EXTRACTION AND CHARACTERISATION OF OIL FROM ANNONA SQUAMOSA SEED

Submitted in partial fulfillment of the requirements for the award of

Master of Science in Chemistry

By

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DEPARTMENT OF CHEMISTRY

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SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

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JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **RANJITHA N (40910009)** who carried out the project entitled **"EXTRACTION AND CHARACTERISATION OF OIL FROM ANNONA SQUAMOSA SEED"** under our supervision from November 2021 to April 2022.

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I wish success in all her future endeavours.

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DECLARATION

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DATE: 11.05.2022 **RANJITHA N**

PLACE: CHENNAI **SIGNATURE OF THE CANDIDATE**

ACKNOWLEDGEMENT

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

I convey my thanks to **Dr. J KARTHIKEYAN, Head of the Department, and Dept. of Chemistry** for providing me necessary support and details at the right time during the progressive reviews.

I take this opportunity to express my profound gratitude and deep regards to **Dr.K.CHENNAKESAVULU PhD**., **Assistant professor, Department of Chemistry**, sathyabama institute of science and technology.

I extremely thanks to Mr.J.JAYAKANTH, Ms.I.CASSANDRA AUSTEN, Sathyabama Institute of Science and technology, Chennai, who helped me greatly throughout my project.

I would like to express my sincere and deep sense of gratitude to my Project Guide **Dr.T.V.VIJAY KUMAR**, **Mrs.N.VASUMATHI principal scientist, Dr.AJITHA scientist,** NML-CSIR, Madras for providing all necessary facilities at CSIR-NATIONAL METALLURGICAL LABORATORY, Madras Centre, Chennai and valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work and I am extremely thanks to all the staff members CSIR- NML for guiding me throughout my project.

My special thanks to Research Interns Mr.RAGUL, Mr.SAIKUMAR, for their help, motivation and suggestion, especially Mr.VAIDHYANADHAN CSIR-NML, who helped me greatly and endure with me in the designing part of my study for the process of the work. It is indeed, a great pleasure for me to express my gratitude to all my family members, friends for their co-operation and timely help throughout this work and to all my other classmates.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Chemistry who were helpful in many ways for the completion of the project.

RANJITHA N

ABSTRACT

The concept of waste to wealth is gaining lot of importance in the recent days. In this context, the present study was undertaken to identify the potential prebiotics present in the Annona squamosa seeds. Among the different components identified, the Low Molecular Weight Carbohydrates (LMWC) were chosen for the study and the extraction was performed using Hexane at two different concentrations (50% and 75%) and different combination of temperature, time and speed of rotation. Custard apple (Annona squamosa) is a nutritional rich fruit and is largely valued for its taste. It is known to have some active ingredients which kills lung, colon, breast and pancreatic cancer cells. Therefore, consumption of this fruit is known to have aided in anti-cancer activities. The investigation is carried out to find the chemical composition, phytochemical properties and evaluation of crust, pulp and the seed of Annona squamosa. The chemical characteristics such as pH, titratable acidity, total sugar and lipids are also verified. The results revealed that seed, pulp and crustcontain adequate amount of vitamin C, citric acid, carbohydrate, protein etc.

The phytochemical analysis revealed that Annona squamosa contains tannins, flavonoids, cardiac glycosides and steroids. Standard GC analysis of seed oils contain certain types of essential oils which are mainly aiding in anti-inflammatory, anti-cancer and anti-microbial properties. Home grown cures were used from old time for the treatment and flourishing of individuals.

A piece of the customary helpful plants is normal to the point that we used them in consistently presence without knowing their supportive importance. Sitaphal is one among of them normally known as custard apple, sugar apple; sweetsop is the product of Annonasquamosa (Annonaceae). Product of Annona squamosa is a tropical organic product principally develop in heat and humidity. The organic products are notable for their tasty taste and are heart moulded with light green skin, a delicate rich white tissue. generally, seeds are separated from seed packaging and dried in sun. When the dried seeds are squashed to separate the oil. Oil separated from Annona squamosa seeds can be utilized as pesticide against various normal irritations like white coarse bug, aphid, termite, and so forth. The oil separated from Annona squamosa seeds contain acetogenin a gathering of strong respiratory restraining harmful parts, which is mindful to go about as a bio-pesticide.

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FTIR	FOURIER TRANSFORM INFRARED SPECTROSCOPY
NMR	NUCLEAR MAGNETIC RESONANCESPECTROSCOPY
GC-MS	GAS CHROMATOGRAPHY- MASS SPECTROMETRY

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CHAPTER - 1

INTRODUCTION

1.1 SAMPLE

The sugar apple or sweet sop (Annona squamosa), regularly called as Sitaphal in conventional language, and is the product of Annonaceae family is a local of tropical America and West Indies. It thrives appropriately in tropical and hotter sub jungles components of India. It fills better in the sandy soil.

The natural product is almost round to oval in shape with thick skin comprising of bumpy fragments in which the seeds are encircled by the mash (Morton, 1987). The mash of the natural product is smooth white, fragrant and sweet in taste which will be stuck to seeds framing individual fragments organized in a uniform layer around the center of the natural product.[1]

Fats and oils are perceived as fundamental supplements in both human and creature slims down [2].

A large number of the natural product handling results and other horticultural buildups are considered as waste and are frequently a reason for concern. A huge sum of unutilized farming materials in the wake of handling is one of the reasons for ecological contamination. Consequently, change of such materials into important items could be a decent commitment in decrease of buildups, squander the board, and ecological wellbeing.

Seeds of a few plant animal groups regularly remain unutilized in many organic product handling enterprises; in any case, some studies have been completed to use them as a wellspring of consumable oil and as biodiesel [8].

It is found sticking to 13-to-16-millimeter-long (0.51 to 0.63 in) seeds framing individual sections organized in a solitary layer around deeply. It is delicate, somewhat grainy, and elusive. The hard, sparkly seeds might number 20-40 or more for each leafy foods have a brown to dark coat, in spite of the fact that assortments exist that are practically seed.

It is broadly developed all through the jungles in India and popularly cultivated in the north eastern pieces of Thailand, principally for its palatable organic product. It contains Dim brown or dark in shading, polished, oval also, smooth. Its seed is notable for killing head lice however there has been no report on the dynamic part.

Its seeds include 30% of its organic product's weight, which is palatable. The oil content of these seeds changes from 14 to 49% having a lot of unsaturated fats, with a higher grouping of oleic corrosive [4].

Its seed oil was accounted for to be use in the cleanser and plasticizer industry as well as in alkyd fabricating, the seeds are bitter and harmful.

It has been accounted for that A. squamosa seeds contain 23% oil of which 9.8% is a hydroxyl corrosive, the oil contains 38.6% soaked unsaturated fats and 61.4% unsaturated fats (29.0% oleic and 32.0% linoleic:), with a saponification worth of 191.8, a refractive record of 1.4826[16], a particular gravity of 0.927, an iodine worth of 88.0 what's more, 1.5% of unsaponifiable matter [17]. Its studies revealed that the different extraction methods of oil produce oils with different physicochemical properties [18].

The tissue is fragrant and sweet, smooth white through light yellow, and looks like and tastes like custard. It is found sticking to 13-to-16-millimeter-long (0.51 to 0.63 in) seeds shaping individual sections organized in a solitary layer around deeply. It is delicate, marginally grainy, and elusive. The hard, sparkling seeds might number 20-40 or more for each food grown from the ground have a brown to dark oat, in spite of the fact that assortments exist that are nearly seedless.

It develops in sweltering dry environments and change a wide range of soil which is hard to a few different plants to its loved ones [19]. Studies have uncovered that the oil of A. squamosa is to some degree viscid in appearance, light yellow in shading and with an industrious smell. The oil is uninhibitedly solvent in oil ether [3].

The seeds are supposed to be abortifacient what's more, great to destroy lice in hair in Yunani medication. Seed yields oil and gum which goes about as cleanser and their powder, is blended in with gram-flour, is a decent hair wash. Seeds are strong aggravation of conjunctiva and produce ulcers in the eye.

Seeds are the essential phase of vegetation cycle; they have solid safeguard component conceivably due to the presence of phytoconstituents adding to cancer prevention agent action.

It has great antifungal properties as seen from on application as customary medication for control of the dermatitis brought about by a lipophilic yeast Malassezia spp [6].

Annona greasy oil can likewise be utilized for eatable purposes after detoxification as the oil showed a high measure of unsaturated fats. Its seed coats can be utilized for manure furthermore, oil free seed cake as in creature feed, to completely use the unutilized seeds.

The seeds of a custard apple are dim brown in shading and are found inside the tissue of the natural product. In each organic product there are various seeds. The seeds contain synthetic compounds known as acetogenins, which are harmful to bugs.

The seeds are harsh, noxious, fill in as fish poison also, insect sprays. The custard seed were found to have insecticidal properties against organic product flies and lies. Seed vermin is utilized in disease medicines. The greater part of the acetogenins were secluded from the seeds of Annona Squamosa (custard apple) also, it displayed noteworthy cytotoxic exercises and as an Abortifacient [7].

The course of action of seeds is in dispersed lines, with the natural product's tissue filling the greater part of the leafy food grooves for the seeds, rather than the tissue's happening just around seeds [5].

In different native and conventional wellsprings of restorative plants have been broadly utilized for medicines. Different pieces of plants like the leaves, organic products, the barks, roots and, surprisingly, the seeds are being utilized for planning of meds [9].

Aside from the basic job of photosynthesis, plants can likewise be made as normal items. Regular items have been utilized to assist human with supporting its wellbeing since the beginning of medication. Over the past century, the phytochemicals and dynamic constituents in plants play had a urgent impact in drug disclosure [10].

1.2. CHEMICAL CONSTITUENTS OF FATTY ACIDS

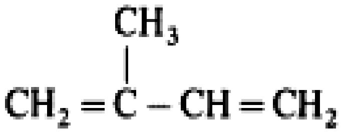
Pure essential oils are mixtures of more than 200 components, normally mixtures of terpenes or phenylpropanic derivatives, in which the chemical and structural differences between compounds are minimal. They can be essentially classified into two groups:

Volatile fraction: Essential oil constituting of 90–95% of the oil in weight, containing the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.

Non-volatile residue: that comprises 1–10% of the oil, containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoids.

1.2.1. HYDROCARBONS

Essential oil consists of chemical compounds that have hydrogen and carbon as their building blocks. Basic hydrocarbons found in plants are isoprene having the following structure.



$$CH_2=\overset{\overset{\displaystyle CH_3}{|}}{C}-CH=CH_2$$

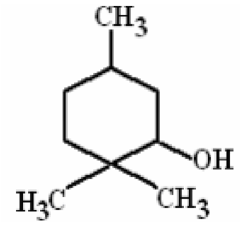
Isoprene

1.2.2. TERPENES

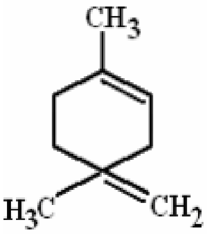
Generally, they end with “ene”. For example: Limonene, Pinene, Piperene, Camphene, etc. Terpenes are anti-inflammatory, antiseptic, antiviral and bactericidal. Terpenes can be further categorized as monoterpenes, sesquiterpenes and diterpenes. Referring back to isoprene units under the hydrocarbon heading, when these isoprene units join head to tail, the result is a monoterpene, when three join then it is a sesquiterpene and if four linked isoprene units are diterpenes.

