

Structural investigation of Hybanthus by using GC-MS FT-IR

**Submitted in partial fulfillment of the requirements for the award of Master of
Science degree in Physics**

By

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SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **JOSELIN PRAGEETHA.J (40590020)** who carried out the project entitled "**Structural investigation of Hybanthus by using GC-MS FT-IR**" under my supervision from **NOVEMBER 2021 TO MARCH 2022**

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I **JOSELIN PRAGEETHA.J (40590020)** hereby declare that the Project Report entitled "**Structural investigation of Hybanthus by using GC-MS FT-IR**" done under the guidance of **DR. AMIRTHA SHER GILL** is submitted in partial fulfillment of the requirements for the award of Master of Science degree in Physics.

A handwritten signature in black ink, reading "Joselin Prageetha", slanted upwards to the right.

DATE:

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SIGNATURE OF THE CANDIDATE

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I am pleased to acknowledge my sincere thanks to the Board of Management of **SATHYABAMA** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

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(JOSELIN PRAGEETHA.J)

Abstract

World Health Organization (WHO), says the goal of health for all cannot be achieved without herbal medicines. In our ancient times, even fatal diseases are cured by the herbal plants grown in our country. Numerous herbal plants used even today originated from India. Most of the biologically active organic compounds were applied as medicine to prevent various diseases in the human body. Plants have been utilized by an individual for ages in traditional medicine because of its therapeutic potential and the inquiry on therapeutic plants has prompted the disclosure of novel drug applicants utilized against different diseases.

In this study, a medicinal plant called *Hybanthus enneaspermus* is characterized. This plant is widely used for the treatment of various acute or chronic diseases with diverse nature of phytoconstituents. In Ayurveda, it is called 'Sthalakamala'. Other names are Ratan purush (Hindi), Numbora (Bengali), Purusharathna (Kannada), Orithalthamara (Malayalam), Orithal thamarai (Tamil), Rathnapurusha (Sanskrit), and Rathanparas (Marathi). It is a perennial medicinal herb which is used in traditional medicine for treating diarrhoea, urinary infections, leucorrhoea, dysuria, inflammation, cholera, and sterility.

Also, in this project, the structural characterization of the plant *Hybanthus* is identified by using GC-MS, and FT-IR. Gas Chromatography-Mass Spectrometry (GC-MS) is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Gas chromatography is a technique used to separate drugs that might be present in a sample. The detector for the GC is the Mass spectrometry detector. A mass spectrometer (MS) is an instrument that serves for the establishment of the molecular weight and structure of both inorganic and organic compounds, and the identification of the components in complex mixtures. FTIR can identify unknown materials and can also determine the quality or consistency of a sample and the number of components in a mixture. Using GC-MS and FT-IR the plant *Hybanthus* is characterized and its structural components are verified.

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CHAPTER 1

1.1 INTRODUCTION

World Health Organisation (WHO), says the goal of health for all cannot be achieved without herbal medicines. As our lifestyle is now cultured in this technological world, we are moving apart from nature. Thereby, medicine and treatment have become business and dealing. But when we look back into our ancient times, even fatal diseases are cured by the herbal plants grown in our country. Numerous herbal plants used even today originated from India. According to Bible data and the book of holy Judah named the Talmud, aromatic plants, were used for various rituals and treatments. Medicinal plants are considered fertile resources of synthetic drugs or substances that can be used in the development of drugs. Apart from that, these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants are considered as an important source of nutrition, and as a result of that, they are suggested for their therapeutic values. As we probably are aware, there are numerous herbs that are taken from nature which we can expand to keep our body's wellbeing. Despite the fact that there have been numerous sorts of research identified with the improvement of therapeutic wellbeing by the concoction treatment, there are as yet numerous individuals who devour the normal drug more than the synthetic ones. Shockingly, since the vast majority of the home grown medicines are done in a conventional matter inside a nearby culture and society of specific nations, numerous individuals in the urban communities don't think about numerous supernatural herbs that may enable them to improve their wellbeing.

Recently, it is estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. As herbs are natural products, they contain no side effects, they are relatively safe and they are environmentally friendly and locally available.

Indian medicinal plants include biological diversity. India is one of the seventeenth identified mega bio-diverse countries of the world. With only 2.4 percent of the total land area of the world, the known biodiversity of India

contributes 8 percent to the known global biodiversity culture and heritage are an integral part of 'Right to life' guaranteed by article 21, Constitution of India. Nature has been a powerful medicine for thousands of years and a number of modern drugs have been extracted and exploited from a natural source, for its use in traditional medicine is still the primary form of treating diseases of the majority of people in developing countries including India. Most of the biologically active organic compounds were applied as medicine to prevent various diseases in the human body. A wide variety of pharmaceutical drugs is used for relieving acute pains and help in controlling mental or physical ailments.

In up-to-date medicine, drugs can be synthesized and developed from medicinal plants. The medicinal plant used for the study is *Hybanthus enneaspermus*(L)F.Muell. This plant is native to the Himalayan region and warmer parts of India. It is also distributed in Sri Lanka, Tropical Asia, Africa, and Australia. It is commonly found in river banks, open grasslands, sandy regions, and waste lands. It is a perennial small herb which is used in Ayurveda, Siddha, and other traditional medicinal for aliments. It is also known as 'spade flower' and 'pink lady slipper'. In Ayurveda, it is called 'Sthalakamala'. Other names are Ratan purush (Hindi), Numbora (Bengali), Purusharathna (Kannada), Orithalthamara (Malayalam), Orithal thamarai (Tamil), Rathnapurusha (Sanskrit), and Rathanparas(Marathi).

The phytochemicals present in the plant include alkaloids, dipeptide, isoarborinol, sitosterol, flavonoids, sugars, tannins, etc. Recent research work proved that the plant possesses medical activities against several dreadful diseases. Annual herbs to 30 cm high; stem woody at base, scabrous. Leaves subsessile, 0.6-3 x 0.3-1 cm, linear-lanceolate or elliptic-lanceolate, base attenuate, margins distantly crenate, crenations scabrous hairy, apex acute, hirsute; stipules 1-2 mm long, linear-lanceolate. Flowers axillary, solitary; pedicel 0.6-1.5 cm long, slender. Sepals 5, subequal, 2-3 x 1 mm, lanceolate, acute, ciliate. Petals 5, pinkish, unequal; lower one 0.8-1.5 cm long, suborbicular, clawed, other 4 smaller, 3-5 mm long, elliptic or triangular-oblong. Stamens 5, c. 2 mm long. Ovary ovoid, 1-celled; ovules many; style clavate; stigma oblique. Capsules 5-8 mm long, ovoid. Seeds many, ovoid.

Traditionally, by ayurvedic this plant is accounted to treat root causes of "Kapha" and "Pitta", vomiting, urethral discharge, blood asthma, cough, urinary calculi, painful dysentery, blood pressure, strangury, epilepsy, burning sensation. Phytochemically, the plant contains a lot of dipeptide alkaloids, aurantiamide acetate, isoarborinol, and β -sitosterol, sugars, flavonoids, steroids, triterpenes, phenols, flavones, catachins, tannins, anthraquinones and amino acids. Pharmacologically, the plant is accounted for to have antidiabetic, antiplasmodial, antimicrobial, anticonvulsant, nephroprotective, aphrodisiac, hepatoprotective, calming, aldose reductase inhibitory and free extremist rummaging exercises. The information will be worthwhile to know the applicability of *H. enneaspermus* for the treatment of various acute or chronic diseases with a diverse nature of phytoconstituents.

1.2 TAXONOMIC CLASSIFICATION OF *HYBANTHUS ENNEASPERMUS*:

Kingdom	-	Plantae
Phylum	-	Tracheophyta
Class	-	Magnoliopsida
Order	-	Malpighiales
Family	-	Violaceae
Genus	-	<i>Hybanthus</i>
Species	-	<i>enneaspermus</i>

The phytochemicals present in *H. enneaspermus* have been extensively investigated. The chief chemical constituents include alkaloids, flavonoids, steroids, terpenoids, phenols, dipeptide, isoarborinol, sitosterol, sugars and tannins. Awobajo et al reported the presence of flavonoids, cardiac glycosides, cyanogenic glycosides, anthraquinone glycosides, saponins, tannins and hexose sugar in the aqueous leaf extract of *H. enneaspermus*. It is a perennial medicinal herb which is used in traditional medicine for treating diarrhoea, urinary infections, leucorrhoea, dysuria, inflammation, cholera and sterility. Gas chromatograph mass spectrometers was used to identify phytochemical compounds present in the extracts of *H. enneaspermus*. Some compounds found in the extracts have been

expressed to display antioxidant, anti-inflammatory, antibacterial and antimicrobial activities. ex: apocynin, n-hexadecanoic acid, trifluoroacetoxy hexadecane, hexadecane,9-nonadecene, 2,4-di-tert-butylphenol, cyclohexadecane and ethyl-cyclodocosane and those were the compounds identified by GC-MS.

1.3 AYURVEDIC USES:

Hybanthus enneaspermus is also used in traditional medicines like the treatment of diarrhoea, urinary infections, leucorrhoea, dysuria, inflammation, cholera, and sterility. For pregnant women, the whole plant is itself a savior. Also, the decoction of leaf and tender stalks are used as a demulcent. Hybanthus enneaspermus leaves are generally used as an external application for the treatment of wounds. Its leaves after drying becomes a life-saving powder for the treatment of asthma. Hybanthus enneaspermus root infusion is used as a diuretic for gonorrhoea and urinary infections. The fruit of it contains antivenom power which is majorly used for snakebite and scorpion sting. In Ayurvedic literature, the plant is used to treat disorders such as painful dysentery, vomiting, urinary calculi, burning sensation, blood troubles, asthma, epilepsy, and also used for breast tone. In Siddha medicine, this divine herb, is used to enhance milk secretion in women. This herb being applied externally can cure ulcers and headache. Hybanthus enneaspermus plant believes to increase libido and is also used to treat stress-related disorders.

