cure infectious hepatitis, liver cirrhosis, jaundice etc. Externally it is used for inflammation, minor cuts and burns. It also used in the treatment of scorpion strings. Leaf juice is very effective in stopping bleeding. It is also known to have various pharmacological properties and is used in the treatment of epilepsy. The water extract of Eclipta alba exhibits the most potent inhibitory activity against HIV-1 integrase. Vedic Guard, a polyherbal formulation contains Eclipta alba as a major ingredient.Chanaka advises taking the juice of Eclipta alba with honey to prevent the onset of senility and its oil as the best medicated massage oils for rejuvenation therapies.

In Ayurveda, the plant is considered as a rasayana for longevity and rejuvenation. In China, as a cooling and restorative herb, this supports the mind, nerves, liver and eyes. Eclipta alba also has traditional external uses, like athlete foot, eczema and dermatitis. The whole plant is used as antiseptic, febrifuge, tonic, deobstruent in hepatic and spleen enlargement and is emetic. In combination with aromatics, the juice is given in anemia, catarrh, cough and jaundice. The plant is also used as scalp tonic for promoting hair growth. The fresh

juice of the leaves is given in the treatment of edema, fevers, liver disorders, eye diseases, asthma, bronchitis, diarrhoea, weak bladder and rheumatic joint pains. It is also used to improve the appetite and to stimulate digestion. The juice is given with honey to treat upper respiratory congestion in children. The hair oil is prepared from boiling the fresh leaves with either coconut or sesame oil renders the hair black and lustrous.

The plant has shown moderate activity against a variety of animal cancers. It is used internally in the treatment of dropsy and liver complaints, anaemia, diphtheria, tinnitus, tooth loss and premature greying of the hair. Externally, it is applied to cuts, bruises and sores in order to stop bleeding and relieve pain. A decoction is used to treat cancer.The ground up leaves are rubbed on the head of infants as a remedy for convulsions, and are rubbed on the skin to make pigmented blotches on infants disappear. The leaves are also used to treat a range of other skin problems including cuts, sores, pimples, rashes and various diseases, including leprosy. Aerial portions of the plant are reputedly effective in a beverage to remedy albuminuria. The roots are emetic and purgative . They are applied externally as an antiseptic to ulcers and wounds, especially in cattle. The flowers are used to treat conjunctivitis.

The compounds obtained from Eclipta alba shows good activity against Staphylococcus epidermidis and Salmonella typhimurium. The active compound 25-beta-hydroxyverazine shows good activity against Candida albicans. The anti-malarial activity was evaluated against Plasmodium berghei ANKA strain in mice. The oral administration of leaves resulted in significant decrease in blood glucose and an increase in the activity of liver hexokinase. The studies shows that hepatoprotective activity of Eclipta alba is by regulating the levels of hepatic microsomal drug metabolizing enzymes. The methanolic extract of leaves and the chloroform extract of rrots of Eclipta alba showed significant activities in reduction of lysosomal enzyme. Protection of neuronal tissues may be possibly due to the immunomodulatory action of Eclipta alba. Therefore it can serve as a potential memory modulator. Methanolic extract of Eclipta alba was estimated for its anticancer activity against Ehrlich Ascites Carcinoma (EAC) in swiss albino mice. A bluish-black dye is obtained from the juice of the leaves and it is used as a hair dye and for tattooing.

CHAPTER 2 – EXPERIMENTAL

The chapter includes the details of the following:

2.1 Sample Preparation
2.2 ICP MS
2.3 Lyophilization
2.4 TLC
2.5 IR Spectroscopy

2.1 SAMPLE PREPARATION

Eclipta alba (kaiyonyum) plant was collected from various places of Ernakulam district in Kerala, India and the whole plant was allowed to undergo shade dry for a few days (4-5 days). After it became completely dry, it was powdered in a grinder to a fine powder. The fine powder weighed around 1.5 kg.

55 grams each of the powdered Eclipta alba powder was put into two 250 ml beakers. To one of the beakers, water (~ 200 ml) was added and to the other one, methanol (~ 200 ml) was added with the intention to prepare the water extract and methanol extract.

Both solution were kept in a shaker machine and shaken well for about 12 hrs. The methanol extract and the water extract was obtained after filtering through a funnel. These were kept in a freezer compartment of the fridge and used for all further studies.

Using the methanol extract, we did the thin layer chromatography in order to get the component compositions of methanol extract. Different components were spotted during the experiment and was analyzed under UV light chamber to get an idea about the component involved. The methanol extract was then transferred into RB flask for further study.

The RB flask containing methanol extract was connected with a rotavapour machine whose prime principle is to vapourize the solvent methanol from the extract and make it dry. To completely remove the solvent, it was connected to a vacuum pump for about 35 min and we obtained a dry paste of the methanol extract devoid of any solvent. The dry paste was scraped out of the RB flask using spatula into a watch glass.

The water extract was lyophilized (works by freezing the material, then reduce the pressure and adding heat to allow the freezer water in the material to sublimate) and the dry sample was also used for further studies.

Anjana kallu was purchased from the local Ayurveda shop and weighed. The chemical composition of Anjana Kallu was detected using ICP MS. To study the bonding nature of Anajana kallu with the Eclipta Alba, infared spectroscopic studies were conducted. For this, an equal amount of anjana kallu (0.113 g) and dry paste of methanol extract (0.116g) were mixed well in a mortar pestle, maintaining a ratio of 1:1.