1.2.2.a. MONOTERPENES [C10H16]

Monoterpenes are naturally occurring compounds, the majority being unsaturated hydrocarbons (C10). But some of their oxygenated derivatives such as alcohols, Ketones, and carboxylic acids known as monoterpenoids.



Menthol



Limonene

The branched-chain C10 hydrocarbons comprises of two isoprene units and is widely distributed in nature with more than 400 naturally occurring monoterpenes identified. Moreover, besides being linear derivatives (Geraniol, Citronellol), the monoterpenes can be cyclic molecules (Menthol – Monocyclic; Camphor – bicyclic; Pinenes (α and β) – Pine genera as well. Thujone (a monoterpene) is the toxic agent found in Artemisia absinthium (wormwood) from which the liqueur, absinthe, is made. Borneol and camphor are two common monoterpenes. Borneol, derived from pine oil, is used as a disinfectant and deodorant. Camphor is used as a counterirritant, anaesthetic, expectorant, and antipruritic, among many other uses.

Example

Camphene and pinene in cypress oil.

Camphene, pinene and thujene in black pepper.

1.2.2.b. SESQUITERPENES

Properties: Anti- inflammatory, antiseptic, analgesic, anti- allergic.

Sesquiterpenes are biogenetically derived from farnesyl pyrophosphate and in structure may be linear, monocyclic or bicyclic. They constitute a very large group of secondary metabolites; some having been shown to be stress compounds formed as a result of disease or injury.

1.2.2.c. DITERPENES

Diterpenes are made of up four isoprene units. This molecule is too heavy to allow for evaporation with steam in the distillation process, so is rarely found in distilled essential oils. Diterpenes occur in all plant families and consist of compounds having a C20 skeleton. There are about 2500 known diterpenes that belong to 20 major structural types. Plant hormones Gibberellins and phytol occurring as a side chain on chlorophyll are diterpenic derivatives. The biosynthesis occurs in plastids and interestingly mixtures of monoterpenes and diterpenes are the major constituents of plant resins. In a similar manner to monoterpenes, diterpenes arise from metabolism of geranyl pyrophosphate (GGPP). Diterpenes have limited therapeutical importance and are used in certain sedatives (coughs) as well as in antispasmodics and antoxiolytics

1.2.3. ALCOHOL

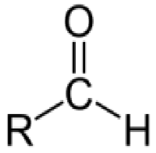
An alcohol is any organic compound in which the hydroxyl functional group (–OH) is bound to a carbon. Alcohols are the compounds which contains hydroxyl compounds. Alcohols exist naturally, either as a free compound, or combined with a terpenes or ester. When terpenes are attached to an oxygen atom, and hydrogen atom, the result is an alcohol. When the terpene is monoterpene, the resulting alcohol is called a mono-terpenol. Alcohols have a very low or totally absent toxic reaction in the body or on the skin. Therefore, they are considered safe to use.

$$R-OH$$

Alcohol

1.2.4. ALDEHYDE

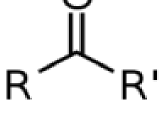
An aldehyde is an organic compound containing a functional group with the structure −CHO, consisting of a carbonyl centre (a carbon double-bonded to oxygen) with the carbon atom also bonded to hydrogen and to an R group, which is any generic alkyl or side chain. The group without R is the aldehyde group, also known as the formyl group. Aldehydes are common in organic chemistry, and many fragrances are aldehydes.



Aldehyde

1.2.5. KETONE

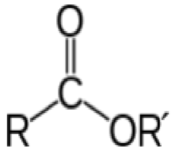
A ketone is an organic compound with the structure R(C=O) R', where R and R' can be a variety of carbon-containing substituents. Ketones and aldehydes are simple compounds that contain a carbonyl group (a carbon oxygen double bond). They are considered "simple" because they do not have reactive groups like −OH or −Cl attached directly to the carbon atom in the carbonyl group, as in carboxylic acids containing −COOH. Many ketones are known and many are of great importance in industry and in biology. Examples include many sugars (ketoses) and the industrial solvent acetone, which is the smallest ketone.



Ketone

1.2.6. ESTER

An ester is a chemical compound derived from an acid (organic or inorganic) in which at least one –OH (hydroxyl) group is replaced by an -O-alkyl (alkoxy) group. Usually, esters are derived from a carboxylic acid and an alcohol. Glycerides, which are fatty acid esters of glycerol, are important esters in biology, being one of the main classes of lipids, and making up the bulk of animal fats and vegetable oils. Esters with low molecular weight are commonly used as fragrances and found in essential oils and pheromones. Phosphoesters form the backbone of DNA molecules. Nitrate esters, such as nitroglycerin, are known for their explosive properties, while polyesters are important plastics, with monomers linked by ester moieties. Esters usually have a sweet smell and are considered high quality solvents for a broad array of plastics, plasticizers, resins, and lacquers. They are also one of the largest classes of synthetic lubricants on the commercial market [11].



Ester

1.2.7. ACIDS

An acid is a molecule or ion capable of donating a hydron (proton or hydrogen ion H+), or, alternatively, capable of forming a covalent bond with an electron pair.

1.2.7.a. AMINO ACIDS

Amino acids are organic compounds containing amine (-NH2) and carboxyl (COOH) functional groups, along with a side chain (R group) specific to each amino acid. The key elements of an amino acid are carbon (C), hydrogen (H), oxygen (O), and nitrogen (N), although other elements are found in the side chains of certain amino acids. About 500 naturally occurring amino acids are known (though only 20 appear in the genetic code) and can be classified in many ways.

They can be classified according to the core structural functional groups' locations as alpha (α-), beta- (β-), gamma- (γ-) or 9 delta- (δ-) amino acids; other categories relate to polarity, pH level, and side chain group type (aliphatic, acyclic, aromatic, containing hydroxyl or sulfur, etc.). In the form of proteins, amino acid residues form the second-largest component (water is the largest) of human muscles and other tissues. Beyond their role as residues in proteins, amino acids participate in a number of processes such as neurotransmitter transport and biosynthesis. Because of their biological significance, amino acids are important in nutrition and are commonly used in nutritional supplements, fertilizers, feed, and food technology. Industrial uses include the production of drugs, biodegradable plastics, and chiral catalysts.

1.2.8. FATTY ACIDS

A fatty acid is a carboxylic acid with a long aliphatic chain, which is either saturated or unsaturated. Most naturally occurring fatty acids have an unbranched chain of an even number of carbon atoms, from 4 to 28. Fatty acids are usually not found in organisms, but instead as three main classes of esters: triglycerides, phospholipids, and cholesterol esters. In any of these forms, fatty acids are both important dietary sources of fuel for animals and they are important structural components for cells.

1.2.8.a. SATURATED FATTY ACID

A saturated fat is a type of fat in which the fatty acid chains have all or predominantly single bonds. A fat is made of two kinds of smaller molecules: glycerol and fatty acids. Fats are made of long chains of carbon (C) atoms. Some carbon atoms are linked by single bonds (-C-C-) and others are linked by double bonds (-C=C-). Double bonds can react with hydrogen to form single bonds. They are called saturated, because the second bond is broken and each half of the bond is attached to (saturated with) a hydrogen atom. Most animal fats are saturated. The fats of plants and fish are generally unsaturated. Saturated fats tend to have higher melting points than their corresponding unsaturated fats, leading to the popular understanding that saturated fats tend to be solids at room temperatures, while unsaturated fats tend to be liquid at room temperature with varying degrees of viscosity (meaning both saturated and unsaturated fats are found to be liquid at body temperature).

Various fats contain different proportions of saturated and unsaturated fat. Examples of foods containing a high proportion of saturated fat include animal fat 10 products such as cream, cheese, butter, other whole milk dairy products and fatty meats which also contain dietary cholesterol. Certain vegetable products have high saturated fat content, such as coconut oil and palm kernel oil. Many prepared foods are high in saturated fat content, such as pizza, dairy desserts, and sausage. Guidelines released by many medical organizations including the World Health Organization have advocated for reduction in the intake of saturated fat to promote health and reduce the risk from cardiovascular diseases. Many review articles also recommend a diet low in saturated fat and argue it will lower risks of cardiovascular diseases, diabetes, or death. However, a smaller number of other reviews have come to different conclusions.

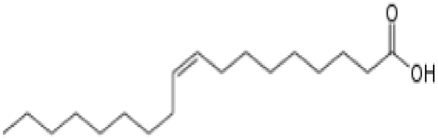
1.2.8.b. UNSATURATED FATTY ACID

An unsaturated fat is a fat or fatty acid in which there is at least one double bond within the fatty acid chain. A fatty acid chain is monounsaturated if it contains one double bond, and polyunsaturated if it contains more than one double bond. Where double bonds are formed, hydrogen atoms are subtracted from the carbon chain. Thus, a saturated fat has no double bonds, has the maximum number of hydrogens bonded to the carbons, and therefore it is saturated with hydrogen atoms. In cellular metabolism, unsaturated fat molecules contain somewhat less energy (i.e., fewer calories) than an equivalent amount of saturated fat. The greater the degree of unsaturation in a fatty acid (i.e., the more double bonds in the fatty acid) the more vulnerable it is to lipid peroxidation (rancidity). Antioxidants can protect unsaturated fat from lipid peroxidation.

1.2.8. b.(i). MONOUNSATURATED FATTY ACID

In biochemistry and nutrition, monounsaturated fatty acids (MUFAs) are fatty acids that have one double bond in the fatty acid chain with all of the remainder carbon atoms being single-bonded. By contrast, polyunsaturated fatty acids (PUFAs) have more than one double bond. Fatty acids are long-chained molecules having an alkyl group at one end and a carboxylic acid group at the other end. Fatty acid viscosity (thickness) and melting temperature increases with decreasing number of double bonds; therefore, monounsaturated fatty acids have a higher melting point than polyunsaturated fatty acids (more double bonds) and a lower melting point than saturated fatty acids (no double bonds). Monounsaturated fatty acids are liquids at room temperature and semisolid or solid when refrigerated resulting in an isotopic lattice structure.

Common monounsaturated fatty acids are palmitoleic acid (16:1 n−7), cis-Vaccenic acid (18:1 n−7) and oleic acid (18:1 n−9). Palmitoleic acid has 16 carbon atoms with the first double bond occurring 7 carbon atoms away from the methyl group (and 9 carbons from the carboxyl end). It can be lengthened to the 18-carbon cis-vaccenic acid. Oleic acid has 18 carbon atoms with the first double bond occurring 9 carbon atoms away from the carboxylic acid group. The illustrations below show a molecule of oleic acid in Lewis's formula and as a space-filling model.