1.4 PHARMACOLOGICAL ASPECTS OF *H.enneaspermus*

Hybanthus enneaspermus possesses antimicrobial activity, antibacterial activity, antifungal activity, antiviral activity, antioxidant activity, antidiabetic activity, anti-arthritic activity, antinociceptive activity, anti-allergic activity, anticonvulsant activity, antihyperlipidemic activity, nephroprotective activity, cardioprotective activity, neuroprotective activity, etc...

1.4.1 Antimicrobial activity:

Antimicrobial activity is the term that prevents the formation of microbial colonies and may destroy micro-organisms in plants. This plant Hybanthus contains the property of antimicrobial activity. Microorganisms are significant sickness-causing specialists which contaminate the two creatures and plants.

Antimicrobial factors follow up on diseases brought about by the microorganisms. Plant-based antimicrobial medications are more effective when contrasted and manufactured drugs. metabolites delivered by the plants have more bioactive mixtures which can be really utilized as antimicrobial factors.

1.4.2 *Antibacterial activity*

The antibacterial action of various concentrates of *H. enneaspermus* was accounted in *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus*.. Most extreme inhibitory impact was found in methanol remove followed by petrol ether and chloroform extricate. The in vitro investigation of *H. enneaspermus* separates against aflatoxin-creating organisms *fumigatus* *A. flavus* and *A.* and human pathogenic growths *Candida albicans* and *Candida tropicalis* showed critical antifungal action . The parasitic spots disengaged from wet garments were refined and the zone of not set in stone by plate dispersion test . The antimicrobial action may be because of the presence of naturally dynamic parts like alkaloids, flavonoids, and so forth. In another review, the ethanolic concentrate of *H. enneaspermus* showed huge antifungal activity against *C. albicans* and *A. niger*.

1.4.3 *Antiviral activity*

Human immunodeficiency infection (HIV) contamination is a lethal illness spreading quickly all through the world. As of late, the counter HIV action of *H. enneaspermus* leaf extricate by invert transcriptase hindrance test. As there is no agreeable remedial medication, more exploration work has been completed in plant-based items. The methanol and hexane removes showed positive outcomes and ethanol, chloroform and petrol ether extricate showed no restraint.

1.4.4 *Antioxidant activity*

There are hundred issues in people including disease like, joint pain, gastritis, cancer and so forth, are because of aggregation of free extremists in the body. They are created by different substance and actual elements. The hydroethanolic concentrate of *H. enneaspermus* alongside paracetamol displayed critical cell reinforcement action and diminished lipid peroxidation action in rodents. They are created by different substance and actual elements. Cell reinforcements go about as likely specialists to treat the free extremist interceded infections. The in vitro investigation of alcoholic concentrate of *H. enneaspermus*

showed higher cell reinforcement action with great lessening power against responsive oxygen species like DPPH revolutionary, nitric oxide, hydrogen peroxide and deoxyribose . The ethyl acetic acid derivation concentrate of *H. enneaspermus* showed solid cancer prevention agent movement because of the presence of phenolic compounds. In another review, the cancer prevention agent capability of field-developed plant was contrasted and in vitro developed calli of *H. enneaspermus*. In all the free revolutionary searching tests, the in vitro-refined examples showed expanded movement than the field-developed plants.

1.4.5 *Antidiabetic activity*

This plant *Hybanthus enneaspermus* contains the antidiabetic activity. Diabetes mellitus is a metabolic problem that occurs all over the world because of deformity in insulin creation. Under extreme circumstances, it prompts confusion in the kidney, eye, sensory system and heart. As of late, more exploration work has been focussed on the treatment of diabetes by phytodrugs. They assessed the alpha-amylase and alpha-glucosidase inhibitory action of different dissolvable concentrates of *Hybanthus enneaspermus*. Ethanol extricate showed most extreme inhibitory impact for both the proteins (alpha-amylase: 200 µg-84.61%; alpha-glucosidase: 200 µg83.02%) when contrasted and oil ether, watery and CH₃)₂CO separates . Blood glucose level was decreased in streptozotocin-actuated diabetic rodent model. Also, improvement in body weight was seen which demonstrated its capacity to lessen hyperglycemia. Patel et al revealed the antidiabetic capability of alcoholic concentrate of *Hybanthus enneaspermus* in rodent hemi-stomach.

1.4.6 *Anti-arthritis activity*

Rheumatoid joint pain is an immune system issue which causes aggravation in a joint. Since the aftereffects and harmfulness of engineered drugs are higher, more successful normal medications are required. Tripathy et al assessed the counter joint capability of alcoholic and fluid concentrates of *H. enneaspermus* on Freund's adjuvant-instigated joint pain in pale skinned person rodents. The paw thickness diminished for the rodents treated with both the concentrates. In ongoing stage, alcoholic concentrate (59.4%) displayed more action than the watery concentrate (57.4%).

1.4.7 *Antinociceptive movement*

The commercially available synthetic analgesic drugs cause several side effects such as kidney damage, constipation, gastrointestinal irritation, etc. Use of plant-derived products reduces the harmful side effects and cost of medication. Afolabi et al evaluated the antinociceptive effect of ethanolic extract of *H. enneaspermus* in albino rats by tail immersion and formalin test. The animals treated with 500 mg/kg and 1000 mg/kg doses of *H. enneaspermus* extract showed increased tail flick latency when compared with the animals treated with the standard drug, acetaminophen. In formalin test, the animals treated with 1000 mg/kg dose of *H. enneaspermus* extract significantly reduced the paw licking time when compared with acetaminophen. In another study, the analgesic activity of *H. enneaspermus* extract was proved by hot plate, radiant heat tail flick and tail immersion methods. In all the tests, ethanol and petroleum ether extracts at 200 mg/kg doses showed more significant antinociceptive activity. The plant extracts increased the stress tolerance capacity of the animals

1.4.8 *Anti-allergic activity*

Allergy is an exaggerated response of the immune system when it encounters a foreign antigen like dust, pollen, mold spores and chemicals. The physiological effects due to allergic reactions lead to various diseases. The in vitro study of ethanol and petroleum ether extracts of aerial parts of *H. enneaspermus* in albino mice proved the antiallergic activity by milk-induced leucocytosis and eosinophilia methods. In both the tests, ethanolic extract exhibited higher level of inhibition at a dose of 200 mg/kg when compared with the petroleum ether extract. The flavonoids and polar constituents in the plant mainly contribute for the anti-allergic activity

1.4.9 *Anticonvulsant activity*

The customary anticonvulsant drug utilized for neurological confusion, epilepsy causes unfavorable incidental effects and neurotoxicity. The plant *H. enneaspermus* has been customarily utilized for the treatment of epileptic fits. Hemalatha et al announced the anticonvulsant action of watery and ethanolic concentrates of *H. enneaspermus* utilizing maximal electrical shock and strychnine-initiated seizure models. Watery concentrate at the dosages of 200

mg/kg and 400 mg/kg showed half and 80% assurance separately. The level of mortality was diminished and no neurotoxicity was seen in creatures.

1.4.10 *Antihyperlipidemic activity*

Hyperlipidemia is the expanded degree of lipid remembering cholesterol and fatty oils for the blood. It expands the gamble of cardiovascular illnesses, diabetes, hypertension, stroke, and kidney harm. Hyperlipidemia was initiated by taking care of the wistar pale skinned person rodents with high-fat eating routine. The hydroalcoholic concentrate of *H. enneaspermus* displayed antihyperlipidemic action at 400 mg/kg portion. In intense poisonousness study, no indication of harmfulness and mortality was seen in creatures treated at a portion of 2000 mg/kg.

1.4.11 *Nephroprotective activity*

The kidneys are the main organ which eliminates the loss from the body. Assuming there is any deformity in the expulsion interaction, the squanders would aggregate in the blood and prompting renal harm. Discharge of many medications and their metabolites through kidney likewise prompts nephrotoxicity. The in vitro investigation of alcoholic and fluid concentrates of *H. enneaspermus* in cisplatin-actuated rodent models demonstrated the nephroprotective action. The heavy drinker (250 mg/kg and 500 mg/kg) and watery concentrates (500 mg/Kg) standardized the raised blood urea, blood protein and serum creatinine. Removes shielded the kidney from lipid peroxidation harm and expansion in body weight was noticed. The action of alcoholic concentrate was more powerful than the fluid concentrate.

1.4.12 *Cardioprotective activity*

Cardiovascular sickness which captures the heart work is the main source of death around the world. Many examination works has been focussed on an elective medication without aftereffects for this imperfection. Therapeutic plants rich in polyphenols may go about as possible specialists to lessen the gamble for coronary illness. Radhika et al announced the cardioprotective movement of *H. enneaspermus* in isoproterenol-incited rodents. Myocardial localized necrosis was instigated in rodents by organization of isoproterenol. When the myocardial infarcted rodents were treated with ethanolic concentrate of *H. enneaspermus*, it

diminished the oxidative pressure by diminished lipid per oxidation, decreased glutathione and standardized the degrees of cardiovascular marker catalysts like creatine kinase, lactate dehydrogenase, serum glutamic oxaloacetic transaminase, serum glutamate pyruvate transaminase and heart protein troponin I in the histological examinations.

1.4.13 *Neuroprotective activity*

Plant-based items like natural oils have been utilized generally for treating focal sensory system (CNS) messes. The vast majority of the depressant medications cause unavoidable secondary effects which influence the other indispensable organs. Kar et al reported the CNS movement of ethanolic and fluid concentrates of *H. enneaspermus* in mice models by labyrinth test (in addition, Y-labyrinth), barbiturate and liquor prompted dozing time, tail suspension test, despair test, head plunge test, locomotor action and engine coordination test. The mice were regulated orally with portions of 250 and 500 mg/kg of every one of the concentrate. Diazepam (1mg/kg), chlorpromazine (5 mg/kg) and imipramine (30 mg/kg) were directed intraperitoneally as standard medications. In every one of the tests, the creatures treated with *H. enneaspermus* separate displayed critical movement than the creatures treated with standard medications.

CHAPTER-2

LITERATURE SURVEY

Sahoo et al reported the antibacterial activity of different solvent extracts of *H. enneaspermus* against urinary tract pathogens (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus aureus*).