The IR spectrum of the resultant mixture was taken using the Thermo Fischer FT IR Spectrometer. The sample (powdered anjana kallu +dry methanol extract) was mixed with pure solid KBr in a mortar and pestle and made into a pellet, for IR measurement.

A brief description on the characterization techniques is given below.

2.2 INDUCTIVELY COUPLED PLASMA MASS SPECTROSCOPY (ICPMS)

Inductively coupled plasma mass spectroscopy (ICP-MS) is a type of mass spectroscopy that uses an inductively coupled plasma to ionize the sample. It atomizes the sample and creates atomic and small polyatomic ions, which are then detected. It is known and used for its ability to detect metals and several non-metal in liquid samples at very low concentrations. It can detect different isotopes of the same element, which makes it a versatile tool in isotopic labeling. It is an analytical technique that can be used to measure elements at trace levels in biological fluids.

Although older techniques such as atomic absorption and atomic emission are still in use by some laboratories, there has been a slow shift toward ICP-MS, particularly in the last decade. ICP Mass Spectrophotometers (ICP-MS) was first introduced by R.S.Houk, A.L. Gray et al. in 1980, then put on the market in 1983, and is now widely used in various fields.

Compared to atomic absorption spectroscopy, ICP-MS has a greater speed, precision and sensitivity. However, compared with other types of mass spectroscopy, such as thermal ionization mass spectroscopy (TIMS) and glow discharge mass spectroscopy (GD-MS), ICP-MS introduces many interfering species: argon from the plasma, component gases of air that leak through the cone orifices, and contamination from glassware and the cones.

ICP-MS is a technique that combines two technologies into one. The high temperature ICP source which is in range between 6000 and 10,000 K converts the atoms of the elements in the sample to ions. An inductively coupled plasma is a plasma that is energized (ionized) by inductively heating the gas with an electromagnetic coil and contains a sufficient concentration of ions and electrons to make the gas electrically conductive. Even a partially ionized gas in which as little as 1% of the particles are ionized can have the characteristics of a plasma (i.e., response to magnetic fields and high electrical conductivity). The plasmas used in spectrochemical analysis are essentially electrically neutral, with each positive charge on an ion balanced by a free electron. In these plasmas, the positive ions are all singly charged and there are few negative ions, so there are nearly equal amounts of ions and electrons in each unit volume of plasma.

What makes Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) unique to other forms of inorganic mass spectroscopy is its ability to sample the analyte continuously, without interruption. This is in contrast to other forms of inorganic mass spectroscopy; Glow Discharge Mass Spectroscopy (GDMS) and Thermal Ionization Mass Spectroscopy (TIMS) that require a two stage process. Disadvantages and weaknesses of the ICP-MS detection are the occurrence of spectral and non-spectral interferences and the high cost.

ICP-MS is used in the semiconductor industry, as ICP mass spectrometry is used as analysis method for quality control of high purity material, where demands increase with the times. Also, it is expected that the method can be applied to analysis of trace amounts of hazardous metals, and recently with various legislations in the environmental field, ICP-MS is used to respond to the stricter environmental and drainage standards.

ICP-MS offers the following features:

*High sensitivity analysis- lower detection limits of most elements in ppt to ppq-order.
*Simultaneous multi-element analysis possible.
*Can determine quality and quantity quickly.
*Wide dynamic range with 8 figures.
*Isotope comparison possible.

2.2.1 PRINCIPLE

As shown in Figure 1, ICP-MS consists of an ion source (ICP), a sampling interface, ion lens, a mass spectrophotometer and a detector.

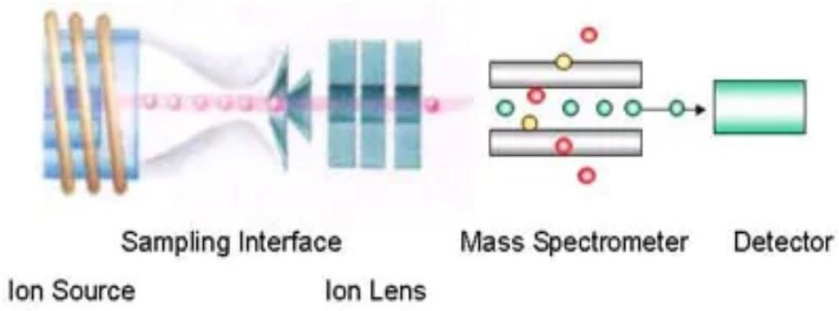


Figure 1: ICP-MS Structure

The ion source, ICP is an ideal ionization source for mass spectroscopy and can ionize over 90% of many elements. Ions produced in the ICP are led through the sampling interface to the mass analysis unit. The sampling interface unit consists of two metallic cones, the sampling cone (orifice radius about 0.5 to 1mm) and the skimmer cone (orifice radius about 0.5 to 1mm), and a rotary gear pump ventilates between the two into several hundreds Pa condition. The path of the ions pulled through by the sampling cone and the skimmer cone converge into the mass spectrophotometer through the ion lens. The ion lens and the mass spectrophotometer unit are ventilated to 10-3 and 10-4 Pa respectively, by the turbo molecular pump. The ions sorted by mass with the mass spectrophotometer are detected by the ion detector.