Oleic acid skeletal formula

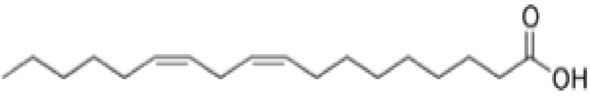
1.*2.8.b.(ii) LIST OF MONOUNSATURATED FATTY ACIDS*

Common name	**Lipid name**	**Chemical name**
Myristoleic acid	14:1 (n-5)	cis-Tetradec-9-enoic acid Palmitoleic acid
Palmitoleic acid	16:1 (n-7)	cis-Hexadec-9-enoic acid
cis-Vaccenic acid	18:1 (n-7)	cis-Octadec-11-enoic acid
Vaccenic acid	18:1 (n-7)	trans-Octadec-11-enoic acid
Paullinic acid	20:1 (n-7)	cis-13-Eicosenoic acid
Oleic acid	18:1 (n-9)	cis-Octadec-9-enoic acid
Elaidic acid (trans-oleic acid)	18:1 (n-9)	trans-Octadec-9-enoic acid
11-Eicosenoicacid (gondoic acid)	20:1 (n-9)	cis-Eicos-11-enoic acid
Erucic acid	22:1 (n-9)	cis-Tetracos-15-enoic acid
Brassidic acid	22:1 (n-9)	trans-Tetracos-15-enoic acid

Nervonic acid	24:1 (n-9)	cis-Tetracos-15-enoic acid
Sapienic acid	16:1 (n-10)	cis-6-Hexadecenoic acid
Gadoleic acid	20:1 (n-11)	cis-9-Icosenoic acid Petroselinic acid
Petroselinic acid	18:1 (n-12)	cis-Octadec-6-enoic acid

1.2.8. b (iii). POLYUNSATURATED FATTY ACID

Polyunsaturated fats are fats in which the constituent hydrocarbon chain possesses two or more carbon–carbon double bonds. Polyunsaturated fat can be found mostly in nuts, seeds, fish, seed oils, and oysters. "Unsaturated" refers to the fact that the molecules contain less than the maximum amount of hydrogen (if there were no double bonds). These materials exist as cis or trans isomers depending on the geometry of the double bond. Saturated fats have hydrocarbon chains which can be most readily aligned. The hydrocarbon chains in trans fats align more readily than those in cis fats, but less well than those in saturated fats. In general, this means that the melting points of fats increase from cis to trans unsaturated and then to saturated. See the section about the chemical structure of fats for more information.



Chemical structure of the polyunsaturated fat linoleic acid

The position of the carbon-carbon double bonds in carboxylic acid chains in fats is designated by Greek letters. The carbon atom closest to the carboxyl group is the alpha carbon, the next carbon is the beta carbon and so on. In fatty acids the carbon atom of the methyl group at the end of the hydrocarbon chain is called the omega carbon because omega is the last letter of the Greek alphabet. Omega3 fatty acids have a double bond three carbons away from the methyl carbon, whereas omega-6 fatty acids have a double bond six carbons away from the methyl carbon. The illustration below shows the omega-6 fatty acid, linoleic acid. While it is the nutritional aspects of polyunsaturated fats that are generally of greatest interest, these materials also have non-food applications. Drying oils, which polymerize on exposure to oxygen to form solid films, are polyunsaturated fats. The most common ones are linseed (flax seed) oil, tung oil, poppy seed oil, perilla oil, and walnut oil. These oils are used to make paints and varnishes.

1.2.8.b.(iv) LIST OF POLYUNSATURATED ACIDS

Common name	**Lipid name**	**Chemical name**
Linoleic acid (LA)	18:2 (n-6)	all-cis-9,12-octadecadienoic acid
Gamma-linolenic acid (GLA)	18:3 (n-6)	all-cis-6,9,12-octadecatrienoic acid
Eicosadienoic acid	20:2 (n-6)	all-cis-11,14-eicosadienoic acid
Dihomo-γ-linolenic acid (DGLA)	20:3 (n-6)	all-cis-8,11,14-eicosatrienoic acid
Arachidonic acid (AA)	20:4 (n-6)	all-cis-5,8,11,14-eicosatetraenoic acid
Docosadienoic acid	22:2 (n-6)	all-cis-13,16-docosadienoic acid
Adrenic acid (AdA)	22:4 (n-6)	all-cis-7,10,13,16-docosatetraenoic acid
Docosapentaenoic acid (Osbond acid)	22:5 (n-6)	all-cis-4,7,10,13,16-docosapentaenoic acid
Tetracosatetraenoic acid	24:4 (n-6)	all-cis-9,12,15,18-tetracosatetraenoic acid
Tetracosapentaenoic acid	24:5 (n-6)	all-cis-6,9,12,15,18-tetracosapentaenoic acid

1.3. METHODS OF EXTRACTING OIL

The essential oils are obtained from plant raw material by several extraction methods. The timid technologies about essential oils are processing with the abundant significance and are still overused in the copious parts of the globe. They are Hydro Distillation (HD), Supercritical Fluid Extraction (SFE), Microwave - Assisted Hydro Distillation (MAHD), Ultrasound - Assisted Extraction (UAE), Soxhlet Extraction Method, Steam Distillation, Solvent Extraction method, Water Distillation, Cold Expression etc.

1.4. TROPICAL PLANT

The plant kingdom still holds different unknown species, containing chemical constituents of therapeutic and nutraceutical value, which have yet to be revealed. Fruits and vegetables are a ‘treasure house’ for a catalogue of nutritional compounds. The modern pharmaceutical industries need a large quantity of genuine plants for the manufacturing of drugs. Extraction of active constituents and production of drug formulations is a sophisticated technology and assets intensive with attractive remuneration.

Different parts of plant like fruit, leaves, bark, and root are used in the treatment of various diseases and famed for its medicinal and nutraceutical values. It is a fast-growing tree producing compound, 6 - 10 cm diameter fruits with a thick, scaly or knobby skin that gives them a pine-cone appearance.

Fig:1.1. Annona squamosa ***fig:1.2. Annona squamosa seed***

It is a terrestrial coarse-gained, woody, deciduous, perennial tree with characteristic odor rough, ash grey colored bark with visible leaf scars and smooth to slightly fissured into plates, inner bark light yellow and slightly bitter, twigs become brown with light brown dots.

The flowers have several conglomerated and spirally arranged stamens below and around an upper globes shaped dome of numerous united carpels. The fruit flesh is fragrant, sweet, and white to light yellow, with the texture. Fruits are divided into 20 - 38 segments, each generally containing a hard, shiny brownish-black, seed, enmeshed in the flesh, although some trees produce seedless fruit.

The fruits are generally eaten fresh, or used to make juice beverages or sorbet, and are a good source of iron, calcium, and phosphorus. Seeds are shiny brownish-black, black color with ovoid shape, numerous scattered over the white pulp [12].

The family is concentrated in the tropics, with few species found in temperate regions. About 900 species are Neo-tropical, 450 are Afro-tropical, and the other species Indo Malayan [13].

Under Annonaceae family 130 genera are available, out of that Genera are widely available Annona, Anonidium, Rolliania, Uvaria, Melodorum, Asimina, Stelechocarpus [14]. At present, many commercial fruit products are existed in the market hence the present review will probably act as connection between nutraceutical food and industrial pharmaceutical potentials of Annona squamosa [15].

The crushed leaves are applied on ulcers and wounds and a leaf decoction is taken in cases of dysentery. The bark decoction is given as a tonic and to halt diarrhea. Throughout tropical America, a decoction of the leaves is imbibed either as an emmenagogue, febrifuge, tonic, cold remedy, digestive, or to clarify urine. The leaf decoction is also employed in baths to alleviate rheumatic pain. Sitopaladi churna is an ayurvedic medicine for cough and cold and sneezing nose.

The bark leaves and roots of some species are used in folk medicines. The strong bark is used for carrying burdens in the Amazon Rainforest and for wooden implements, such as tool handles and pegs. The wood is valued as firewood yellow and brown dyes.

Administration of the aqueous extract of the leaves also improved the activities of plasma insulin and lipid profile and reduced the levels of blood glucose and lipid peroxidation, indicating that the high levels of triglyceride and total cholesterol associated with diabetes can also be significantly managed with the extract.

1.5. MINERAL PROCESSING

Production of metals is one of the oldest applied sciences, and it continues to be a fundamental industry that builds our civilisation. Metals are found naturally in native form or more often as minerals contained in ore bodies in the crust of the earth. Most ores are mixtures of extractable minerals and waste gangue materials (Wills, 2005). Extracting metals from their ores can be done by three methods; hydrometallurgy, electrometallurgy, and the most common pyrometallurgy. However, the pyrometallurgical method, i.e., smelting, requires a large amount of energy. It is therefore of interest to reduce the amount of gangue that is sent to the smelter with the minerals. Mineral processing is a series of physical processes during which valuable minerals are firstly liberated from the gangue, then concentrated to produce an enriched product containing the majority of the valuable materials (the concentrate). Mineral processing reduces both smelter energy cost and metal losses (Wills, 2005).

In the liberation stage, valuable minerals are freed from surrounding gangue by crushing and grinding the mined ore to particles. This process of size reduction is called comminution (Fig.1.3.). The size of the particles is important as particles need to be small enough so that the minerals can be liberated but not too small to avoid wasting energy in grinding. The particle size also affects the concentration process.

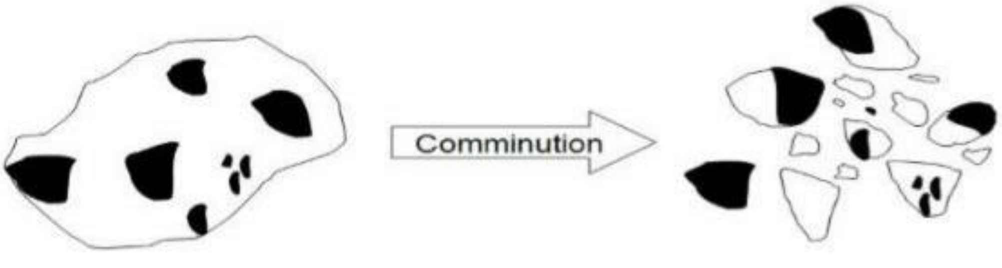


Fig.1.3. Breaking of ore to smaller fragments results in particles with varying degrees of liberation. The shaded area represents the valuable minerals.

The concentration of minerals is carried out using the differences in physical properties between minerals and gangue, such as appearance, density, electrical conductivity, magnetic and surface properties. One of the most important methods of concentration is froth flotation, which exploits differences in surface properties of mineral and gangue species.

1.6. FROTH FLOTATION

Froth flotation is a highly versatile method for physically separating particles based on differences in the ability of air bubbles to selectively adhere to specific mineral surfaces in a mineral or water slurry. The particles with attached air bubbles are the carried to the surface and removed, while the particles that remain completely wetted stay in the liquid phase. Froth flotation can be adapted to a broad range of mineral separations, as it is possible to use chemical treatments to selectively alter mineral surfaces so that they have necessary properties for the separation. It is currently in use for many diverse applications, with a few examples being, separating sulphide minerals from silica gangue and from other sulphide minerals; separating potassium chloride (sylvite); from sodium chloride (halide); separating coal from ash – forming minerals; removing silicate minerals from iron ores; separating phosphate minerals from silicates; and even non – mineral applications such as de- inking recycled newsprint. It is particularly useful for processing fine- grained ores that are not amenable to conventional gravity concentration.

Some of the components that involves the flotation system are:

Chemistry component: Collectors, Frothers, Activators, Depressants, pH.

Equipment component: Cell design, Agitation, Air flow, Cell bank configuration.

Operation component: Feed rate, Mineralogy, Particle size, Pulp density, Temperature.