Anbalagan et al reported the anti-HIV activity of *H. enneaspermus* leaf extract by reverse transcriptase inhibition assay. The methanol and hexane extracts showed positive result and ethanol, chloroform and petroleum ether extracts showed no inhibition.

Patel et al reported the antidiabetic potential of alcoholic extract of *H. enneaspermus* in rat hemi-diaphragm. Blood glucose level was reduced in streptozotocin-induced diabetic rat model. Moreover, improvement in body weight was observed which indicated its ability to reduce hyperglycemia.

Tripathy et al evaluated the anti-arthritic potential of alcoholic and aqueous extracts of *H. enneaspermus* on Freund's adjuvant-induced arthritis in albino rats. The paw thickness decreased for the rats treated with both the extracts. In chronic phase, alcoholic extract (59.4%) exhibited more activity than the aqueous extract (57.4%).

Afolabi et al evaluated the antinociceptive effect of ethanolic extract of *H. enneaspermus* in albino rats by tail immersion and formalin test. The animals treated with 500 mg/kg and 1000 mg/kg doses of *H. enneaspermus* extract showed increased tail flick latency when compared with the animals treated with the standard drug, acetaminophen. In formalin test, the animals treated with 1000 mg/kg dose of *H. enneaspermus* extract significantly reduced the paw licking time when compared with acetaminophen.

Hemalatha et al reported the anticonvulsant activity of aqueous and ethanolic extracts of *H. enneaspermus* using maximal electrical shock and strychnine-induced convulsion models. Aqueous extract at the doses of 200 mg/kg and 400

mg/kg showed 50% and 80% protection respectively. The percentage of mortality was decreased and no neurotoxicity was observed in animals.

Radhika et al reported the cardioprotective activity of *H. enneaspermus* in isoproterenol-induced rats. Myocardial infarction was induced in rats by administration of isoproterenol. When the myocardial infarcted rats were treated with ethanolic extract of *H. enneaspermus*, it reduced the oxidative stress by decreased lipid per oxidation, reduced glutathione and normalised the levels of cardiac marker enzymes like creatine kinase, lactate dehydrogenase, serum glutamic oxaloacetic transaminase, serum glutamate pyruvate transaminase and cardiac protein troponin I in the histological studies.

Kar et al reported the CNS activity of ethanolic and aqueous extracts of *H. enneaspermus* in mice models by maze test (plus, Y-maze), barbiturate and alcohol-induced sleeping time, tail suspension test, despair test, head dip test, locomotor activity and motor coordination test. The mice were administered orally with doses of 250 and 500 mg/kg of each of the extract. Diazepam (1mg/kg), chlorpromazine (5mg/kg) and imipramine (30mg/kg) were administered intraperitoneally as standard drugs. In all the tests, the animals treated with *H. enneaspermus* extract exhibited significant activity than the animals treated with standard drugs.

CHAPTER 3

MATERIALS AND EXTRACTION METHOD

3.1 *PLANT COLLECTION*

The plant and leaves of *Hybanthus enneaspermus* were collected from senji, Villupuram district.



Fig 3.1 plant sample

3.2 *Materials and Extraction Method*

The shade dried *Hybanthus* leaves and *cassia auriculata* flowers need to be coarsely powdered, using a pestle and mortar, to provide a greater surface area. The plant material should be sufficient to fill the porous cellulose thimble (in our experiments we use an average of 14 g of thyme in a 25- x 80-mm thimble). Subsequently, these extracts were used for further investigation of the present study. Placed in the 120xhlet extractor for the extraction of bioactive compounds. A Soxhlet extractor has three main sections: a percolator (boiler and reflux) which circulates the solvent, a thimble (usually made of thick filter paper) which retains the solid to be extracted, and a siphon mechanism, which periodically empties the thimble. The source material containing the compound to be extracted is placed inside the thimble. The thimble is stacked into the main chamber of the Soxhlet extractor. The extraction solvent to be utilized is placed in a distillation flask. The flask is placed on the heating element. The

Soxhlet extractor is placed atop the flask. A reflux condenser is placed atop the extractor.

The solvent (250 ml of ethanol) is added to a round bottom flask, which is attached to a Soxhlet extractor and condenser (Fig.2.2) on an isomantle. The crushed leaf or flowers of Hybanthus is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The sidearm is lagged with glass wool. The solvent is heated using the reflux and solvent vapor travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapor cools, and drips back down into the chamber housing the solid material. The condensate at that point drips into the reservoir containing the thimble. Once the level of solvent achieves the siphon it pours back into the flask and the cycle starts once more. During each cycle, a portion of the non-volatile compound dissolves in the solvent, after many cycles the desired compound is concentrated in the distillation flask. The procedure should keep running for a sum of over hours or days once it has set up the extraction it can be left to run without direct supervision. After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound.



Fig 3.2 soxhlet apparatus

When the procedure has been completed, the ethanol will be evaporated utilizing a rotary evaporator, leaving a small yield of extracted plant material (about 2 to 3 ml) in the glass bottom flask. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave a residue

when evaporated. The non-soluble portion of the extracted solid remains in the thimble is discarded.



Fig 3.3 EXPERIMENT PROCEDURE IN IMAGES

3.4 PHYTOCHEMISTRY

Phytochemistry is the study of chemicals present in the plant. Furthermore, it expresses a large range of secondary metabolites present in plants which are by products of metabolic pathways. Phytochemicals include different classes of compounds like terpenoids. Anthocyanins alkaloids, flavonoids, coumarins etc. These Biomarkers are quantitatively and qualitatively analyzed by sophisticated analytical techniques and measure these compounds

3.5 *Phytochemistry in medicinal plants*

It was first proposed by French scientists Caventou and Pelletier after the discovery of quinine from Cinchona bark. This leads the world to an interest in plants of terrestrial and marine origins in the search of new medicines for the treatment of diseases. Laboratory of Professor J.B. de Harbonney has become center for the isolation of the active principles of medicinal plants from around the globe.

The therapeutic activity of the medicinal plant lies in the organic molecules possessing pharmacological properties that are produced by them. Many chemical constituents isolated from plants possess different activities depending on the attachment of functional groups on their structures. Many traditionally used medicinal plants are claimed to exert shows their activity based on the chemical constituents present in that particular medicinal plant. If a plant has different type of compound and thus different activity then overall effect of plant may modify the major active component. Bioactive constituents from plants are important component of R&D (research and development) projects in the Pharmaceutical industry. Natural products play a vital role in the discovery of new compounds which is pharmacological active that means which are clinically useful drugs, to cure ailments and diseases. Herbs provide bio-active products as well as the starting material for the new synthetic or semi synthetic drug entity. Chemical modification of natural compounds is done by applying various chemical approaches and it will be a promising line for lead generation.

Recently the active principles of medicinal plants were found as alkaloids, glycosides which have significant pharmacological properties therefore, they has received special attention, and constituted as major principal plant drugs of

allopathy. It is now realized that there are so many constituents other than alkaloids and glycosides, associated with plants may responsible for medicinal properties which provides a wide scope of natural plant metabolites for detailed investigation.

Regular advancement in synthetic organic chemistry provides many opportunities to identify key chemical molecules and to novel compounds. Many new drugs have produced via this route and now days have been used to treat cancer and heart related diseases, infections and blood pressure conditions weather high or low. Many drugs have been emerged via random screening of synthesized chemicals; many others resulted from sharp observations of scientists (happy chance). The phytochemical investigation thus involves the isolation of bioactive compound, characterization of the isolated compounds through spectroscopy and further quantitative evaluation and their biosynthetic pathways investigation

ISOLATION AND PURIFICATION:

- A. Collection of plants and its identification by our Taxonomy Division
- B. Solvent extractions of the plant materials using different solvents
- C. Partitioning of the extract into different parts according to the nature of compounds viz. terpenes, phenolics, glycosides, alkaloids etc.
- D. Assay of the extracts wherever possible for different biological activities.
- E Separation and purification of the compounds by column chromatography, and instrumental methods wherever necessary.

Natural products and their derivatives represent over 60% of all drugs clinically used worldwide where natural products from medicinal plants alone contribute to 25% of total drugs. Natural products and related drugs are reportedly used for example as antibacterial, anticancer, anticoagulant, antiparasitic and immunosuppressant agents to treat 87% of all categorized human diseases. More than 28% of new chemical entities introduced into the market are derived from natural products. More than 100 compounds particularly, anticancer and anti-infective, which are derived from natural products, are undergoing clinical trials at present and at least 100 natural products-derived compounds (Primarily plant or

microbial sources) are in preclinical development presently The value of natural products in this regard can be assessed using 3 criteria:

The rate of introduction of new chemical entities of wide structural diversity, including serving as templates for semi-synthetic and total synthetic modification.

The number of diseases treated or prevented by these substances.

Their frequency of use in the treatment of disease.

3.6 3,7,11,15-Tetramethyl-2-hexadecen-1-ol:

A chemical structure of a molecule includes the arrangement of atoms and the chemical bonds that hold the atom together. The 3,7,11,15-Tetramethyl-2-hexadecen-1-ol. Chemical formula of title compound C₂₀H₄₀O and its molecular weight 296.307917 and IUPAC name is 3,7,11,15-tetramethylhexadec-2-en-1-ol.

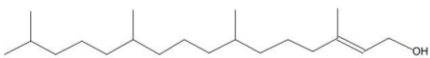
COMPOUND NAME	STRUCTURE	MOLECULAR FORMULA	MOLECULAR WEIGHT
3,7,11,15-Tetramethyl-2-hexadecen-1-ol		C ₂₀ H ₄₀ O	296.307917

Table 3.6 3,7,11,15-Tetramethyl-2-hexadecen-1-ol

CHAPTER – 4

SPECTROSCOPY INSTRUMENTATION TECHNIQUES OF FT-IR AND GC-MS

4.1 INTRODUCTION OF SPECTROSCOPY

Spectroscopy deals with interaction of matter with electromagnetic energy and spectroscopies harvest wealth of information about the matter from this interaction. It is an excellent technique to study atoms and molecules. Different spectroscopic techniques give different kinds of information, Molecular spectroscopy aims to understand the interaction of molecular energy with electromagnetic radiation. A molecule possesses various forms of energy due to its different kinds of motion and intermolecular interactions. Vibrational spectroscopy has been used to make significant contribution in many areas of physics and chemistry as well as in other areas of science. It's important applications are in the study of intra molecular and inter molecular forces, molecular structure determinations, computation of degree of association in condensed phases, elucidation of molecular symmetries, identification and characterization of new molecules, deducing thermodynamical properties of molecular systems, etc.,. Vibrational spectroscopy has also contributed significantly to the growth of other areas of chemistry such as polymer chemistry, catalysis, fast reaction dynamics, charge-transfer complexes, etc.