2.2.2 APPLICATIONS

* One of the largest volume uses for ICP-MS is in the medical and forensic field, specifically, toxicology.
* Another primary use for this instrument lies in the environmental field. Such applications include water testing for municipalities or private individuals all the way to soil, water and other material analysis for industrial purposes.
* Industrial and biological monitoring has presented another major need for metal analysis through ICP-MS. Urine and blood analysed for metal toxicity in individuals working in factories exposed to metals such as in battery factory.
*ICP-MS is also used widely in the geochemistry field for radiometric dating, in which it is used to analyze relative abundance of different isotopes, in particular uranium and lead.
*In the pharmaceutical industry, ICP-MS is used for detecting inorganic impurities in pharmaceuticals and their ingredients.

2.3 LYOPHILIZATION

Lyophilization also known as cryodesiccation or freeze-drying is a process whereby a product is dried by removing the water under low temperature and pressure. It is a water removal process typically used to preserve perishable materials, to extend shelf life or make the material more convenient for transport. Primary application of freeze-drying include biological, biomedical, food processing and preservation.

2.3.1 THE TECHNICAL EXPLANATION OF LYOPHILIZATION

Lyophilization involves the removal of water or other solvents from a given product by a process called sublimation. This occurs when the ice of a frozen product converts directly to the gaseous state without passing through the liquid phase. This enables the preparation of a stable product that is easy to use and store at ambient temperature.

A low pressure environment is pre-requisite to allow this process to take place. In order to start the removal of water, the pressure inside the freeze dryer must be below the triple point value for the product,whilst also maintaining the temperature of the sample below its freeze point in the lyophilization process.

Lyophilization consists of three major stages. They are:

i) Pre-freezing

ii) Primary drying

iii) Secondary drying

i) PRE-FREEZING

The sample is frozen, which means the water in the product is converted to ice, thereby the phase has changed from liquid to solid.

Slow pre-freezing will produce larger ice crystals, which are easier to lyophilize, whilst fast pre-freezing results in smaller crystals.

ii) PRIMARY DRYING

In the second stage of lyophilization, the sublimation process starts. The ice formed in the pre-freeze step is removed from the sample by the direct transition of the solid ice to vapour without passing through a liquid phase. The resultant vapour is collected by the condenser, which has a lower temperature and pressure than the product. The vapour is thus converted back to ice on the condenser surface. The energy required for this process to occur is provided by a gentle heating of the sample, which will start the sublimation process and eventually the sample will dry.

If too much energy (heat) is applied to the sample during this stage, the condenser of the lyophilizer may not be able to condense the volume of vapours fast enough, the ice condenser

temperature will subsequently rise along with its vapour pressure, thus increasing the risk of the sample melting.

iii) SECONDARY DRYING

Finally, ,any residual water present, which is strongly bound to the molecules of the sample,is converted to vapour and removed from the sample.

This sample has invariably a vapour pressure lower than that of water in its free form.

Removal of the water in this final stage of lyophilization will be performed at higher product temperatures, consequently any biological activity of the sample will not be impaired or affected. This usually involves increasing the temperature and lowering the pressure to provide enough energy to break down the molecular bonding.

2.3.2 ADVANTAGES OF LYOPHILIZATION

The principal advantages of lyophilization as a drying process are:

i) Minimum damage and loss of activity in delicate heat-liable materials.

ii) Speed and completeness of rehydration.

iii) Possibility of accurate, clean dosing into final product containers.

iv) Porous, friable structure.

2.4 THIN LAYER CHROMATOGRAPHY(TLC)

Thin layer Chromatography is a chromatographic technique used to isolate the components of the nonvolatile mixtures. It was developed by Izmailov in the year 1938 considering Mikhail Tswett's description on column chromatography. The experiment is conducted on a sheet of aluminum foil, plastic or glass which is coated with a thin layer of adsorbent material. The material usually used is aluminum oxide, cellulose or silica gel. On completion of the separation, each component appears as spots separated vertically.

Each spot has a retention factor (Rf) expressed as : -

Rf = Distance traveled by the sample / Distance travelled by the solvent

Factors affecting Rf factor are the solvent system, amount of the material spotted, adsorbent and the temperature. Thin layer chromatography is one of the fast, least expensive, simple and easiest chromatographic technique.

2.4.1 COMPONENTS OF TLC

Thin layer chromatography experiment is conducted with the help of different components. They are : -

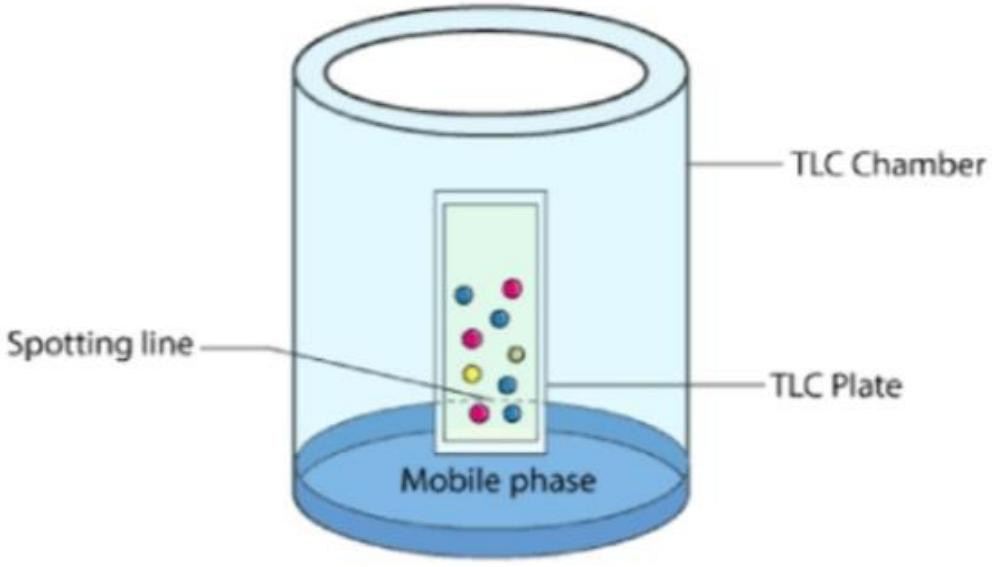
TLC plate - ready made plates are used which are chemically inert and stable. Stationary phase is applied on its surface in the form of a thin layer. Stationary phase on the plate has a fine particle size and also has a uniform thickness. One can also use a TLC paper and it is moistened in the mobile phase.