It is important to take all of these factors into account in froth flotation operations. Changes in the settings of one factor will automatically cause or demand changes in other parts of the system. As a result, it is difficult to study the effects of any single factor in isolation and compensation effects within the system can keep process changes from producing the expected effects (Klimpel, 1995). This makes it difficult to develop predictive models for froth flotation, although work is being done to develop simple models that can predict the performance of the circuit from easily- measurable parameters such as solids recovery and tailings solid content (Rao et al., 1995)

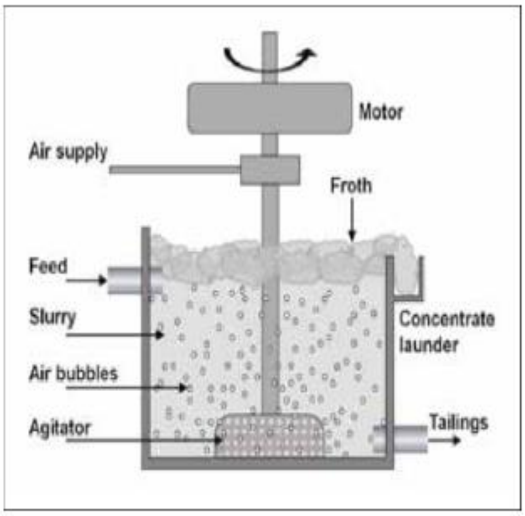


Fig.1.4. Flotation cell

1.7 COAL FLOTATION

Coal flotation is a complicated interaction including a few stages (particles, oil beads and air bubbles). These stages all the while cooperate with one another and with different species like the atoms of an advancing reagent and broke down particles in water.

Coal is a black or brownish-black sedimentary rock that can be burned for fuel and used to generate electricity. It is composed mostly of carbon and hydrocarbons, which contain energy that can be released through combustion (burning).

coal, one of the main essential petroleum derivatives, a strong carbon-rich material and most frequently happens in separated sedimentary stores. Carbon, which burns to form carbon dioxide and some water from the residual hydrogen present, and gives off heat energy.

Coal is generally carbon with variable measures of different components; mostly hydrogen, sulfur, oxygen, and nitrogen. Coal is framed when dead plant matter rots into peat and is changed over into coal by the hotness and strain of profound internment north of millions of years.

Coal contains the energy put away by plants that lived a huge number of years prior in marshy backwoods. Layers of soil and rock covered the plants north of millions of years. The subsequent strain and hotness transformed the plants into the substance we call coal.

Estimating assumes an imperative part in the exhibition of coal planning plants. Among the different sorts of screens accessible, vibrating screens are prevalently utilized for various sizes of coal, while sifter twists and characterizing tornadoes are explicitly utilized for fine and ultrafine coal.

There are three scattered stages that establish buoyancy mash: coal particles, oil beads and air bubbles. These stages connect in water as the medium through different sub-processes during buoyancy which are distinguished.

The target of washing is to redesign crude coal to deliver various items through various tasks. The washing strategies rely upon the nature of coal and the functioning rule of unit tasks.

coal is made out of macerals, discrete minerals, inorganic components held microscopically by the natural matter, and water and gases contained in submicroscopic pores. Naturally, coal comprises essentially of carbon, hydrogen, and oxygen, and lesser measures of sulfur and nitrogen.

The collaboration of various boundaries of foam buoyancy is examined, including the utilization of reagents, hydrodynamics of the assortment cycle, energy and delivery investigation.

Oxidation brings about warming and self-ignition of coal on open capacity. For each coal, there is a basic temperature (~60°C) past which the warming rate pointedly increases and self-start becomes conceivable.

Low positioning bituminous and endured (oxidized) coal might require an amount of frother as incredible as 2.0 kg/t of crude coal. As a rule, how much frother required differs from 0.2 to 0.5 kg/t of crude coal.

Authorities are utilized related to frothers and are expected to support drifting those coals which are less hydrophobic in nature and in this manner less promptly drifted.

The essential motivation behind a gatherer is to deliver the surfaces of the particles of coal more hydrophobic to such an extent that the particles of coal and the rising air bubbles which are covered with the frother have more noteworthy contact and bond.

The authority is by and large particular in that it specifically sticks to and specially wets the surfaces of the particles of coal however not the particles of contaminations and other matter contained in the slurry.

Authorities are generally a hydrocarbon oil. Diesel fuel, fuel oil and lamp oil are the most generally utilized. Endeavors have been made to work on the viability of the gatherer.

In coal buoyancy, just authorities and frothers are by and large utilized as compound reagents. The authority upgrades the hydrophobic property of coal. Buoyancy of coal fines has become progressively significant as a partition and cleaning process where there is a bringing down in both the molecule size and grade of the coal being recuperated from mining activities.

The capacity to eliminate the coal fines from coal washery waters or tailings is additionally invaluable to recuperate coal fines missed by different strategies of coal recuperation.

Physical and substance collaborations between fine coal particles could prompt collection, particularly for high position coals. Non-particular molecule total could be supposed to be the primary justification for the selectivity issues in coal buoyancy.

It ought to be tended to by functional preparing or substance (advertisers) pretreatment previously or during buoyancy. Albeit the associations between the oil beads and coal particles are really preferred, adjustment of the oil drops by modest quantities of fine hydrophobic particles might prompt an abatement in selectivity and an expansion in oil utilization.

coal is a non-clastic sedimentary stone. They are the fossilized remaining parts of plants and are in combustible dark and caramel dark tones. Its primary component is carbon, yet it can likewise contain various components like hydrogen, sulfur and oxygen. Dissimilar to coal minerals, it doesn't have a decent synthetic synthesis and precious stone construction.

Contingent upon the kind of plant material, fluctuating levels of carbonization and the presence of contaminations, various sorts of coal are framed. There are 4 perceived assortments. Lignite is the most minimal grade and is the mildest and least scorched. Sub-bituminous coal is dull brown to dark. Bituminous coal is the most bountiful and is frequently singed for heat age. Anthracite is the most noteworthy grade and most transformed type of coal.

It contains the most elevated level of low-emanation carbon and would be an optimal fuel if not for nearly less. Coal is chiefly utilized as a fuel. Coal has been utilized for millennia, yet its genuine use started with the development of steam motors after the modern transformation. Coal gives two-fifths of power creation worldwide and coal is utilized as the primary fuel in iron and steel creation offices.

CHAPTER 2

LITERATURE SURVEY

2.1 TITLE - Characterization of custard apple seeds for the separation of prebiotic low molecular weight carbohydrates (LMWC).

AUTHOR - Kiran Vitalenahalli Rajegowdaa and Suresh Kumar Kalakandan.

YEAR OF PUBLISHED - 21-12-2020

INFERENCE - The idea of waste to abundance is acquiring parcel of significance in the new days. In this unique circumstance, the present study was embraced to distinguish the potential prebiotics present in the Annona squamosa. Among the various parts recognized, the Low Molecular Weight Carbohydrates (LMWC) were picked for the study and the extraction was performed utilizing Ethanol at three unique fixations (half, 75% and 95%) and different blend of temperature, time and speed of revolution. The greatest yield of 9.16% was acquired with half Ethanol, 37 0C, 72h and 60 rpm. The probiotic living being Lactobacillus bulgaricus showed a positive development of 7.54±0.04 log cfu/ml on LMWC over a time of 24 hours which affirmed the prebiotic usefulness of LMWC. Checking Electron Microscopy and FTIR investigation were done to affirm the morphological attributes. Hence, every one of the outcomes got showed that LMWC can be utilized as potential prebiotics in the food business and the business usage of Annona squamosa.

2.2 TITLE- Extraction of volatile and non-volatile components from custard apple seed powder using supercritical CO2 extraction system and its inventory analysis.

YEAR OF PUBLISHED- Jan 2021

AUTHOR-Dhanashree panadre

INFERENCE - at developing an intensified method to extract volatile and non-volatile components from custard apple seeds using supercritical CO2 extraction (SC−CO2) with detailed process optimization so as to establish conditions for maximum yield

2.3 TITLE- Analysis and Applications of Custard Apple

AUTHOR- Ajitha Priya Neeraja Jammala, Ahlam 'Abdallah Salim Al-Shibli, Eman Hamad Zahran Al-Shuraiqi, Shahla Soud Abdullah Alrahbi and Zianab Mahmood Nasser Al Qassabi

YEAR OF PUBLISHED - 13 February 2019

INFERENCE- The examination is completed to track down the synthetic structure, phytochemical properties and assessment of covering, mash and the seed of Annona squamosa. the substance qualities like pH, titratable acridity, all out sugar and lipids are additionally confirmed. The outcomes uncovered that seed, mash and hull contain sufficient measure of L-ascorbic acid, citrus extract, sugar, protein and so on the phytochemical examination uncovered Annona squamosa contains tannins, flavonoids, heart glycosides and steroids. Standard GC examination of seed oils contain certain sorts of natural oils which are for the most part helping with calming, hostile to disease. what's more, against microbial properties. The cancer prevention agent action is assessed utilizing DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) measure. The presence of anthocyanins may additionally offer enemy of malignant growth, mitigating and against viral advantages of Annona squamosa.

2.4 TITLE - Annona squamosa L. Phytochemicals and Biological Activities

AUTHOR - Chengyao Ma, Yayun Chen, Jianwei Chen, Xiang Li and Yong Chen

YEAR OF PUBLISHED - 29 June 2017

INFERENCE - A wide scope of ethno-therapeutic purposes has been connected with various bits of A. squamosa, like tonic, apophlegmatisant, cool medication, abortient and heart narcotic. Various exploration projects on A. squamosa have observed that it has anticancer, against oxidant, antidiabetic, antihypertensive, hepatoprotective, antiparasitic, antimalarial, insecticidal, microbicidel and molluscicidal exercises. Phytochemistry examinations on A. squamosa have considered annonaceous acetogenins (ACGs), diterpenes (DITs), alkaloids (ALKs) and cyclopeptides (CPs) as the principle of constituents. Until 2016, 33 DITs, 19 ALKs, 88 ACGs and 13 CPs from this species were accounted for. Based on the numerous explores on A. squamosa, this audit endeavors to incorporate accessible data on its phytochemicals, old stories utilizes and bioactivities, expecting to advance a superior comprehension of its restorative qualities.

2.5 TITLE- Phytochemical analysis of Annona squamosa seed extracts

AUTHOR - P. Remani

YEAR OF PUBLISHED - January 2013

INFERENCE- Annona squamosa seed removes were read up for their phytochemical constituents, complete phenolics and flavonoid substance. For this reason of various trial of oil ether, chloroform, ethyl acetic acid derivation and methanol concentrates of Annona squamosa were removed and thought. The primer phytochemical screenings of Annona squamosa for its phytochemical constituents were performed involving commonly acknowledged research centre method for subjective conclusions. Significant phytochemical. were available in the concentrates. The presence of apparent to direct measures of phytochemicals, for example, flavanoids, coumarines, alkaloids, terpenoids can be corresponded with the conceivable huge clinical capability of the plant.

2.6 TITLE- Fatty Oil and Fatty Acid Composition of Annona squamosa Linn. Seed Kernels

AUTHOR- Virendra S. Rana

YEAR OF PUBLISHED - 15 Sep 2014.

INFERENCE - The yield and greasy oil parts of the seed bits of Annona squamosa Linn. (Family) not entirely settled by a dissolvable extraction technique and gas chromatography-mass spectrometry (GC/MS). The seed parts were found to contain about 22.2% of rough greasy oil on a dry weight premise. The rough greasy oil was changed over into methyl esters and examined by GC/MS. Eleven mixtures, establishing 99.8% of the oil, were recognized what's more, the primary mixtures were oleic corrosive (47.4%), linoleic corrosive (22.9%), stearic corrosive (13.6%), and palmitic corrosive (12.1%). Oleic corrosive and linoleic corrosive together establish 70.3% of the unsaturated unsaturated fats (UFAs) trailed by the immersed unsaturated fats (25.7%), basically palmitic corrosive and stearic corrosive. Heneicosanoic corrosive (2.3%), 11-eicosanoic corrosive (0.2%), 17-methyloctadecanoic corrosive (0.1%), what's more, dihydrostereculic corrosive (0.1%) have not been accounted for before from the oil of A. squamosa.