In order to "view" a molecule, we must use light having a wavelength smaller than the molecule itself (roughly 1 to 15 Å). Such radiation is found in the X-ray region of the electromagnetic spectrum and the field of X-ray crystallography yields remarkably detailed picture of the molecular structures amenable to examination. The chief limiting factor here is the need for high quality crystals of the compound being studied. The spectroscopic techniques described below do not provide a three-dimensional picture of a molecule, but instead yield information about certain characteristic features.

4.2 FOURIER TRANSFORM INFRARED SPECTROSCOPY

The conversion of time domain to the frequency domain is known as Fourier Transformation. FTIR can identify unknown materials and can also

determine the quality or consistency of a sample and the amount of components in a mixture. FTIR is the production of path difference between the two optical pathway which results in interference which may be constructive or destructive and this path difference is directly proportional to the intensity of radiation which is passing into the sample, which is converted into a wave number. Path difference is related to wave number of radiations that is passing into the sample and this process is controlled with respect to time. So we can accurately measure the wave number of the radiations passing through the sample that makes the FTIR to achieve more accuracy.

4.2.1 *PRINCIPLE:*

Based on the principle of interferometry and interferogram will be obtained which is a complex signal occurs in wave like pattern. Interferogram obtained after these analysis is plotted between intensity vs time and is calculated by mathematical equation which is known as Fourier transform. FTIR spectra will be generated between intensity vs frequency.

4.2.2 *INSTRUMENTATION:*

The basic components used in FTIR are, Radiation source, Interferometer, He-Ne laser, Detector and Sample. As shown in. Figure 4.1

RADIATION SOURCE:

The source useful for mid IR region are Globar, Nernst Glower. The source useful for near IR region is tungsten halogen lamp. The source useful for far IR region is mercury vapour lamp. This will produce IR radiation and then it will go to the beam splitter.

SAMPLE:

Solid, liquid and gaseous samples can be used.

DETECTOR:

It will detect the transmitted radiation and produce interferogram in the form of waves. Since FTIR is highly accurate and precise also having the high speed of detection, we have to select the detector which is fast acting detector. The most commonly used detectors are Pyroelectric detector which is made up of Deuterium Triglycine Sulphate and Photon detector which is made up of Mercury Cadmium Telluride.

4.3 HELIUM – NEON LAZER:

It is imperative that the position of the moving mirror is known at any given moment. The intensity of the laser beam is measured at two points in the interferometer. As the mirror moves the intensity at these two points will rise and fall due to the enhancement and cancellation of the He- Ne beam paths, producing a sine wave of intensity vs. mirror position. The number of fringes in the sine wave allows the instrument to know exactly how far the mirror has moved and the relative phase of sine wave tells the instrument in which direction the mirror is moving. It is responsible for accuracy of FTIR.

INTERFEROMETER:

One of the main component in FTIR is Michelson Interferometer. It consists of three components they are beam splitter, moving mirror and fixed mirror.

BEAM SPLITTER:

Beam splitter splits the incident radiations into two equivalent radiations. It is angled at 45° and is made up of germanium coated on KBr which can cleave the IR into two equivalent components.

MIRRORS:

Static and movable mirror are present in FTIR. Mirrors in an FTIR are generally made of metal. The mirrors are polished on the front side and may be gold coated to improve corrosion resistance. Commercial FTIRS use a variety of flat and curved mirrors to move light within the spectrometer, to focus the source onto the beam splitter, and to focus light from the sample onto the detector.

4.4 WORKING:

Source produces IR radiation, the radiations will reach beam splitter. The beam splitter will split the radiation into two equal parts. One part of the radiation will directly go through the beam splitter and reach movable mirror, while the other part of the radiation gets deflected and reach stationary mirror. The movable and stationary mirror reflects the radiation and will recombine again at beam splitter. The beam splitter splits the radiation into two halves. One part goes to the radiation source and other part of the radiation goes to the sample. Sample absorbs the radiation and it will reach detector. Then the detector will detect the transmitted radiation and will produce interferogram in the form of waves. The

interferogram will be calculated by the mathematical calculations or by the Fourier transforms equations and will be calculated by computer. The computer will give the result in the form spectra.

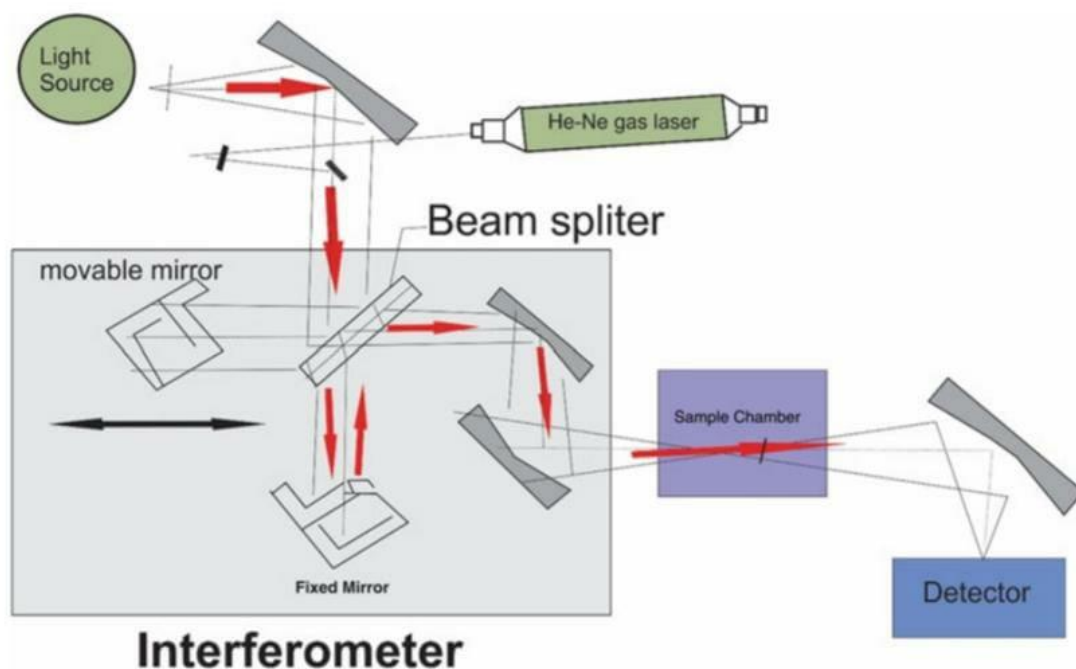


Figure 4.1 Instrumentation of the double-beam (top) and single-beam (bottom) IR spectrophotometers.

4.5 ADVANTAGES:

- One of the major advantages of FTIR technique is rapid scanning. It is capable of scanning and producing the result within seconds.
- The simultaneous data collection helps one to investigate the spectrum of transient species such as unstable molecules or intermediaries in a chemical reaction and for the analysis of environmental samples, etc.
- Other advantages of this technique are improved signal to-noise ratio, mechanical simplicity, high sensitivity which helps one to investigate species absorbed on metal oxides or other metal catalysts.
- High resolution of spectral information can be obtained.

4.6 DISADVANTAGES:

- It is difficult to analyse aqueous solutions.

- Mounted samples obstruct the IR beam. So only small samples can be studied.

4.7 APPLICATIONS:

- FTIR technique is used to identify unknown materials. It is used for obtaining extremely fast measurements. It can determine the quality and consistency of a sample.
- It is used to determine the amount of components present in a mixture.
- It is used for determining the structure and properties of materials.
- It can detect impurities in a compound.
- It is used to study complex molecules and certain chemical reactions.

4.8 GAS CHROMATOGRAPHY- MASS SPECTROMETRY

4.8.1 INTRODUCTION:

Gas Chromatography-Mass Spectrometry (GC-MS) is an analytical method that combines the features of gas- liquid chromatography and mass spectrometry to identify different substances within a test sample. Gas chromatography is a technique used to separate drugs that might be present in a sample. The detector for the GC is the Mass spectrometry detector. • Mass spectrometer (MS) is an instrument that serves for establishment of the molecular weight and structure of both inorganic and organic compounds, and the identification and determination of analysing component in complex mixtures.

4.8.2 PRINCIPLE:

GC-MS instrument separates chemical mixtures (the GC component) and identifies the components at a molecular level (the MS component). GC works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas. • As the separated substances emerge from the column opening, they flow into MS. Mass spectrometry identifies compounds by the mass of the analysing molecule.

OR

- ✓ The Gas Chromatography/Mass Spectrometry (GC/MS) instrument separates chemical mixtures (the GC component) and identifies the

components at a molecular level (the MS component). It is one of the most accurate tools for analysing environmental samples.

- ✓ The GC works on the principle that a mixture will separate into individual substances when heated. The sample is injected into the GC inlet where it is vaporized and swept into a chromatographic column by the carrier gas (helium).
- ✓ The sample flows through the column and the compounds comprising the mixture of interest are separated by virtue of their relative interaction with the coating of the column (stationary phase) and the carrier gas (mobile phase).
- ✓ The latter part of the column passes through a heated transfer line and ends at the entrance to the ion source where compounds eluting from the column are converted to ions. A beam of electrons ionizes the sample molecules resulting in the formation of molecular ions and smaller ions with characteristic relative abundances that provide a fingerprint for that molecular structure. The mass analyser separates the ions and is then detected.