TLC chamber - chamber is used to develop the plates. It prevents the solvent evaporation and keep the entire process dust - free.

TLC mobile phase - mobile phase is the one that moves and consists of a solvent mixture or a solvent. This phase should be particulate - free. Higher the quality of purity, the development of spots is better.

2.4.2 PRINCIPLE

Like other chromatographic technique, thin layer chromatography depends on the separation technique. The separation relies on the relative affinity of compounds towards both the phase. The compounds in the mobile phase move over the surface of stationary phase. The movement occurs in such a way that the compounds which have a higher affinity to the stationary phase move slowly while the other compounds travel fast. Therefore, the separation of the mixture is attained. On completion of the separation process, the individual components from the mixture appear as a spot at respective levels on the plates. Their character and nature are identified by suitable detection techniques.



2.4.3 APPLICATIONS

* The qualitative testing of various medicines such as sedatives, local anesthetics, analgesics, anti - convulsant tranquilizers, steroids, hypnotics is done by TLC.

* TLC is extremely useful in biochemical analysis such as separation or isolation of biochemical metabolites from its blood plasma, body fluids, serum etc.

* It is widely used in separating multicomponent pharmaceutical formulations.

* TLC can be used to identify natural products like essential oils or volatile oils, glycosides, waxes, alkaloids etc.

* It is used to purify of any sample and direct comparison is done between the sample and authentic sample.

* It is used in food industry, to separate and identify colours, sweetening agent and preservations.

* It is used in cosmetic industry.

2.5 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Infrared spectroscopy is basically vibrational spectroscopy. The absorption of infrared radiation by molecules results in vibrational energy transitions. The vibrational energy levels in a molecule are quantized and this energy is very small compared to the energy required for electronic transitions and therefore vibrational transitions can be brought about by infrared radiations in the frequency range 4000 cm^{-1}- 400 cm^{-1}.

Fourier transform infrared spectroscopy (FTIR) is a technique 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 wavelengths at a time.

The term Fourier transform infrared spectroscopy originates from the fact that a fourier transform (a mathematical process) is required to convert the raw data in to the actual spectrum.

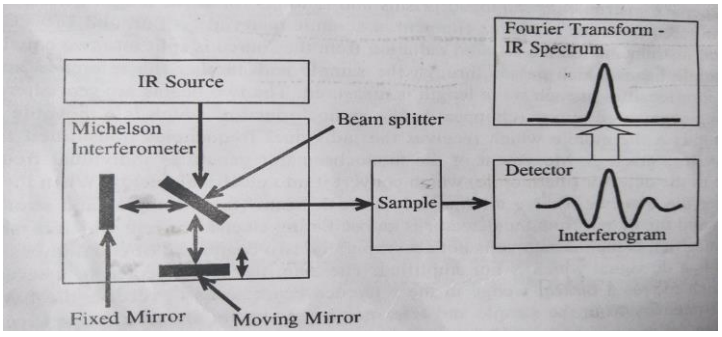
2.5.1 PRINCIPLE

The heart of fourier transform infrared spectrometer is a Michelson interferometer. In an FT instrument, the spectrum is obtained as an interferogram, consisting of a complex interacting signals which is a time domain spectrum and this information is a function of time, which is of

no use to a chemist. A more useful information can be sought by converting it in to a frequency domain spectrum by a mathematical process known as fourier transformation by using a computer. The intensity versus frequency, spectrum is identical to the infrared spectrum obtained from a dispersive instrument.

FTIR instrument operates in a single beam mode and use a computer. The interferometer processes the infrared energy put in to the sample. In the interferometer, the beam splitter is a mirror placed at 45 degree angle to the incoming IR radiation which allows the incoming radiation to pass through and separates it in to two perpendicular beams, one undeflected and the other orienting at an angle 90 degree. The 90 degree beam falls on a fixed mirror and returns to the beam splitter. The path length of the second beam varies as a result of the motion of the mirror. When these two beams meet at the beam splitter their recombination produces constructive and destructive interference.

The combined beam consisting of all these interference pattern is called interferogram and contains a wide range of wavelengths. This interferogram is directed towards the sample by the beam splitter. Thus the sample absorbs all the wavelengths simultaneously and the modified interferogram that reaches the detector carry information about the energy absorbed at every frequency which is compared to a standard laser beam. A fourier transform of the final interferogram (a time domain signal) produces a frequency domain infrared spectrum.



2.5.2 ADVANTAGES OF FTIR INSTRUMENTATION

1) Data acquisition needs very small time even less than one second and the spectra can be added

2) Better signal to noise ratio

3) Great speed and better sensitivity compared to a dispersive instrument

4) High wavelength accuracy is maintained by He - Ne laser calibration and resolution even less than 0.1per cm^{-1} can be achieved.