2.7 TITLE- Study of Diethanolamide from Custard AppleSeed Oil (Annona Squamosa L)

AUTHOR- Lokhande A. R., Patil V. S., Wani K. S.

YEAR OF PUBLISHED - September – 2013

INFERENCE - Diethanolamides are nonionic emulsifiers broadly utilized in businesses like beauty care products and cleanser enterprises as a froth sponsor. Diethanolamides were arranged from Annona squamosa seed oil and in view of the test results an unsaturated fat to diethanolamine proportion of 1:2 (w/w) for example 1:5 (5.37) (on molar premise) and a temperature of 150 OC were seen as reasonable for the blend of unsaturated fat alkanolamides of Annona squamosa seed oil. The diethanolamide have laid out their places as a significant crude materialofproducts going from clothing cleanser to better quality shampoos on the grounds that of their different cleanser properties.

CHAPTER – 3

AIM AND SCOPE OF THE PRESENT INVESTIGATION

3.1 OBJECTIVE OF THE STUDY

3.1.1 GENERAL OBJECTIVE

The general objective of this study was production and characterization of locally available Annona squamosa seed extract for its possible application to extract pure oil.

3.1.2. SPECIFIC OBJECTIVE

The specific objectives of this study were

- To determine the proximate analysis of the Annona squamosa seeds.
- To extract pure oil from Annona squamosa seed
- To analyze the physicochemical characteristics of Annona squamosa seed extract.
- To study and determine the optimum operating extraction parameters (extraction time, temperature to annona squamosa seed ratio).

3.1.3 SCOPE OF THE STUDY

The principle focal point of this exploration was coordinated towards the creation from normal plant seed (Annona squamosa) inside specific boundaries: extraction time, extraction temperature and hexane to annona squamosa seed proportion with (2, 3 and 4 hours), (30, 40 and 50) and (5:1, 7:1 and 9:1v/w) individually.

The general examination of the Annona squamosa seed were investigated with standard strategies. The item has been portrayed by FTIR examination, GC-MS investigation and NMR.

The measurable meaning of each trial not set in stone and the relating numerical expectation model was created.

3.1.4 SIGNIFICANCE OF THE STUDY

This study is an experimental study of Soxhlet extraction method from Annona squamosa seed.

- This study is to introduce the application and possibility of Annona squamosa seed as pure oil which is natural material.
- Provide a means to exploit and manage local available resources
- To create jobs for those will be engaged in planting/cultivating of the fruit as well as establishing to producing plants.

3.2 AIM OF THE STUDY

Plants require specific things to develop effectively. They need biotic things like water, light, supplements, a substrate to fill in, and frequently an appropriate temperature. On the off chance that these are not ideal for a plant, then, at that point, its capacity to develop can be decreased. They can likewise be impacted by abiotic factors such as contamination.

Seeds safeguard and support the undeveloped organism or youthful plant. They normally give a seedling a quicker start than a sporeling from a spore, due to the bigger food holds in the seed and the multicellularity of the encased incipient organism.

The principal land plants developed around 468 million years ago,[20] they imitated utilizing spores. The most established seedbearing plants were which had no ovaries to contain the seeds, emerging at some point during the late Devonian time frame (416 million to 358 million years ago) [21].

CHAPTER 4

MATERIALS AND METHODS

4.1. MATERIALS

Soxhlet apparatus with heating mantle and condenser was used for the extraction of oil. n-Hexane was used as a solvent for the extraction of oil from the aromatic plant. Leishman’s solution, isopropyl alcohol, Sodium hydroxide (NaOH), Alcoholic potassium hydroxide (KOH), Hydrochloric acid (HCl) and phenolphthalein indicator were used for the characterization and determination of oil from aromatic plant. Coal for used for the froth flotation process.

4.2. CHARACTERIZATION TECHNIQUES

4.2.1. GAS CHROMATOGRAPHY- MASS SPECTROMETRY (GC-MS)

4.2.1.a. INSTRUMENTATION

The GC-MS is composed of two major building blocks: the gas chromatograph and the mass spectrometer. The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g.,5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture and their relative affinity for the stationary phase of the column will promote separation of the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute (come off) from the column at different times (called the retention time), and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass-to-charge ratio.

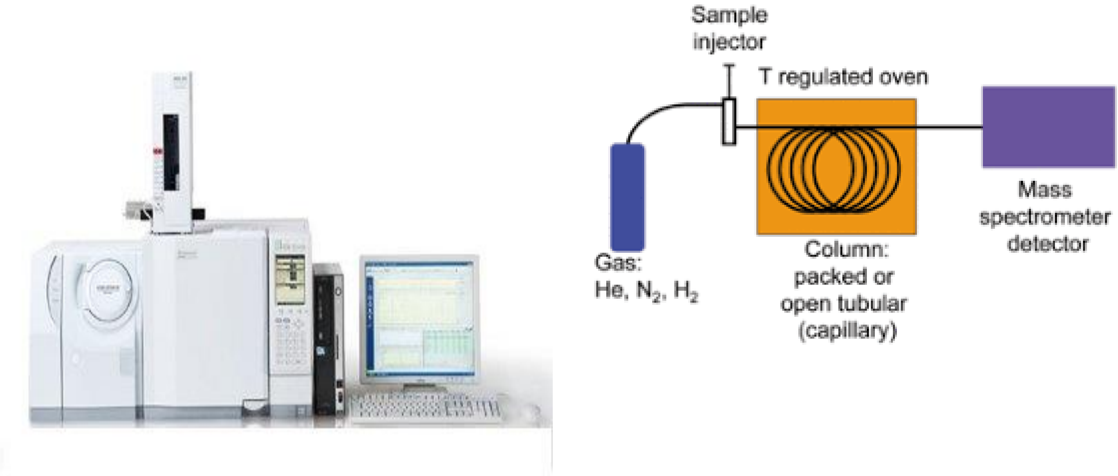


Fig: 4.1. GC-MS instrument

These two parts, utilized together, permit a lot better level of substance distinguishing proof than either unit utilized independently. It is beyond the realm of possibilities to expect to make an exact recognizable proof of a specific particle by gas chromatography or mass spectrometry alone.

The mass spectrometry process typically requires an extremely unadulterated test while gas chromatography utilizing a conventional locator (for example Fire ionization indicator) can't separate between numerous atoms that happen to invest in some opportunity to go through the segment (for example have something very similar maintenance time), which brings about at least two particles that co-elute. Here and there two unique atoms can likewise have a comparative example of ionized sections in a mass spectrometer (mass range). Joining the two cycles lessens the probability of blunder, as it is incredibly far-fetched that two unique particles will act similarly in both a gas chromatograph and a mass spectrometer.

In this manner, while a recognizing mass range shows up at a trademark maintenance time in a GC-MS examination, it normally increments sureness that the analyte of interest is in the example.

For the examination of unstable mixtures, a cleanse and trap (P&T) concentrator framework might be utilized to present examples. The objective analytes are separated by blending the example in with water and cleanse with dormant gas (for example Nitrogen gas) into an impenetrable chamber, this is known as cleansing or sparging. The unstable intensifies move into the headspace over the water and are drawn along a strain inclination (brought about by the presentation of the cleanse gas) out of the chamber.

The unpredictable mixtures are drawn along a warmed line onto a 'trap'. The trap is a section of adsorbent material at encompassing temperature that holds the compounds by returning them to the fluid stage. The snare is then warmed and the test compounds are acquainted with the GC-MS section by means of a volatiles interface, which is a parted bay framework. P&T GC-MS is especially fit to unstable natural compounds (VOCs) and BTEX compounds (fragrant mixtures related with petrol).

A quicker elective is the "cleanse shut circle" framework. In this framework the idle gas is risen through the water until the groupings of natural mixtures in the fume gradually ease is at balance with focuses in the fluid stage. The gas stage is then investigated straightforwardly.

4.2.1.b. TYPES OF MASS SPECTROMETER DETECTORS

The most widely recognized sort of mass spectrometer (MS) related with a gas chromatograph (GC) is the quadrupole mass spectrometer, at times alluded to by the Hewlett-Packard (presently Agilent 1200) trademark "Mass Selective Detector" (MSD). Another somewhat normal locator is the particle trap mass spectrometer. More over one might observe an attractive area mass spectrometer, but these specific instruments are costly and massive and not ordinarily found in high throughput administration labs. Different finders might be experienced like time of flight (TOF), couple quadrupoles (MS-MS) (see underneath), or on account of a particle trap MS where n demonstrates the number mass spectrometry stages.

4.2.1.c. APPLICATONS

Gas chromatography-mass spectrometry (GC-MS) is an insightful strategy that consolidates the elements of gas-chromatography and mass spectrometry to recognize various substances inside a test. Utilizations of GC-MS incorporate medication recognition, fire examination, explosives examination, and ID of obscure examples, including that of material examples got from planet Mars during test missions as soon as the 1970s. GC-MS can likewise be utilized in air terminal security to distinguish substances in baggage or on individuals.

Moreover, it can distinguish minor components in materials that were recently thought to have crumbled past recognizable proof. Like fluid chromatography-mass spectrometry, it permits investigation and location even of minuscule measures of a substance.

GC-MS has been viewed as a "best quality level" for scientific substance recognizable proof since it is utilized to play out a 100 percent explicit test, which decidedly distinguishes the presence of a specific substance. A vague test simply shows that any of a few in a classification of substances is available. Albeit a vague test could genuinely propose the character of the substance, this could lead to misleading positive recognizable proof [25].

4.2.2. FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Infrared spectroscopy takes advantage of the way that atoms retain frequencies that are normal for their design. These retentions happen at thunderous frequencies, for example the recurrence of the retained radiation matches the vibrational recurrence. The energies are impacted by the state of the sub-atomic potential energy surfaces, the majority of the iotas, and the related vibronic coupling. Specifically, in the Born-Oppenheimer and symphonious approximations, for example when the sub-atomic Hamiltonian relating to the electronic ground state can be approximated by a symphonious oscillator in the neighborhood of the harmony sub-atomic math, the full frequencies are related with the typical modes comparing to the sub-atomic electronic ground state potential energy surface.

The thunderous frequencies are additionally connected with the strength of the bond and the mass of the particles at one or the flip side of it. In this way, the recurrence of the vibrations is related with a specific typical method of movement and a specific bond type.