4.8.3 INSTRUMENTATION:

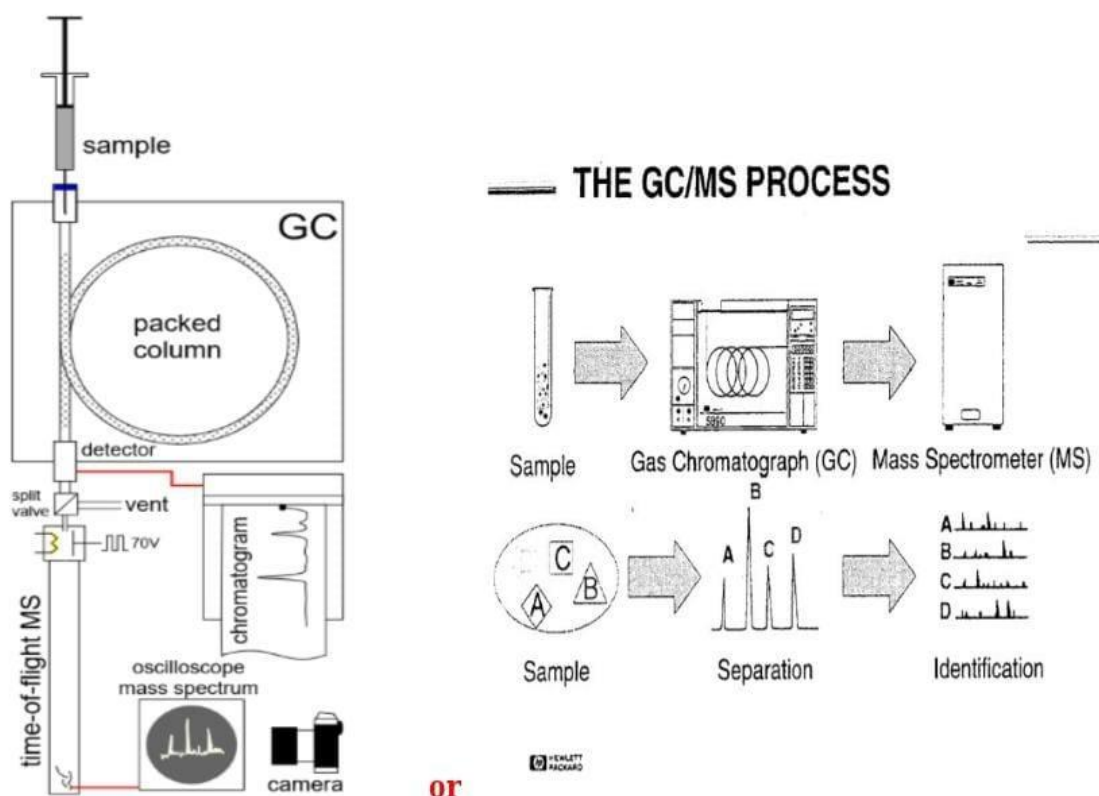


Figure 4.8.3 The schematic shows the major components of an early gas chromatography-mass spectrometer.

The GC-MS is composed of two major building blocks:

- Gas chromatography
- Mass spectrometer
- The molecules are retained by the capillary column and elute from the column at different times.
- The Mass spectrometer capture, ionize, accelerate, deflect and detect the ionized molecules separately by breaking each molecule into ionize fragments and detecting these fragments and detecting these fragments using their mass to charge ratio. As Shown Figure

4.8.4 WORKING AND COMPONENTS:

- ✓ Carrier gas
- ✓ Flow regulators and Flow Meters
- ✓ Injection devices
- ✓ Columns

- ✓ GC-MS interface
- ✓ Ionization (Ion source)
- ✓ Mass analyser
- ✓ Detectors

CARRIER GAS:

- Inertness
- Suitable for the detector
- High purity
- Easily available
- Cheap
- Should not cause the risk of fire

1. *Hydrogen*

- Better thermal conductivity.
- disadvantage: it reacts with unsaturated compounds & inflammable

2. *Helium*

- Excellent thermal conductivity
- It is expensive

3. *Nitrogen*

- Reduced sensitivity
- It is inexpensive

Flow regulators & Flow meters: -

Deliver the gas with uniform pressure/flow rate - Rota meter & Soap bubble flow meter

✓ **Rota meter:** - placed before column inlet, it has a glass tube with a float held on to a spring, the level of the float is determined by flow rate of carrier gas.

✓ **Soap Bubble Meter:** -Similar to Rota meter & instead of a float, soap bubble formed indicates the flow rate.

Injection Devices: -

- ✓ The injection port consists of a septum through which a syringe needle is inserted to inject the sample.

- ✓ The sample is injected into a stream of inert gas usually at an elevated temperature by a micro syringe.
- ✓ The vaporised sample is carried into a column packed with the stationary phase.
- ✓ To ensure rapid & complete solute volatilization temp of injector 30-50 degree Celsius >column temp

The injection port is a hollow, heated, glass-lined cylinder: -

- ✓ The injector is heated so that all components in the sample will be vaporized.
- ✓ If the temperature is too low, separation is poor and broad spectral peaks should result or no peak develops at all.
- ✓ If the injection temperature is too high, the specimen may decompose or change its structure.
- ✓ The temperature of the sample port is usually about 50°C higher than the boiling point of the least volatile component of the sample.

COLUMNS:

- ✓ Column is one of the important parts of GC which decides the separation efficiency. Columns are made up of glass and stainless steel.
- ✓ Is where the chromatographic separation of the sample occurs.
- ✓ Several types of columns are available for different chromatographic applications.
- ✓ The heart of the system.
- ✓ It is coated with a stationary phase which greatly influences the separation of the compounds

4.8.5 GC-MS INTERFACE:

The GC/MS interface is a device for transporting the effluent from the gas chromatography column to the mass spectrometer. For capillary columns, the exit end of the column is inserted directly into the ion source. The capillary column between the GC and the ion source must be heated to eliminate cold spots where analysing compound could condense. The interface temperature is set above the boiling point of the highest-boiling component of the sample.

The exhibit is a GC/MS interface from the MD 800 single quadrupole detector manufactured by Fisons in 1992. It consists of a piece of 1/8" O.D. glass lined stainless steel tube with a 0.8 mm internal bore. The capillary column was placed inside the tube and a vacuum-tight seal was made with fitting inside the GC oven. This tube was inserted inside a second larger tube, which was fitted with a thermocouple and spirally wound heater. The heating element was supplied with 28 V DC. To insulate the hot components from the user, an outer stainless steel tube formed a vacuum housing around the interface.

IONIZATION:

The standard ionisation technique in GC-MS is **electron impact ionisation (EI)** with electrons of 70 eV kinetic energy. EI is a universal ionisation method, since all organic compounds are ionised with a comparable efficiency. Organic compounds are usually heavily fragmented by the ionisation process.

MASS ANALYSER:

- ✓ To separate the ions produced in the ion source acc. to their mass/charge ratio.
- ✓ Ideally mass analyser should be capable of distinguishing small mass differences.
- ✓ It should also allow passage of a sufficient number of ions to yield radially measurable ion

DETECTOR:

The ion beam that emerges from the mass analyser, have to be detected and transformed into a usable signal. The detector is an important element of the mass spectrometer that generates a signal from incident ions by either generating secondary electrons, which are further amplified, or by inducing a current (generated by moving charges) .

4.8.6 APPLICATIONS:

- ✓ Petrochemical and hydrocarbons analysis
- ✓ Geochemical research
- ✓ Forensic (arson, explosives, drugs, unknowns) Environmental analysis
- ✓ Pesticide analysis and Food safety
- ✓ Pharmaceutical and drug analysis

- ✓ Clinical toxicology Food and fragrance
- ✓ High end research GC-MS.
- ✓ Service and institution GC-MS analysis

4.8.7 *ADVANTAGES:*

- ✓ It can identify trace elements in materials that were previously thought to have disintegrated beyond identification.
- ✓ Like liquid chromatography–mass spectrometry, it allows analysis and detection even of tiny amounts of a substance.
- ✓ GCMS has been regarded as a “gold standard” for forensic substance identification because it is used to perform a 100% specific test, which positively identifies the presence of a particular substance.
- ✓ A nonspecific test merely indicates that any of several in a category of substances is present. Although a nonspecific test could statistically suggest the identity of the substance, this could lead to false positive identification.

4.8.8 *DISADVANTAGES:*

- ✓ Non-volatile compounds are not usually ionized with good efficiency.
- ✓ The pinhole is prone to blockage and therefore the system must be kept completely free of solid materials.
- ✓ Only a small proportion of the flow from a conventional HPLC column is able to enter the source of the mass spectrometer and sensitivity is consequently low.

4.9 *EXTRACTION TECHNOLOGY:*

- 1.Collection and authentication of plant material & drying
- 2.Size reduction
- 3.Extraction
- 4.Filtration
- 5.Concentration
- 6.Drying & reconstitution

Quality of an extract is influenced by several factors such as, plant parts used as starting material, solvent used for extraction, extraction procedure, and plant material: solvent ratio, etc. From laboratory scale to pilot scale all the parameters are optimized and controlled during extraction. Extraction techniques separate the soluble plant metabolites through selective use of solvents.

Soxhlet extraction

Soxhlet extraction was named after Baron Von Soxhlet, who introduced this method in the mid-nineteenth century. It has been the most widely used method until other extraction techniques were developed in the 1980s. A schematic diagram of a typical soxhlet apparatus is shown in figure 1. There are three components in the system: (1) The top part is a solvent vapor reflux condenser. (2) In middle are a thimble holder with a siphon device and a side tube, (3) The thimble holder connects to a round bottom flask at the bottom. Because the sample is extracted with cooled, condensed solvents, soxhlet is slow and can take 6 to 48 h. Soxhlet is: rugged, well established technique that is often used as the benchmark for comparing other methods. Few parameters like long extraction time and relatively large solvent consumption are the main drawbacks of this method. (Mitra, 2003). The higher concentration of nerolidol from *Piper gaudichaudianum* Kunth was achieved in the soxhlet extraction with petroleum ether (Peres et al., 2006). The percentage extraction of all tanshinones from *Salvia miltiorrhiza* Bunge increased with the increase of Soxhlet extraction time from 30 to 90 min (Pan et al., 2002). The extracts of *Ficus bengalensis* Linn. (hanging roots), *Eugenia jambolana* Lam. (bark), *Ficus racemosa* Linn. and *Leucas lavandulaefolia* Rees smaller volumes of extraction solvent, this method has not been as widely used as the Soxhlet and sonication due to the lower extraction efficiency and unsatisfactory quantitative results. Comparable results were only attainable with long shaking times to extend the contact time with solvent (Lau et al., 2010), ERT was practiced for extraction of phytochemicals from root of *Salvia miltiorrhiza* Bunge with an appropriate solvent (Pan et al., 2002). Induced diarrhoea and the extracts was prepared by Soxhlet method (Mukherjee et al., 1998). Extraction of phenolic compounds from fruits of bitter melon (*Momordica charantia*) was done with Soxhlet extraction (Budrat and Shotipruk, 2008). Soxhlet extraction technique is employed for the extraction and separation of chemical constituents in

the medicinal plant, Herbal.conuri (Ahmad et al., 2010). Soxhlet extractions were carried out in order to determine the best solvent was methanol, used for Andrographis paniculata diterpenoid lactones extraction (Kumoroa et al., 2009).