5) Works with low sample concentration.

6) When a spectrum is recorded infrared active gases like carbon dioxide and water vapour present in the atmosphere also absorb and produce their own absorption bands. This can be eliminated first by recording a background spectrum before the sample is examined and the subtraction of the background spectrum from the sample spectrum yields a spectrum obtained similar to that from a double beam dispersive instrument.

2.5.3 APPLICATIONS OF FTIR SPECTROSCOPY

1) Quality verification of incoming / outgoing materials.

2) Deformulation of polymers, rubbers, and other materials through thermogravimetric infra-red (TGA-IR) or gas chromatography infra-red (GC-IR) analysis.

3) Microanalysis of small sections of materials to identify contaminants.

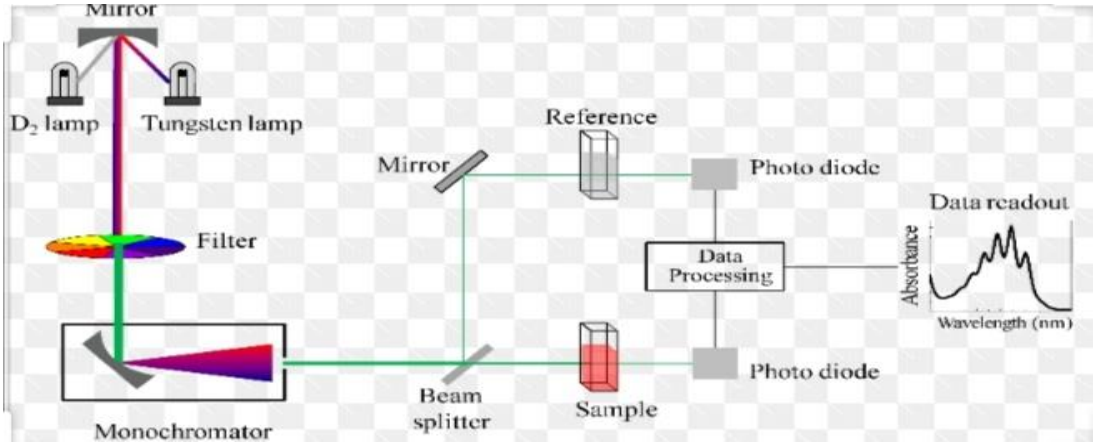
4) Analysis of thin films, coatings and failure analysis.

5) Monitoring of automotive or smokestack emissions.

UV SPECTROSCOPY

Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed or emitted when the molecules or atoms or ions of a sample moves from one energy state to another energy state. UV spectroscopy is type of absorption spectroscopy in which light of ultra violet region (200-400nm) is absorbed by the molecule, which results in the excitation of the electrons from the ground state to higher energy state. Such transitions can be studied extensively to understand the binding energy of corresponding electrons that undergoing transtions. When the wave length of transition exceeds the uv range, based on the same principle, even the colors of the molecules can be explained on the basis of absorption of the visible light.

PRINCIPLE



UV-Visible is often called a general technique because most molecules will absorb in the UV-Visible wavelength range. The UV extends from 100-400 nm and the visible spectrum from 400-700nm. The 100-200 nm range is called the deep UV. Light sources are more difficult to find for this range, so it is not routinely used for UV-Visible measurements. Typical UV-Visible spectrometers use a deuterium lamp for the UV that produces light from 170-375 nm and a tungsten filament lamp for visible, which produces light from 350-2,500 nm.

When a photon hits a molecule and is absorbed, the molecule is promoted into a more excited energetic state. UV-visible light has enough energy to promote electrons to a higher electronic state, from the highest occupied molecular orbital(HOMO) to the lowest unoccupied molecular orbital(LUMO). The energy difference between HOMO and the LUMO is called the band gap. Typically these orbital are called bonding and anti-bonding. The energy of the photon must match the band gap for the photon to be absorbed. Thus, molecules with different chemical structures have different energy band gaps and different absorption spectra. The most transitions that fall in UV-visible range are π-π^* and n-π^*. Pi orbitals arise due to double bonds, and n orbitals are for non bonding electrons. π^* are antibonding pi orbitals. Thus the best UV-visible absorption is by molecules that contain double bonds. Pi orbitals adjacent to each other that are connected, called conjugation, typically increases absorption. Sigma* transitions associated with single bonds, are higher energy and fall in the deep UV, so they are less useful for routine use. The appearance of broad bands or shoulders on the UV-visible structure is due to the numerous vibrational and rotational states of a molecule, which lead to separate energy bands gaps of slightly different energies.

APPLICATION

* Detection of impurities

- It is one of the best methods for determination of impurities in organic compounds.

* Structural elucidation of organic compounds.

- It is useful in detecting the presence or absence of unsaturation and presence of hetero atoms.

* Quantitative determination of compounds.

* UV absorption spectrum can characterize those types of compounds which absorbs uv radiation, thus useful in qualitative determination of compounds

* This technique is used to detect the presence or absence of functional group in the compounds.

* Kinetics of the reaction can also be studied using uv spectrometer.

* Molecular weight of the compounds can be measured spectrometrically by preparing suitable derivatives of these compounds.

* UV spectrophotometer may be used as a detector for HPLC.

CHAPTER 3- RESULTS AND DISCUSSION

1. ICPMS

ICPMS of Anjanakallu was done and the sample was analysed for its heavy metal content.