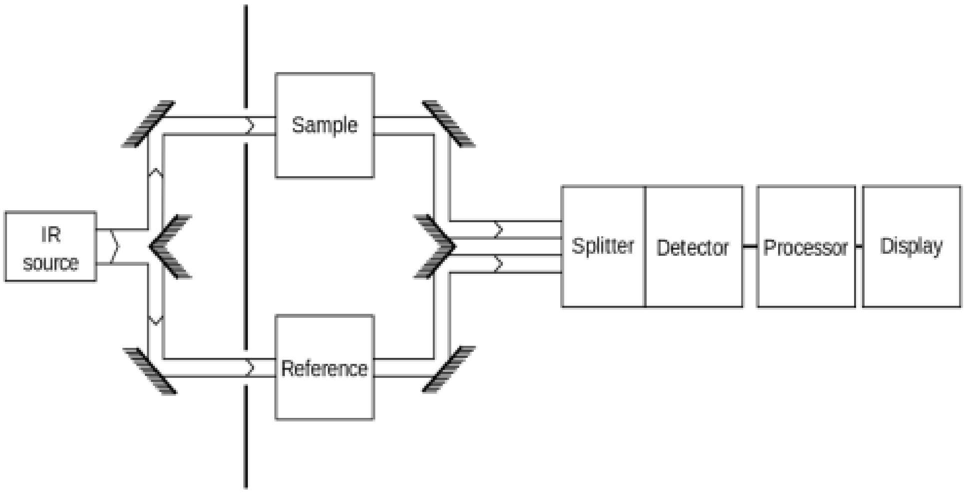


Fig.4.2. FTIR two beam absorption spectrometer

In order for a vibrational mode in a sample to be "IR active", it must be associated with changes in the dipole moment. A permanent dipole is not necessary, as the rule requires only a change in dipole moment. Six of these vibrations involve only the CH2 portion: symmetric and anti- symmetric stretching, scissoring, rocking, and twisting [26], [27].

4.2.3. NUCLEAR MAGNETIC RESONANCE (NMR)

4.2.3 a. INSTRUMENTATION

When white light is given to the surface of an atom, molecule, or other object, spectrum is seen from it which is called spectroscopy. [29][30]. we can say that the spectrum that is found here depends entirely on the defined atom and molecule or some other substance. And spectroscopy is also used in physics, chemistry and biology. Are and the frequency is the lowest. While violet have the shortest wavelength and the highest frequency [31][32].

When it comes from the sun's rays it is an ultraviolet wave. The shortest wavelength from above is that of gamma radiation (1 picometer). A total of 22 types of the spectroscopy are found. Of which here we will talk about nuclear magnetic resonance spectroscopy [33][34].

strong state atomic attractive reverberation (NMR) is a spectroscopic technique that gives results delicate to the nearby atomic climate and is utilized to concentrate on a wide assortment of carbon materials, including peat, humic substances, coal, coke and charcoal. It consequently an itemized comprehension of the synthesis of plasma blacks at the nuclear scale is accordingly of interest focused on their likely viable applications and the ionization improvement of their creation strategies. Subsequently the more extensive test approach utilized in the NMR investigation of these carbon substances utilizes cross polarization (CP) to build the polarization of the carbon cores by their collaboration with them bountiful 1 H cores. Commitments are effectively separated in light of or because of gatherings, normally in the scope of 0 to 90 ppm for aliphatic and 110-160 ppm for sweet-smelling gatherings.

Cross Polarization experiments are less time consuming compared to experiments based on the direct polarization (DP) of the 13C nucleus, as the relative longitudinal relaxation time in CP is associated with an abundant 1 H nucleus (T1H). Which means that the spectral intensity in the 13C NMR spectrum recorded with CP will be affected by differences in the rate of polarization transfer in the chemically differentiated 13C nucleus from 1 H [35].

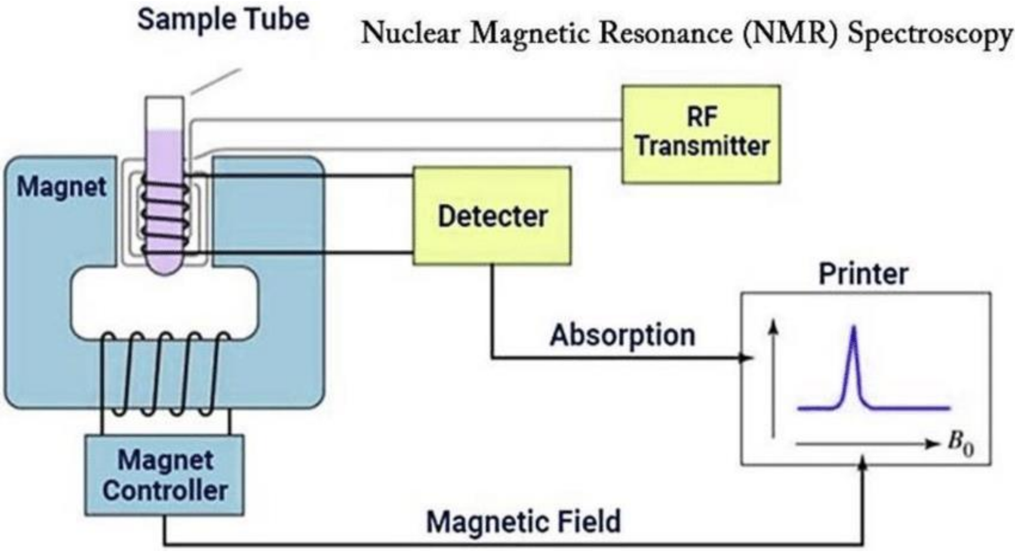


Fig.4.3. NMR spectroscopy instrumentation

It possible to determine the fraction of carbon atoms with sp2 or sp3 hybridization, observation of chemical reduction of graphene oxide, study of the relationship between chemical transformation in heat-treated carbon and structural parameters, the study of relaxation processes of 13C nuclei interacting with paramagnetic centres in nanocarbons and many others. the solid-state 13C NMR spectroscopy was used to study plasma black and carbon deposits produced by thermal cracking of natural gas using different types. i.e., different types of plasma reactors.

Assume there is a core wherein a proton is available while the proton addresses the twist second. This makes the proton go about as a little magnet. There should be a clear rule for deciding if atomic attractive reverberation is dynamic for a particle. the atomic attractive reverberation will be dynamic provided that the twist quantum number of a particle is more noteworthy than nothing. What's more, if the quantum number becomes zero then atomic attractive reverberation won't be initiated. This will be conceivable when the nuclear number and the nuclear mass become equivalent for example their twist quantum will be zero. Consequently, one might say that atomic attractive reverberation will be initiated just when the nuclear number and the nuclear mass are not equivalent.

This recommends that the core ought to contain all the data relating to the core that has protons and neutrons in which the proton has a positive charge and the neutron doesn't have a charge. At the point when the electron spins around the core and the actual electron additionally rotates in its place for example the actual electron has some twist. The actual electron can be clockwise or against clockwise twist second. While the other word is attractive, it infers that the attractive field additionally has an impact.[36][37].

4.2.3.b. TYPES OF NMR

(1) H is used to determine the type and number of atoms in an atom.

(2) Used to determine the types of carbon atoms in an atom.

Atoms are tested on the basis of hydrogen atoms in hydrogen nuclear magnetic resonance. When an atom is tested on the basis of a carbon atom in a carbon nuclear magnetic resonance. Nuclear magnetic resonance is techniques that determine the structure of various compounds.

4.2.3.c. PRINCIPLE OF NMR

When energy in the form of radio frequency is applied when applied frequency is equal to processional frequency absorption of energy occurs nucleus is in resonance and nuclear magnetic resonance signal is recorded.

(1) Sample holder

(2) Permanent magnet

(3) Probe

(4) Sweep generator

(5) Radio frequency transmitter

(6) Radio frequency receiver

(7) Read out system

4.2.3.d. NMR APPLICATION

Atomic attractive reverberation is utilized as quality control of iotas, particles or different surfaces. Discussing its utilization here, atomic attractive reverberation is additionally used to test the immaculateness of an example too as its extents and the organization of the beginning. It is likewise used to decide the atomic construction of a strong state atomic attractive spectroscopy strong.

It can likewise be utilized for the assurance of protein structure. Hence, NMR spectroscopy is a spectroscopy procedure utilized by physicists and natural chemists to research the properties of natural particles however can be applied to an example that has a twist containing core. Subsequently, once the fundamental construction is known, NMR is utilized to decide the sub-atomic profile in arrangements as well as to concentrate on the actual properties at the atomic level, for example, physical trade, stage change, dissemination and dissolvability.[38]. For example, NMR can perform quantitative analysis of mixtures containing known compounds. NMR can be used to either match spectral libraries or to apply the basic structure directly to unfamiliar combinations.

Nuclear magnetic resonance is a most powerful analytical technique in terms of the range of system that can be studied and the nature of information and can be obtained regarding the system of interest. Provide qualitative & quantitative data. Only require few milligram samples.

4.3. EXTRACTION METHODS

For the extraction of oil Soxhlet apparatus was used. A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. Typically, Soxhlet extraction is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material.

4.3.1. DESCRIPTION

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.

4.3.2. ASSEMBLY

The source material containing the compound to be extracted is placed inside the thimble. the thimble is loaded into the main chamber of the Soxhlet extractor. The extraction solvent to be used 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.

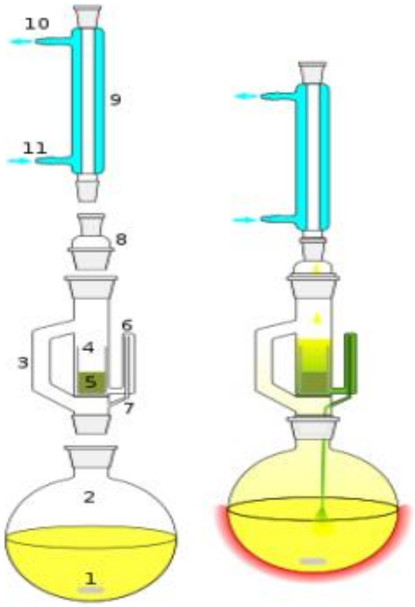


Fig: 4.4. Soxhlet apparatus

A schematic representation of a Soxhlet extractor

- Stirrer bar
- Still pot (the still pot should not be overfilled and the volume of solvent in the still pot should be 3 to 4 times the volume of the Soxhlet chamber)
- Distillation path
- Thimble
- Solid
- Siphon top
- Siphon exit
- Expansion adapter
- Condenser
- Cooling water out
- Cooling water in.

4.3.3. OPERATION

4.3.3.a. SOXHLET EXTRACTION METHOD

The collected seeds were weighed (298 g) and dried in oven at 75°C for about 3 hours. This dried material was weighed (267g). Once dried, the seed was ground using pulverisers for half an hour. Then the size reduction was made for the pulverized material using different particle size sieve. From this 10g of the sample was introduced into the thimble chamber. Soxhlet apparatus was connected to the distilled flask containing 30 mL of n-hexane (1:3 ratio). The solvent is heated to reflux. The solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound dissolves in the warm solvent. When the Soxhlet chamber is almost full, the chamber is emptied by the siphon. The solvent is returned to the distillation flask. The thimble ensures that the rapid motion of the solvent does not transport any solid material to the still pot. This cycle may be allowed to repeat many times, over hours.

Fig.4.5. Soxhlet apparatus with the sample

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 advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded. The extracted oil was directly used as the bio-collector for coal flotation.

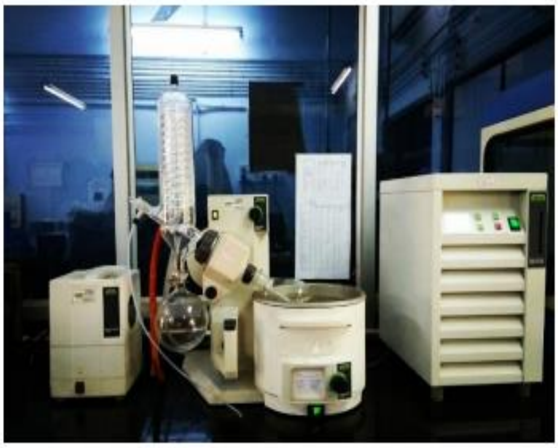


Fig. 4.6. Rotary Evaporator

For each cycle two factors were considered which plays an important role throughout the experiment. Time and temperature are the two factors. Each cycle takes 30 minutes for completion. For each 30 minutes, the temperature of water in the water bath and the solvent with the oil in the round bottom flask varies. For all these variations the oil yield was calculated and the bulk was regenerated. This bulk of oil was used as an application for the froth flotation method.