4.10 Plant materials

Fresh plant/plant parts were collected randomly from Tamil Nadu region, India (Thiruvallur dist.). The details of the plant/plant parts screened, their families, Plants were compared with Department of Biotechnology, Madras University, Chennai, Tamil Nadu, India. Fresh plant materials were washed in tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Extraction

10 g of air-dried powder was taken in 100 ml of methanol in a conical flask, plugged with cotton wool, and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 h, the supernatant was discarded and petroleum ether was evaporated from the powder. This dry powder was then taken in 100 ml of solvent (methanol or acetone) in a conical flask, plugged with cotton wool, and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 h, the extracts were centrifuged at 5000 g for 10 min, the supernatant was collected, solvents were evaporated, and the dry extract was stored at 40°C in airtight bottles. The extraction was done at least three times for each plant and the mean values of extractive yields are presented (Vaghasiya and Chanda 2007).

Advantages of the Process

- Unlike other processes that employ high temperatures, the phytonics process is cool and gentle and its products are never damaged by exposure to temperatures in excess of ambient. No vacuum stripping is needed which, in other processes, leads to the loss of precious volatiles.
- The process is carried out entirely at neutral pH and, in the absence of oxygen, the products never suffer acid hydrolysis damage or oxidation. The technique is highly selective, offering a choice of operating conditions and hence a choice of end products.
- It is less threatening to the environment.

- It requires a minimum amount of electrical energy. It releases no harmful emissions into the atmosphere and the resultant waste products (spent biomass) are innocuous and pose no effluent disposal problems.
- The solvents used in the technique are not flammable, toxic, or ozone-depleting.
- The solvents are completely recycled within the system.

APPLICATIONS:

The phytonics process can be used for extraction in biotechnology (e.g for the production of antibiotics), in the herbal drug industry, in the food, essential oil and flavor industries, and in the production of other pharmacologically active products. In particular, it is used in the production of top quality pharmaceutical-grade extracts, pharmacologically active intermediates, antibiotic extracts and phytopharmaceuticals. However, the fact that it is used in all these areas in no way prevents its use in other areas. The technique is being used in the extraction of high-quality essential oils, oleoresins, natural food colors, flavors, and aromatic oils from all manner of plant materials. The technique is also used in refining crude products obtained from other extraction processes. It provides extraction without waxes or other contaminants. It helps remove many biocides from contaminated biomass.

Chapter-5

RESULTS AND DISCUSSION

5.1 *Experimental: Structure and Spectra*

Several analytical techniques for the identification of pigments have been in use for many years; examples include. Fourier-transform infrared spectroscopy (FT-IR), UV–visible absorption

5.1.2 *Computational Details*

The DFT calculations of OZ and CZ molecules were carried out using the Gaussian 09W and the output files are visualized by Gauss View 5.0 software. The optimized structure, vibrational frequencies and HOMO-LUMO energies are calculated using B3LYP and HSEH1PBE methods with 6-311++G(d,p) set. To obtain the good agreement, the computed data of wavenumbers are compared with the experimental data. The two methods are scaled down by using scaling factors 0.961 and 0.958. From Veda 4 program the detailed vibrational assignments of OZ and CZ have done. The NBO analysis was performed by utilizing NBO 3.1 program in the Gaussian 09W at B3LYP/6-311++G(d,p) method. To investigate the reactive sites, molecular electrostatic potential (MEP) is evaluated using the above level of calculation. The molecular docking calculation was performed with Autodock software. The protein-ligand complex and intermolecular interactions were viewed by Pymol.

5.1.3 *GCMS RESULT*

Gas chromatograph mass spectrometers was used to identify phytochemical compounds present in the extracts of (B. disticha bulb). Some compounds found in the educational program extracts have been expressed to display antioxidant, anti-inflammatory, antibacterial and antimicrobial activities. ex: apocynin, n-hexadecanoic acid, trifluoroacetoxy hexadecane, hexadecane, 9-nonadecene, 2,4-di-tert-butylphenol, cyclohexadecane and ethyl- cyclodocosane were this compounds identified by GC-MS.

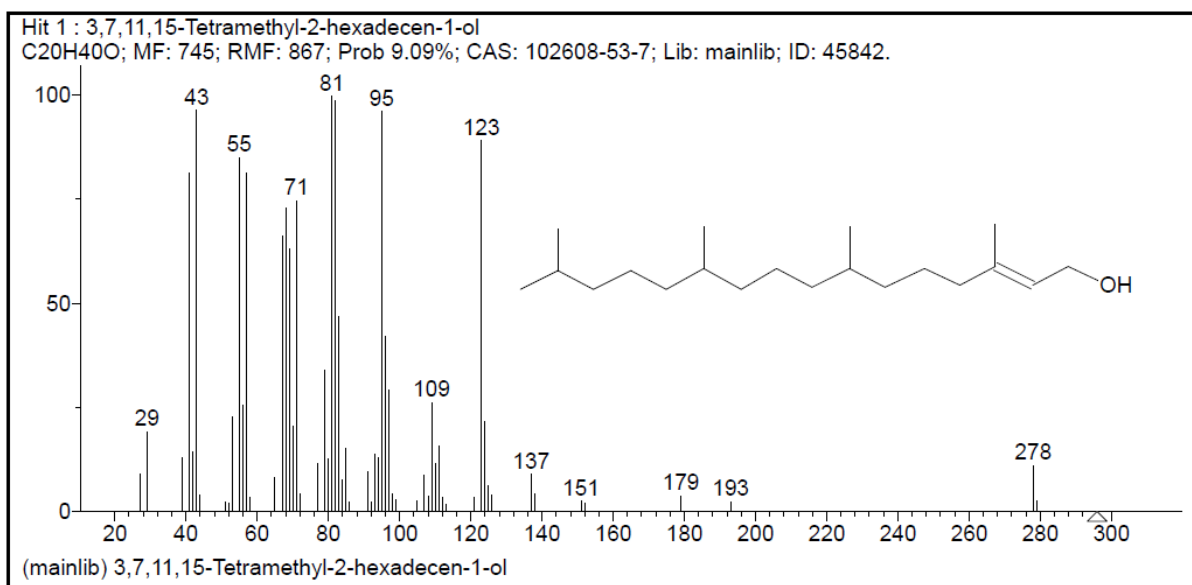


FIG 5.1.3 GCMS RESULT

FT-IR:

Fourier transform infrared microscopy experiments details were commenced to avoid data bias due to available specimen degradation via radiation illness by a synchrotron hard X-ray microprobe. In doing so, a Thermo Nicolet 8700 FT-IR spectrometer, coupled to a Nicolet Continuum IR Microscope, were used in a transmission mode, involving an 80 × 80 μm aperture (32x condenser/objective) with the step of 100 μm in both directions. The spectral range was constrained to the mid-IR range of 3900–900 cm.

5.1.4 FT-IR RESULT

FTIR can identify unknown materials and can also determine the quality or consistency of a sample and the amount of components in a mixture.

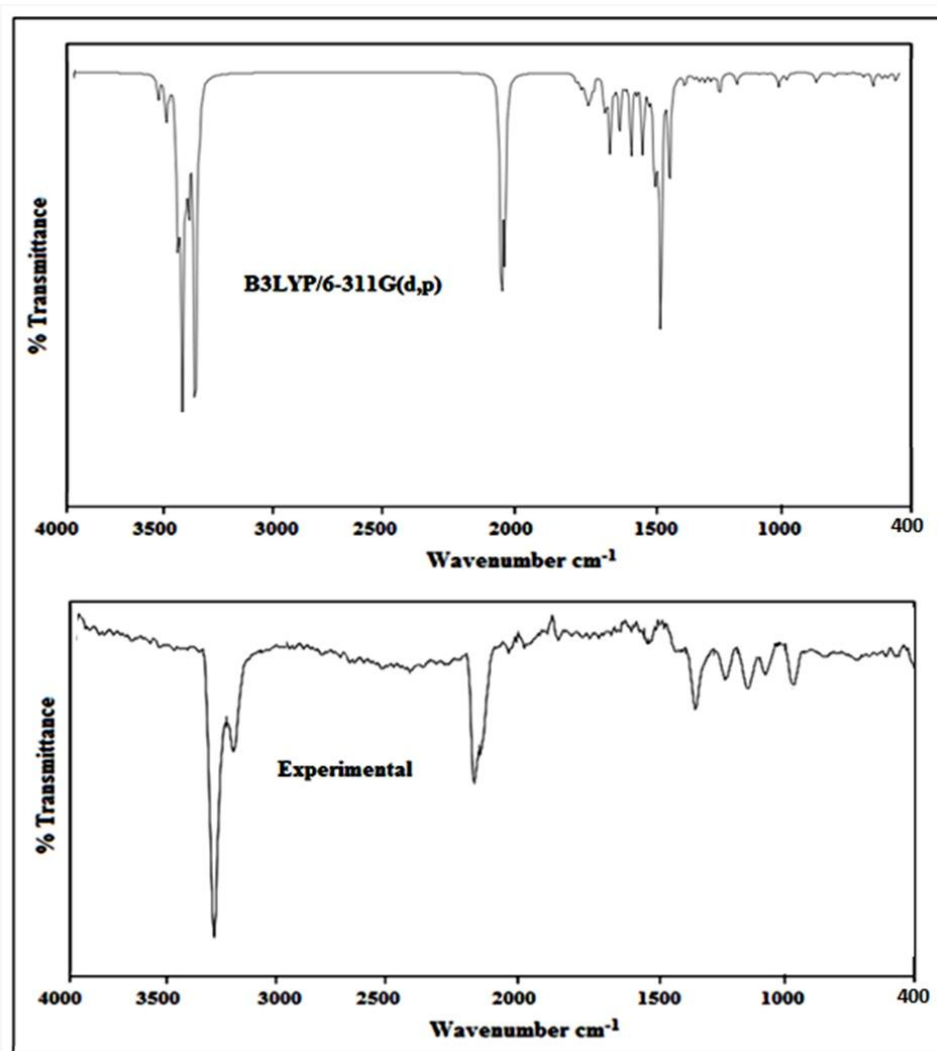


Fig 5.1.4 FT-IR RESULT

5.1.5 FT-IR TABLE

WAVENUMBER(Cm)	BOND	FUNCTIONAL GROUP	APPEARANCE
3500-3200 nm	O-H, H	Alcohols, Phenols	Strong
2260-2100nm	$-C\equiv C-$	Alkynes	Medium
1500-1400nm	C-C	Aromatics	Weak
1470-1450nm	C-H	Alkanes	Weak
1250-1020nm	C-N	Aliphatic amines	weak