ICPMS analysis result is given in the table below.

S.No.	Heavy Metal	Test Result
1.	Lead	83.35%
2.	Arsenic	0.61 ppm
3.	Cadmium	24.72ppm
4.	Mercury	Below Detection Level

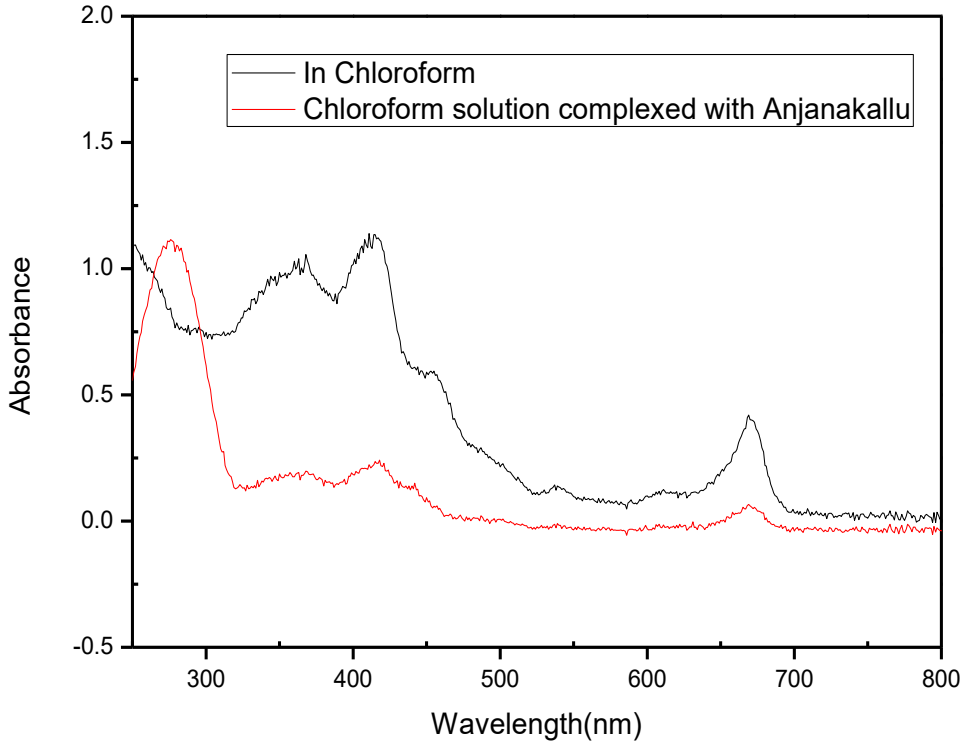
Table 3.1 The percentage composition of Anjanakallu.

This proves that Anjanakallu has predominantly Pb as the heavy metal element present in it.

2. UV-Visible Spectrum

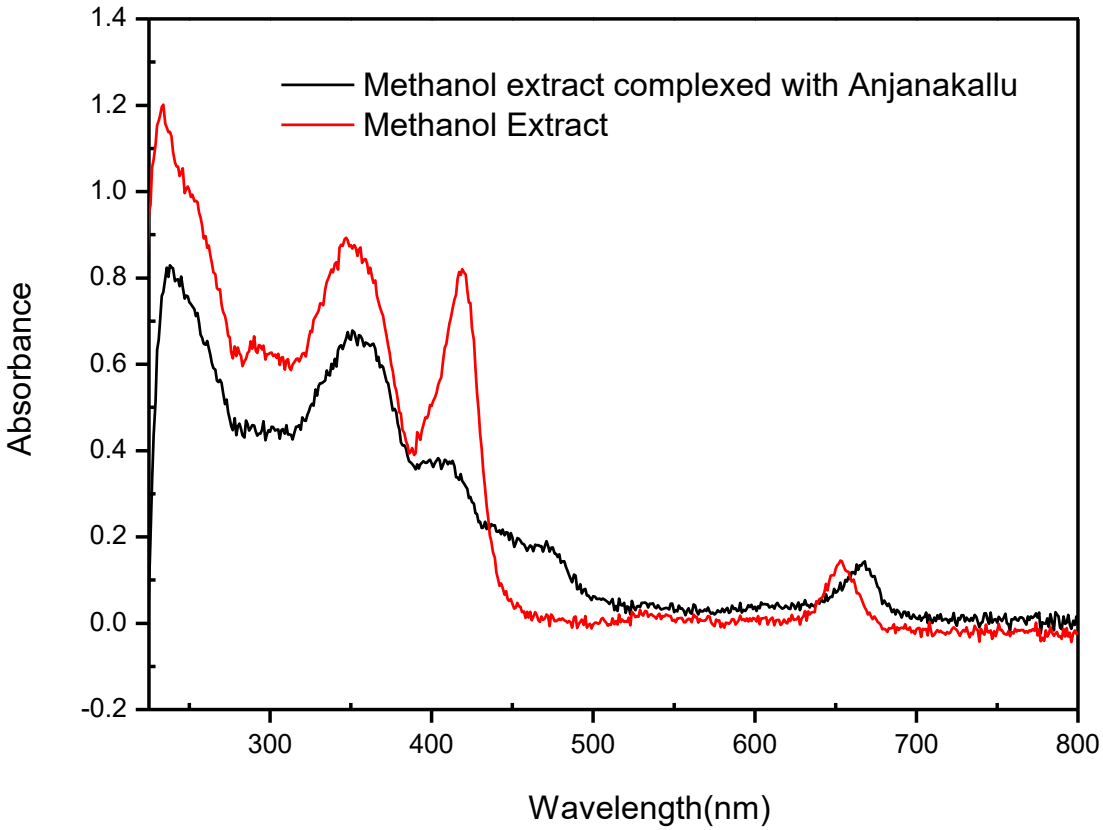
The UV Visible spectrum was recorded in two solvents, one in chloroform and the other in methanol.