4.4. APPLICATION OF FLOTATION METHOD

4.4.1. DESCRIPTION OF EQUIPMENT

In the present study, the particle size distribution was carried out using a set of BSS screens or sieves. A set of 350, 600, 850 and 1000 μm screens were shown in the fig.4.7. Size reduction of ore is the important parameter which enhances the flotation process and hence grinding of Graphite ore was carried out in Ball Mill with 3.4Kg ball charge. A Hot air oven (fig.4.11.), muffle furnace (fig.4.12) and crucibles are used for determining % moisture and % ash, % volatile matter respectively.

The flotation process is perhaps the most efficient, widely applicable, and for a variety of ores used by the mining industry. Theoretically, it can be applied to any mixture of particles that are essentially free from one another and that are fine enough to be lifted by rising gas bubbles. Bench scale conventional flotation tests were performed in a standard D12 Denver Flotation machine (fig.4.10) manufactured by the Denver Equipment company. Flotation cells of various sizes ranging from 1.5 to 5 litres were available. In the present study 2.5 lit capacity cell has been used for beneficiation of coal ore (- 0.2 mm). the apparatus provides recirculation and agitation to maintain thorough mixing and solid/liquid suspension.

The vertical position of the agitator can be adjusted by pulling out the stop knob on the left side of the unit and turning the lever on the right-hand side. The rotation speed (rpm) of the agitator is varied by turning the knob at the top of the motor and at the back of the unit. An rpm tachometer is located at the top of the unit. Aeration is controlled using a needle valve.

The equipment used for this experiment are given below,

- Sieves (350,600,850 and 1000 μm)
- Riffle sampling Apparatus
- Ball mill
- D-12 Denver Flotation Cell
- Oven, Muffle Furnace and Crucibles.

Fig.4.7. Sieves

Fig. 4.8. Riffle sampling

Fig.4.9a. Ball milling machine

Fig.4.9b Grinding media(balls)

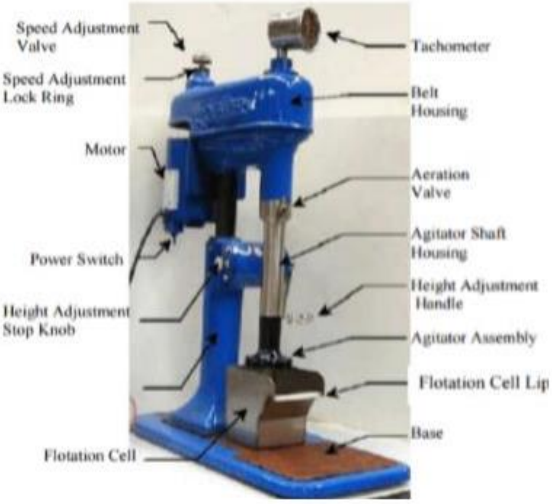


Fig.4.10. D-12 Denver flotation cell

Fig. 4.11. Hot air oven

Fig. 4.12. Muffle furnace

4.4.2. EXPERIMENTAL PROCEDURE

Major steps involved in the experimentation procedure for the beneficiation process are given below:

1. Preparation of sample for laboratory studies includes
   - a. Conning and Quartering
   - b. Grinding
   - c. Proximate analysis
2. Wet screen analysis
3. Flotation
4. Ash analysis.

4.4.3. PREPARATION OF SAMPLE FOR LABORATORY STUDIES

First of all, coal ore was subjected to the crushing and grinding to reduce the size of the bulk sample for experimentation convenience. Afterwards, coning and quartering analysis was done to get the desired amount of sample for further analysis.

4.4.4. PROXIMATE ANALYSIS

The proximate analysis of coal separates the products into four groups:

1. Moisture,
2. volatile matter consisting of gases and vapours driven off during pyrolysis,
3. Fixed carbon, the non-volatile fraction of coal, and
4. Ash, the inorganic residue remaining after combustion.

The proximate analysis of coal is presented as a group of test methods that has been used widely as the basis for coal characterization in connection with coal utilization. The proximate analysis of coal was done according to an Indian standard IS 14852: 2000.

(a)Moisture content

Determination of moisture is carried out by placing a 1 gm sample of powdered coal material in a tared porcelain dish and covers it with a watchglass. Place the dish in an air oven maintained at temperature 150 + 1°C and remove the watch-glass from it. Heat the sample for two hours in Hot air oven and cover the dish with the watch-glass before taken it out of the oven. Cool the dish in a desiccator and weigh.

The loss in weight represents moisture

$$\text{Moisture }\% = \frac{weight\ of\ moisture}{weight\ of\ sample} \times 100$$

(b)Volatile Matter (VM)

Introduce in a weighted silica crucible with tightly fitted lid about one gram of the moisture free sample. Heat the crucible in a muffle furnace maintained at a temperature of 925 + 25°C for 7 min. The bottom of the crucible shall not rest on the muffle floor. Remove the crucible from the muffle after 7 min; Allow it to cool in a desiccator and weigh.

The loss of weight is taken as weight of volatile matter.

$$\text{Volatile matter}\% = \frac{weight\ of\ volatile\ matter}{weight\ of\ original\ sample} \times 100$$

(c) Ash content

Weigh accurately about 1 to 2 g of the moisture free material in a silica crucible, keep in a muffle furnace and heat to 500 + 10°C within one hour and 775 + 10°C in two hours. A slow stream of air is maintained through the muffle furnace. When the carbon is completely removed as indicated by the absence of black particle upon stirring with a platinum wire, the temperature is further increased to 950 + 25°C and-kept for one hour. Cool in a desiccator and weigh.

The loss of weight is taken as weight of Ash content.

$$\text{Ash \%} = \frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100$$

(d) Fixed carbon (FC)

Fixed Carbon or FC is derived by subtracting from 100 the value of moisture, volatile matter and ash.

$$\text{Fixed Carbon \%} = 100 - (\text{Moisture \%} + \text{Volatile Matter \%} + \text{Ash \%})$$

4.5. COAL GRINDING

Size reduction of ore is the important parameter which enhances the flotation process and hence grinding of coal ore carried out in Ball Mill with 3.4Kg ball charge, 66% solid percent and depressant dosage of Sodium silicate in variation of time preliminary experiments were performed to optimize the grinding process with varying grinding time and sodium silicate dosage followed by flotation. Grinding was performed for 3 minutes for optimizing grinding time as established in previous experiments conducted by NML was taken into consideration.

4.5.1.WET SIEVE ANALYSIS

Wet sieving is a procedure used to evaluate particle size distribution of a granular material or aid in particle size analysis by removing fines that may impede the separation process. 100g of the sample feed is been grinded in ball mill for 3 min were taken for wet screening consisting of 350,600,850 and 1000 mesh screens. After wet sieving analysis, 0.5g of representative sample was collected from the oversize of each screen of the sieve set to determine the ash content. Average overall ash has been computed from wet sieve analysis data.

4.5.2. LABORATORY FLOTATION STUDIES (PRELIMINARY EXPERIMENTS)

267g of grounded coal sample mixing with water and was allowed to conditioned for 2 minutes at the impeller speed of 1250 rpm for each batch flotation. 37.5mL of (1%) Sodium Silicate was added as a depressant to the pulp and conditioned for five minutes. After conditioning required amount of Collector (extracted oil) was added and allowed the mixture for three minutes for uniform mixing of Collector within the pulp. Now water was added to fill the cell up to the marked height to make the pulp density at 19%. Air inlet valve was opened to allow the air to introduce into the system from the bottom of flotation cell. These air generated air bubbles which carried the coal particles up to the top of the surface as froth. This froth was collected as a concentrate for four minutes whereas the remaining parts collected as tailings. The flotation concentrate and filtered tailing were dried, weighted and analysed for ash content [28].

4.5.3. D-12 DENVER SUB-AERATION FLOTATION CELL – CONDITIONS

Flotation tests were carried at specified conditions:

Size of the feed particle : below 0.5 mm

PH of the slurry : Neutral

Impeller speed : 1250 rpm

Solid concentration of pulp during preparation of coal slurry solid : 40%
Concentration of pulp during conditioning : 33%
Solid concentration of pulp during frothing : 19%
Wetting time : 2 minutes
Depressant conditioning time : 5 minutes
Collector conditioning time : 3 minutes
Froth Collection time : 4 minutes

Fig.4.13 coal flotation machine

4.6. REAGENTS USED FOR FLOTATION

According to the test conditions given above, the reagents were used.

Two tests were done:

Test-1:

1). 1% Sodium Silicate as depressant

2). Extracted oil from aromatic seed using Soxhlet extraction method (Reagent) was used as the collector.

Test-2:

1). 1% Sodium Silicate as depressant.

2). Oil was used as the collector.

3). Extracted oil from aromatic seed using Soxhlet extraction method (Reagent) was used as the frother.

Dosages of the reagents was varied for each test.

CHAPTER 5

RESULTS AND DISCUSSION

As a result of this review, the extraction of oil from sweet-smelling plant was performed by Soxhlet extraction technique. This technique is done utilizing a chosen natural dissolvable which is n-Hexane and boundaries like time, temperature and decrease of the molecule size. The outcomes are organized and the diagrams are plotted for every boundary. The assurance and portrayal of oil from sweet-smelling plant are additionally illustrated in this review.

5.1. DESIGN OF EXPERIMENT (DOE)

Design of experiment techniques, you can determine the individual and interactive effects of various factors that can influence the output results of your measurements. It is a structured approach for collecting data and making discoveries. To determine whether a factor, or a collection of factors, has an effect on the response. the factorial guideline, randomization, replication and hindering. Creating and examining these plans depended fundamentally close by estimation before, up to this point experts began utilizing PC produced plans for a more powerful and effective DOE. To run trials that span the potential experimental region for our factors. Design of experiments (DOE) is defined as a branch of applied statistics that deals with planning, conducting, analysing, and interpreting controlled tests to evaluate the factors that control the value of a parameter or group of parameters. DOE is a powerful data collection and analysis tool that can be used in a variety of experimental situations.