The sample hybanthus's strong peak lies in the range of 3500-3200, which indicates the presence of alcohols and phenols.

5.2 Global and Local Reactivity Descriptors

The energy gap between HOMO and LUMO is a critical parameter to determine molecular electrical transport properties. By using HOMO and LUMO energy values for a molecule, the global chemical reactivity descriptors of molecules such as hardness, chemical potential, softness, electronegativity and electrophilicity index as well as local reactivity are being defined [Boyle et al 2008; Parr et al 1999; Chattaraj et al 2003; Parr et al 1978; Parr et al 1983; Parr et al 1991]. Pauling introduced the concept of electronegativity as the power of an atom in a molecule to attract electrons to it. Hardness (η), chemical potential (μ) and electronegativity (χ) and softness are defined follows.

$$\chi = -\mu = -\left(\frac{\partial E}{\partial N}\right) V(r)$$

where E and $V(r)$ are electronic energy and external potential of an N -electron system respectively. Softness is a property of molecule that measures the extent of chemical reactivity. It is the reciprocal of hardness.

$$S = \frac{1}{\eta}$$

Using Koopman's theorem for closed-shell molecules η , μ and χ can be defined as

$$\eta = \frac{I-A}{2}$$

$$\mu = \frac{-(I+A)}{2}$$

$$\chi = \frac{I+A}{2}$$

where A and I are the ionization potential and electron affinity of the molecules respectively. The ionization energy and electron affinity can be expressed through HOMO and LUMO orbital energies as $I = -E_{\text{HOMO}}$ and $A = -E_{\text{LUMO}}$. Electron affinity refers to the capability of a ligand to accept precisely one electron from a donor. The ionization potential calculated by B3LYP/6-31++G (d, p) methods for Metaxalone is 6.3090 eV. Considering the chemical hardness, large HOMO-LUMO gap means a hard molecule and small HOMO-LUMO gap means a soft molecule. One can also relate the stability of the molecule to hardness, which means that the molecule with least HOMO-LUMO gap means it is more reactive. Recently Parr et al. [Parr et al 1999] have defined a new descriptor to quantify the global electrophilic power of the molecule as electrophilicity index (ω), which defines a quantitative classification of the global electrophilic nature of a molecule Parr et al. [Parr et al 1999] have proposed electrophilicity index (ω) as a measure of energy lowering due to maximal electron flow between donor and acceptor. They defined electrophilicity index (ω)

as follows: $\omega = \frac{\mu^2}{2}$. Using the above equations, the chemical potential, hardness and electrophilicity index are being calculated for Metaxalone and their values as shown in **Table 5.2**. The usefulness of this new reactivity quantity has been recently demonstrated in understanding the toxicity of various pollutants in terms of their reactivity and site selectivity [Parthasarathi et al 2004; Padmanabhan et al 2004; Parthasarathi et al 2003]. The calculated value of electrophilicity index describes the biological activity of Metaxalone.

Table 5.2 molecular properties of the sample Hybanthus

Molecular Properties	Formula	B3LYP/6311++G(d,p)
Ionisation potential (I)	$I = -(\text{HOMO})$	6.6651
Electron affinity (A)	$A = -(\text{LUMO})$	0.2560
Energy gap	$E_g = \text{HOMO} - \text{LUMO}$	6.3090
Electronegativity(χ)	$\chi = \frac{I + A}{2}$	3.4105
Chemical potential(μ)	$\mu = \frac{-(I + A)}{2}$	-3.4105
Chemical hardness(η)	$\eta = \frac{I - A}{2}$	3.1545
Chemical softness (S)	$S = \frac{1}{\eta}$	0.3170
Electrophilicity index (ω)	$\omega = \mu^2/2\eta$	1.8436

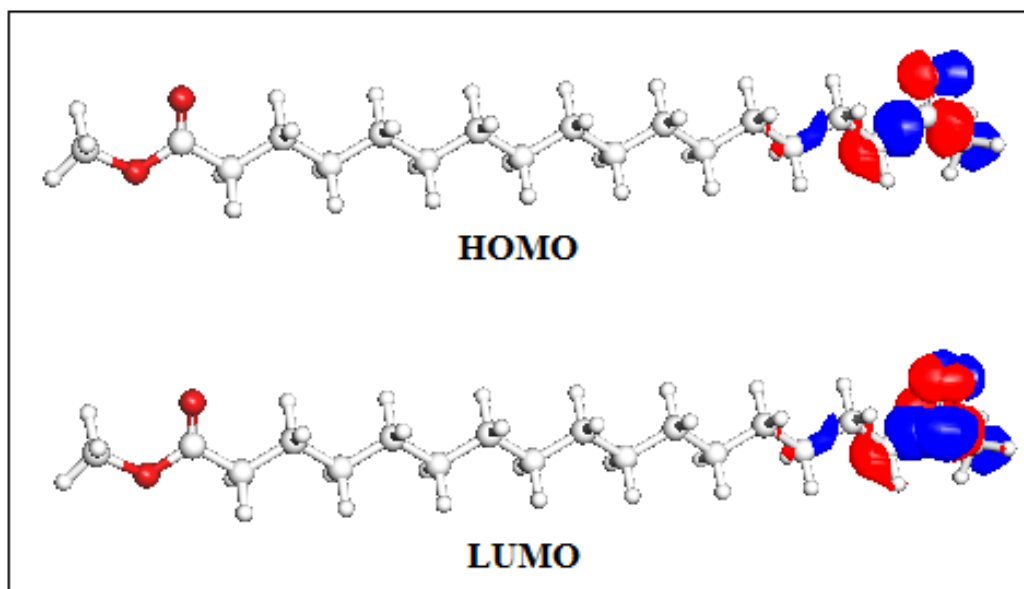


Fig.5.2 HOMO-LUMO energy of title calculated at B3LYP/6-311++G (d, p)

CHAPTER 6

SUMMARY AND CONCLUSIONS

The spectrum of coverage of medicinal plants is broad, encompassing methods and techniques relevant to the detection, extraction, separation, purification, identification, quantification, and characterization of the molecular structure of compounds in phytoconstituents. All forms of physical, chemical, biochemical, spectroscopic, radiometric, electrometric, chromatographic, metabolomic, and chemometric investigations of plant products include monomeric species as well as polymeric molecules such as nucleic acids, proteins, alkaloids, steroids, lipids, and carbohydrates which are included within the study of phytochemistry. Therefore the structural elucidation of the secondary metabolites is important for understanding the applications of phytochemicals. It is necessary to identify the non-side effect alternatives for drugs and new kinds of molecules for various applications.

Spectroscopy is one of the pioneering fields of research and is mainly employed for molecular structural elucidations. In physicochemical analysis, the application of spectroscopy is highly useful and informative in the molecular structural studies of secondary metabolites such as steroids, flavonoids, and alkaloids. Molecular spectroscopies have played a significant role in understanding the various physical-chemical phenomena. The development in science and technology paved the way for improvements in more precise spectroscopic measurements. The improvements in instrumentation and efficient data handling are in software used as the basis for new applications of molecular spectroscopy in different branches of science such as physics, chemistry, pharmaceutical sciences, biological sciences, and research and development sectors. In natural products, the molecular structure can be estimated by the application of spectroscopy and is highly useful and informatics in the analysis of different secondary metabolites particularly alkaloids, steroids, and flavonoids. The principle of the gas chromatography-mass spectrometer (GC-MS), Ultra Violet-Visible spectroscopy (UV-Vis), Fourier transforms infrared spectroscopy (FT-IR),

Fourier transforms spectroscopy are used for analyzing the molecular structure of the compounds. Gas chromatography-mass spectroscopy is a method used to identify different substances within a sample. In medicine the gas chromatography-mass spectroscopy is used to identify the composition of different kind of compounds.

Density functional theory (DFT) is a computational quantum mechanical modeling method used to investigate the molecular structures, vibrational frequencies, atomization energies, ionization energies, and electric and magnetic properties in particular atoms and large molecules. Since the vibrational motions of such large molecules are complicated, the normal modes of these molecules are also computed by using classical and quantum theory. The DFT method is generally accepted for interpretation of the spectral data of the molecules and has been adapted to study molecular vibrational analysis. FT-IR spectral investigation of some selected biologically significant organic compounds has been undertaken and its properties were studied. The Gaussian 09W program developed such as DFT calculations have been used in this work to calculate the vibrational frequencies and other parameters of certain important biologically significant organic molecules. The various optimized molecular geometrical parameters such as bond length, bond angle, and dihedral angles were calculated, and Frontier Molecular orbitals (HOMO-LUMO) analysis and molecular electrostatic potential interpretation were carried out.

The future studies on the identified alkaloids is to find the physical parameters and trace out the physical applications such as electronic, electrolytic, magnetic, etc., and also find the bioactivities on the antibody disease.