In Chloroform:

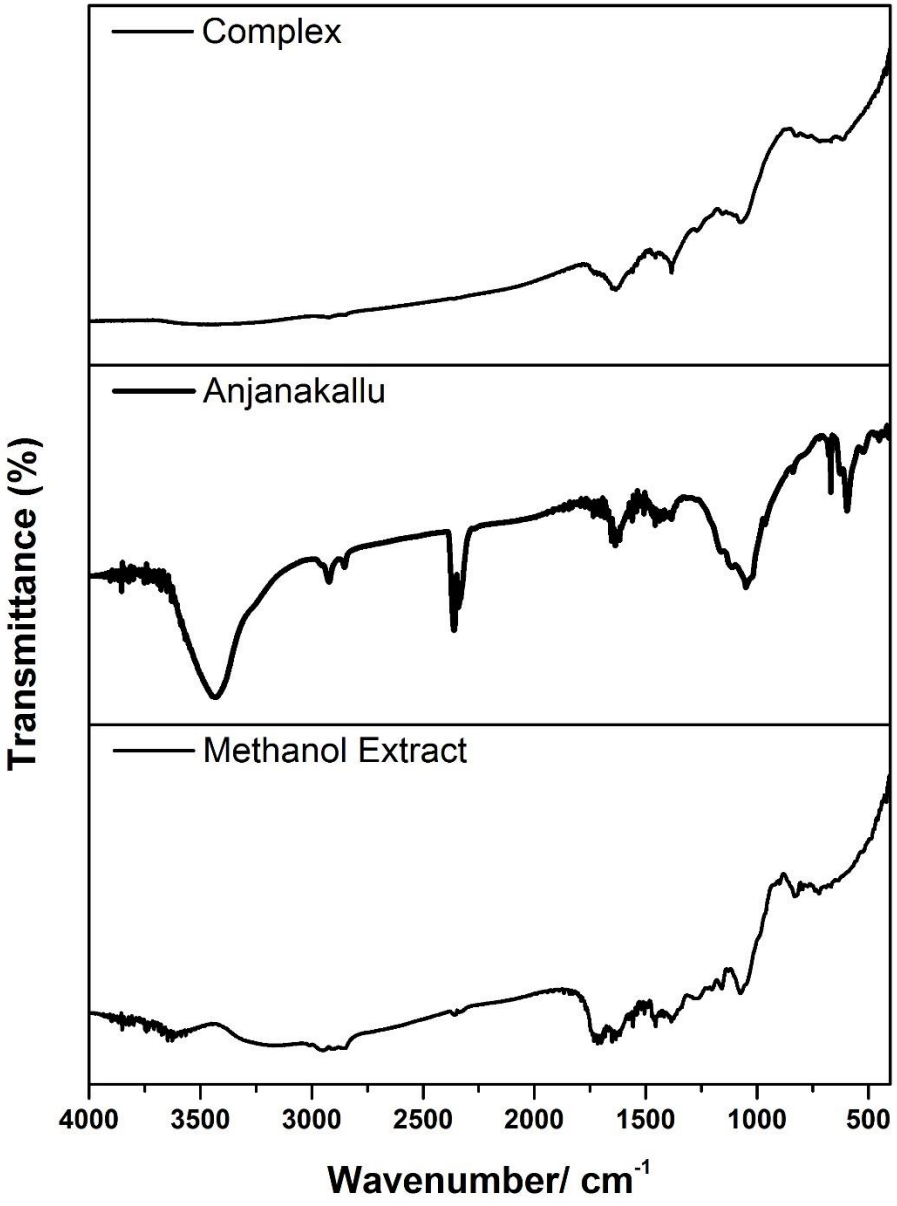


The compound isolated from the methanol extract was dissolved in chloroform and a UV Visible Spectrum was recorded. Another spectrum of the sample after mixing and grinding with Anjanakallu was recorded in Chloroform. One find that the intensity of two peaks at 368 nm and 415 nm has decreased on complexation and a new peak has emerged at 276 nm, a clear indication that there is a complexation of the Eclipta Alba taking place with Pb. A peak at 275 nm is generally reported for Pb-S system, which indicate that Pb has complexed with Eclipta Alba.

In Methanol:



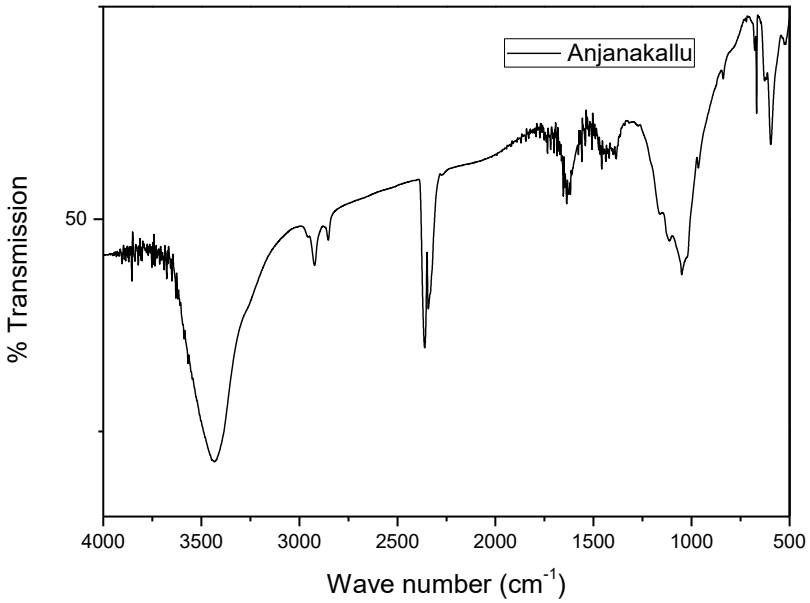
UV spectrum was recorded of the sample and the sample complexed with Anjanakallu was done in methanol. There is a distinct decrease in the intensity of the 415 nm peak when it is complexed with Anjanakallu.

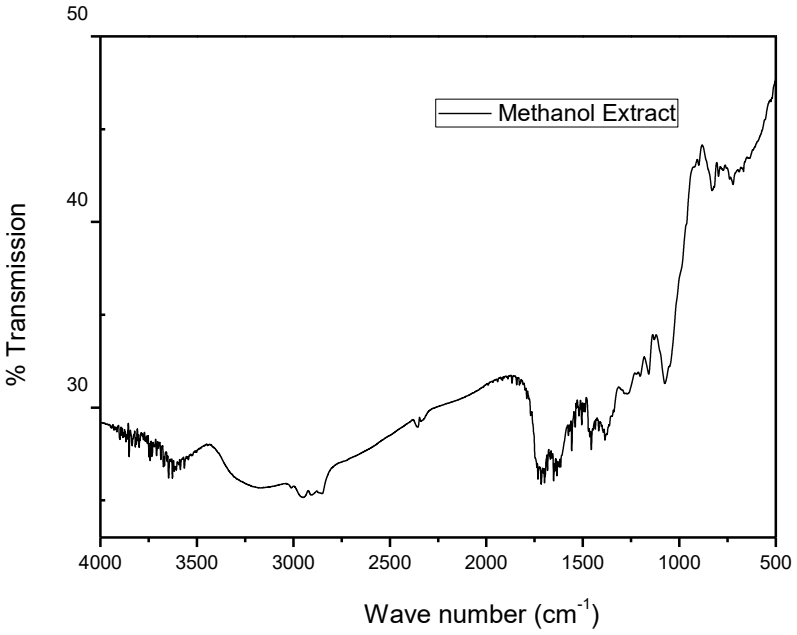


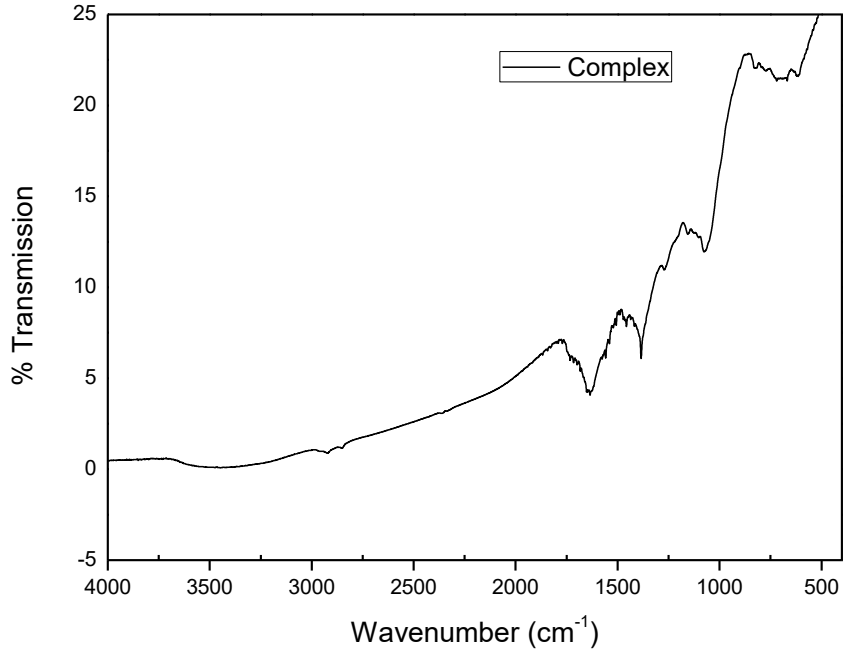
3. IR DATA

The FTIR Spectrum of Anjanakallu show peak at 3433 cm^{-1} might be due to the presence of free hydroxyl ions. The peaks at 808 cm^{-1} and 780 cm^{-1} are indicative of the Pb-S band.

The individual plots are depicted below for more clarity.


Anjanakallu
% Transmission
50
4000
3500
3000
2500
2000
1500
1000
500
Wave number (cm-1)


Methanol Extract
% Transmission
50
40
30
4000
3500
3000
2500
2000
1500
1000
500
Wave number (cm-1)



The Pb-S bond peak at 808 and 780 cm^{-1} has weakened and has less intensity in the complex. A new sharp and strong peak at 1284 cm^{-1} needs to be assigned.

Conclusions

A investigation into the interaction of Anajanakallu with Eclipta Alba has been carried out using IR and UV. The results has indicated that there is some binding of the lead with the components of Eclipta Alba. Both UV and IR datas are in agreement with this conclusion.

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