REFERENCES

1. Akkas SB., SevercanM. ,Yilmaz., J.foodchem (2007) 105,1281– 1288.
2. Anderson SD., Smith CM., Osmotic challenges in the assessment of bronchialhyperresponsiveness, Am Rev Resoir Dis. 142 (2007) 43-46.
3. Andraud C., Brotin T., Garcia C., Pelle V, Goldner P., Bigot B., Collet A., J.Am.Chem. Soc. 116 (1994) 2094.
4. Arjunan V.,Saravanan I.,Ravindran P.,Spectrochim.Acta A (2009)375–384.
5. ArunBalaji, Molecular Conformational studies on some anti conversantdugs, 2008.
6. AshfieldLJ.,Cowley AR., Dilworth JR.,Inorg. Chem. 43(2004) 4121–4123.
7. Atalay Y., Avil D., Basaoglu A., Struct. Chem. 19 (2008) 239–243.
8. Ataly Y., Avci D., BaSoglu A., Struct. Chem.19 (2008) 239-246.
9. Auda, SH.; Mrestani, Y.; Ahmed, AMS.;Neubert RHH. Characterization of the interaction of cefadroxil with different metal ions using capillary electrophoresis. Electrophoresis 2009, 30,1066–1070.
10. Bair EJ., Introduction to Chemical Instrumentation, McGraw-Hill, New York, 1962.
11. Baker EN.,Blundell TL.,Cutfield JF.,CutfieldSM.,DodsonEJ.,Dodson GG., Crowfoot
12. Balachandran V., Parimala K., J.Mol. Struct. 1003(2012)136-145.
13. Baldini V, Belicchi-Ferrari M., Bisceglie F., Pelosi G., Pinelli S., TarasconiP., Inorg. Chem. 42 (2003) 2049–2055.
14. Bao, X., Thelen, J.J., Bonaventure, G. and Ohlrogge, J.B. Characterization of cyclopropane fatty-acid synthase from Sterculia foetida. J. Biol. Chem., 278, 12846-12853 (2003) (DOI: 10.1074/jbc.M212464200).
15. Becke AD., J. Chem. Phys. 97 (1992) 9173–9177.
16. Becke AD., J. Chem. Phys. 98 (1993) 5648–5652.
17. Becke AD., J. Chem. Phys. 98 (1993) 5648–5653.
18. Bellamy LJ.,The Infrared Spectra of Complex Molecule, Chapman& Hall, London,1935.

19. Bernhard Schrader, Infrared and Raman Spectroscopy, New Age International (P) Ltd, Pub, New Delhi (1996).
20. Bevan Ott J., Boerio-Goates J., Calculations from statistical Thermodynamics, Academic Press, 2000.
21. Blomstrand E., Eliasson J., Karlsson HKR., Nutr. 136 (2006) 269S-233S.
22. Botez EC., Stephens WP., Crystal structure of anhydrous D-Mannitol. Powder Diffraction. 18 (3) (2003).
23. Boyle NM., tenderholt AL., J. Comput. Chem. 29 (2008) 839–845.
24. Burdass D, Grainger J, Hurst J, editors. Basic practical microbiology. Society for General Microbiology, Reading; United Kingdom: 2006.
25. Carel G. M., Meskers Alfred C., Marieke M. L., Rich Jurriaan H., de Groot Jasper Schuurmans J. H. Arendzen Exp Brain Res (2010) 201:681–688.
26. Casas JS., Castellano EE., Ellena J., Garcia Tasende MS., Sanchez A., Sordo J., Vidarte M.J., Inorg. Chem. 42 (2003) 2584–2595.
27. Cavanagh HMA, Wilkinson JM. Biological activities of lavender essential oil. Phytotherapy Res. 2002;16(4):301–308. doi: 10.1002/ptr.1103.
28. Chattaraj PK., Maiti B., Sarkar V, J. Phys. Chem. A 107 (2003) 4973–4975.
29. Christie, W.W. Cyclopropane and cyclopropene fatty acids. In: 'Topics in Lipid Chemistry' Vol. 1, pp. 1-49 (edited by F.D. Gunstone, Logos Press, London) (1970).
30. Chu CK., Beach JW., Jeong LS., Choi BG., Comer FI., Alves AJ., Schinazi RF., J. Org. Chem. 56 (1991) 6500-6503.
31. Clothup NB., Daly LH., Wiberley SE., Introduction to Infrared and Raman Spectroscopy, Academic Press, New York, 1990.
32. Colthub N B., Daly L.H. and Cross P.C., Molecular Vibrations Dimension to Quantum Chemistry: Analytic Derivative Methods in Ab Initio G.A. Segal, Ed, Modern theoretical Chemistry, Plenum, New York (1977).
33. D. Sajan, J. Binoy, B. Pradeep, K. Venkatakrishnan, V.B. Kartha, I.H. Joe, V.S. Jayakumar, Spectrochim. Acta 60A (2004) 173–180.
34. Dennington R., Keith V., J. Millam, GaussView 5.0.9, Semichem. Inc., Shawnee Mission, KS, 2009.
35. Frisch, A., Nielsen, A.B., Holder, A.J. 2003. Gauss view Users' Manual, Gaussian Inc.

36. G. Aruldas, Molecular Structure and Spectroscopy, Prentice Hall, India, (2001).
37. Gas Chromatography Mass Spectrometry(GCMS) Applications | AxisPharm
<https://www.ms-museum.org/exposition>.
38. G.Sharmila, V.S.Nikitha, S.Ilayarasi, K.Dhivya, V.Rajasekar, N.Manojkumar, K.Muthukumaran, C.Muthukumaran, Industrial crops and Products 84(2016) 13-21
39. Guerere M. Mondon J. M. Pujaniaye A. Physicochemical composition of some seeds from plants of the isle of Re-union. Ann. Falsif. Expert Chim. Toxicol. 78: 1985, 281-286.
40. Gunasekaran, S., Ponnambalam, U., Muthu, S., Anand, G. 2004. Indian J. Pure Appl. Physics, 46: 162.
41. Gunstone F. D. Vegetable Oil, Edible oil and fat products, chemistry and properties, volume I, 6-7
42. Haitham Q. Antibacterial activity in vitro of Thymus capitatus from Jordan. Pak. J. Pharm. Sci. 2009;22(3):247–251.
43. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 1999;86(6):985–990. doi: 10.1046/j.1365-2672.1999.00780.x. [PubMed]
44. Hawthorne SB, Krieger MS, Miller DJ. Analysis of flavor and fragrance compounds using supercritical fluid extraction coupled with gas chromatography. Analytical Chem. 1988;60(5):472–477. doi: 10.1021/ac00156a020.
45. Hodgkin DM., Hubbard RE., Isaacs NW., Reynolds CD., Philos. Trans. R. Soc. London 319 (1988) 369-456.
46. J. Kuhnau. The flavonoids, A class of semi-essential food components; Their role in human nutrition. In Bourne G.H. editors, world review nutrition Diet-Basel; Switzerland; 1976, 117-120.
47. Jamroz, M.H. 2004. Vibrational Energy Distribution Analysis VEDA 4, Warsaw.
48. K. Nakanishi, P.H. Solomon, Infrared Absorption Spectroscopy, 2nd ed., Holdenday, San Francisco, (1977).
49. K. Lewis and F.M. Ausubel, 2006. Prospects for plant-derived antibacterial. Nat. Biotechnol., 24; 1504-1507.
50. K.R. Kirtikaran & B.D. Basu, Indian Medicinal plants vol 2. (Periodical experts, Delhi), 1993, 887.
51. Leveille A.S. Fischer-Dzoga K. The mitogenic effects of methyl sterulate on aortic smooth muscle cells. Artery 11: 1982, 207- 24.
52. Luque de Castro MD, García-Ayuso LE. Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. Analytica Chimica Acta. 1998;369(1–2):1–10. doi: 10.1016/S0003-2670(98)00233-5

53. M.J. Frisch et al., Gaussian 03, Revision E.01, Gaussian Inc., Wallingford, CT, 2004.
54. Metcalfe J. Culpper guides: herbs and aromatherapy. Bloomsbury Books; London, United Kingdom: 1993.
55. N.B. Colthup, L.H. Daly, S.E. Wiberly, Introduction to Infrared and Raman Spectroscopy, second ed., Academic Press, New York, 1975.
56. N.B. Colthup, L.H. Daly, S.E. Wiberly, Introduction to Infrared and Raman Spectroscopy, second ed., Academic Press, New York, 1975.
57. Niculina Peica, J. Raman Spectroscopy . 40,(2009) 2144–2154.
58. Rajesh, P., Gunasekaran, S., Seshadri, S., Gnanasambandan, T. 2014. Spectrochimica Acta, 132: 249–255.
59. S. Gunasekaran, S.R. Varadhan, K. Manoharan, Asian J. Phys. 2 (1993) 165–172.
60. S. Annie, P.L. Rajagopal, S. Malini effect of *C. auriculata* Linn root extract on cisplatin and gentamicin induced renal injury phytomedicine 12 (2008) 555–560.
61. Salaun J. Cyclopropane derivatives and their diverse biological activities. Top. Curr. Chem. 207: 2000, 1–67.
62. Sharma B. D. Lakshminarasimhan P. Flora of Nashik District, Botanical Survey of India, series 3, September 23, 1991.
63. Singh N. P. Karthikeyan S. Flora of Maharashtra state, Dicotyledons (Ranunculaceae to Rhizophoraceae) Botanical Survey of India, Jan 1, 2000, vol I, series 2, 358–61.
64. Principle instrumentation and applications of Gas Chromatography-Mass spectrometry GC-MS, <https://www.researchgate.net/publication/3541272214>.
65. T. Gnanasambandan, S. Gunasekaran, S. Seshadri, J. Molecular Structure, 1061 (2014) 124–133.
66. Tamagawa, T. Iijima, M. Kimura, J. Mol. Structure. 30 (1976) 243.
67. Wallis T. E. Textbook of Pharmacognosy, fifth edition, S. K. Jain for CBS Publisher and Distributors, Delhi, 1985, 188–195.