RUN NO	FEED SIZE (µm)	TEMPERATURE (ºc)
1	350	70
2	350	75
3	600	70
4	600	75

5	850	70
6	850	75
7	1000	70
8	1000	75

Table: 5.1. DOE method table

5.2 EFFECT OF SIZE AND OIL YIELD

5.2.a. EFFECTS OF SIZE AND OIL YIELD USING AT TEMPERATURE (70ºC)

At temperature 70ºc

OIL YIELD (%)	**FEED SIZE (-µm)**
1.94	350
1.81	600
2.66	850
1.73	1000

Table 5.2. Size variation versus oil yield

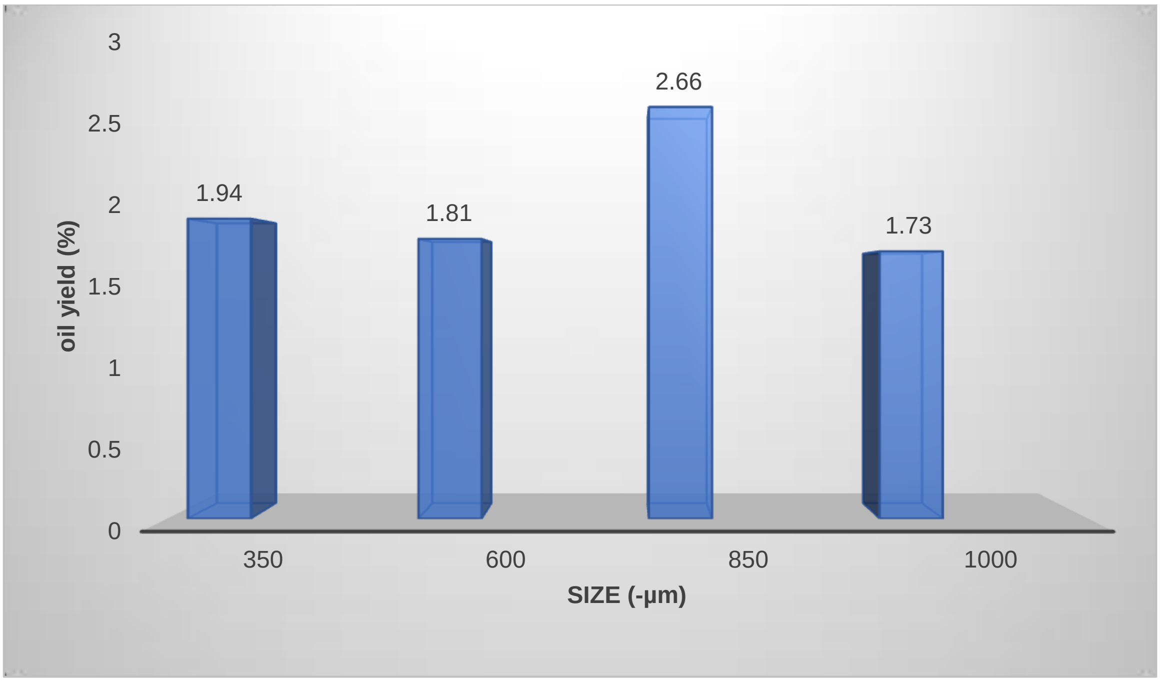


Fig.5.1. Graphical representation of particle size versus oil yield

CALCULATION OF OIL YIELD USING 70ºC

(i) The yield of the oil extracted around 70°C (-350µm) using Soxhlet apparatus was calculated using this below equation,

$$\% \, yield = \frac{weight\ of\ the\ oil\ extracted}{weight\ of\ the\ sample\ feed} \times 100 \qquad = \frac{1.94}{10} \times 100$$

Oil yield = 19.4 %

(ii) The yield of the oil extracted around 70°C (-600µm) using Soxhlet apparatus was calculated using this below equation,

$$\% \, yield = \frac{weight\ of\ the\ oil\ extracted}{weight\ of\ the\ sample\ feed} \times 100$$

$$= \frac{1.81}{10} \times 100$$

Oil yield = 18.1 %

(iii) The yield of the oil extracted around 70°C (-850µm) using Soxhlet apparatus was calculated using this below equation,

$$\% \, yield = \frac{weight \ of \ the \ oil \ extracted}{weight \ of \ the \ sample \ feed} \times 100$$

$$= \frac{2.66}{10} \times 100$$

Oil yield = 26.6 %

(iv) The yield of the oil extracted around 70°C (-1mm) using Soxhlet apparatus was calculated using this below equation,

$$\% \, yield = \frac{weight \ of \ the \ oil \ extracted}{weight \ of \ the \ sample \ feed} \times 100$$

$$= \frac{1.73}{10} \times 100$$

Oil yield = 17.3 %

5.2.b. EFFECTS OF SIZE AND OIL YIELD USING AT TEMPERATURE (75ºC)

At temperature 75ºc

OIL YIELD (%)	**FEED SIZE (-µm)**
2.17	350
1.96	600
2.86	850
1.72	1000

Table 5.3. Size variation versus oil yield

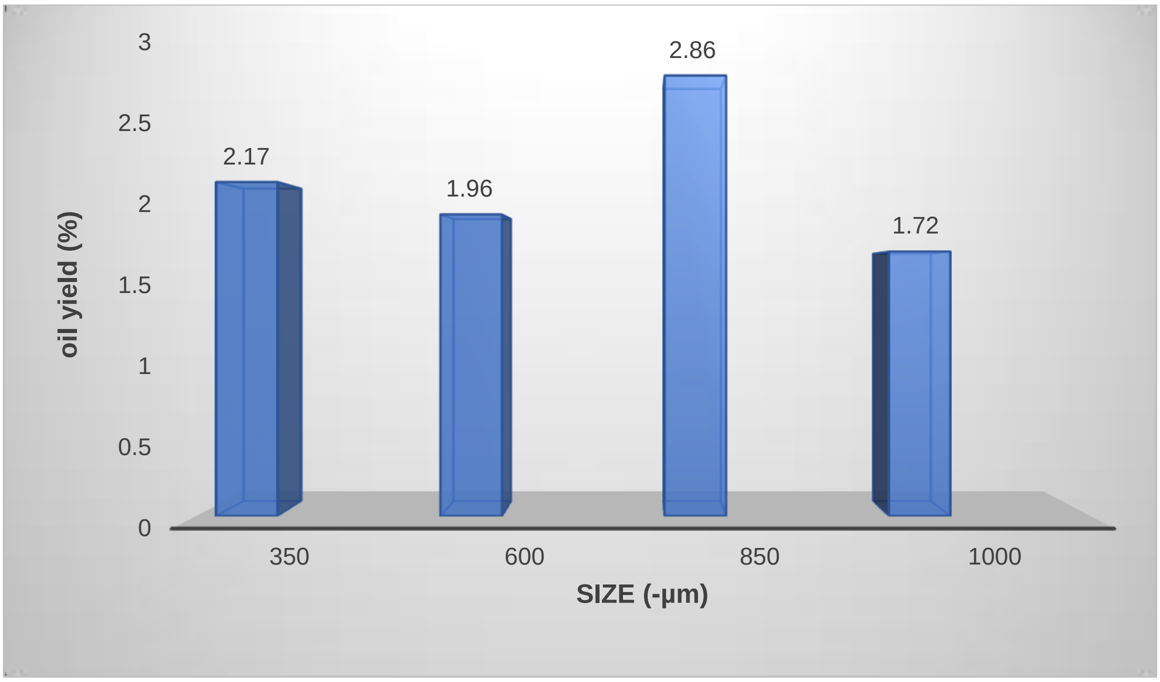


Fig.5.2. Graphical representation of particle size versus oil yield

CALCULATION OF OIL YIELD USING 75ºC

(i) The yield of the oil extracted around 75°C (-350µm) using Soxhlet apparatus was calculated using this below equation,

$$\% \, yield = \frac{weight \; of \; the \; oil \; extracted}{weight \; of \; the \; sample \; feed} \times 100$$

$$= \frac{2.17}{10} \times 100$$

Oil yield = 21.7 %

(ii) The yield of the oil extracted around 75°C (-600µm) using Soxhlet apparatus was calculated using this below equation,

$$\% \, yield = \frac{weight \; of \; the \; oil \; extracted}{weight \; of \; the \; sample \; feed} \times 100$$

$$= \frac{1.96}{10} \times 100$$

Oil yield = 19.6 %

(iii) The yield of the oil extracted around 75°C (-850µm) using Soxhlet apparatus was calculated using this below equation,

$$\% \, yield = \frac{weight \ of \ the \ oil \ extracted}{weight \ of \ the \ sample \ feed} \times 100$$

$$= \frac{2.86}{10} \times 100$$

Oil yield = 28.6 %

(iv) The yield of the oil extracted around 75°C (-1mm) using Soxhlet apparatus was calculated using this below equation,

$$\% \, yield = \frac{weight \ of \ the \ oil \ extracted}{weight \ of \ the \ sample \ feed} \times 100$$

$$= \frac{1.72}{10} \times 100$$

Oil yield = 17.2 %

Results obtained from the above analysis shows that the increase in temperature and time generally favours an increase in the oil yield. This phenomenon is due to the fact that oils are generally more soluble at elevated temperatures (Sen et al.1992). At higher temperatures, the viscosity of the solvent is reduced while the diffusivity, as well as the evaporation rate is increased. This increases the contact time between the solvent and the oil-bearing material (Ayoola et at.2014). From the above result, Soxhlet extraction using solvent as n-hexane with different particle size (-350µm, -600µm, -850µm and -1mm) using two set of temperature are 70°C and 75ºc has obtained the yield.

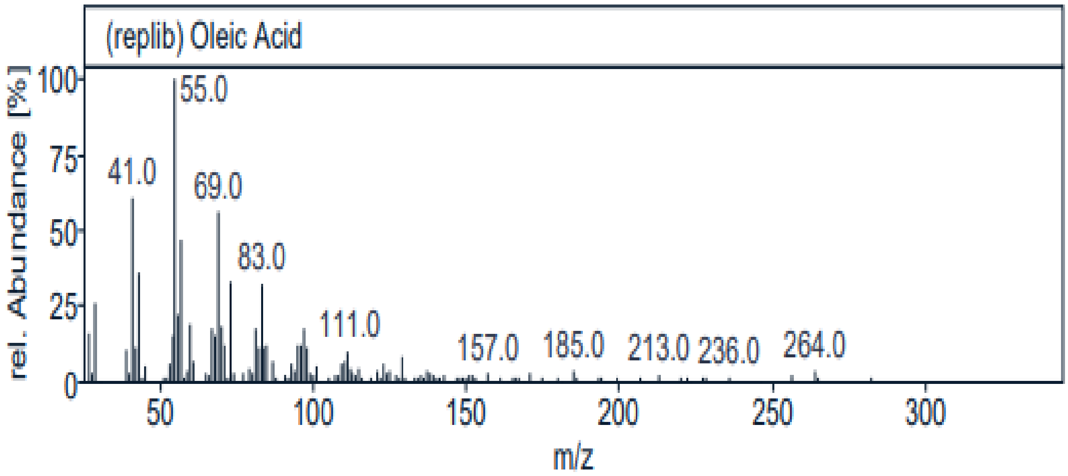
5.3. RESULTS OF GC-MS

Gas chromatography mass spectrometry (GC-MS) is an instrumental technique, comprising a gas chromatograph (GC) coupled to a mass spectrometer (MS), that is carried out by which complex mixtures of chemicals may be separated, identified and quantified. This makes it ideal for the analysis of the hundreds of relatively low molecular weight compounds found in environmental materials. In order for a compound to be analysed by GC-MS it must be sufficiently volatile and thermally stable. In addition, functionalised compounds may require chemical modification (derivatization), prior to analysis, to eliminate undesirable adsorption effects that would otherwise affect the quality of the data obtained. Samples are usually analysed as organic solutions consequently materials of interest (e.g.: soils, sediments, tissues etc.) need to be solvent extracted and the extract subjected to various 'wet chemical' techniques before GC-MS analysis is possible.

The sample solution is injected into the GC inlet where it is vaporized and swept onto a chromatographic column by the carrier gas (usually 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 ion source where compounds eluting from the column are converted to ions.

The GC-MS results of the extracted oil is observed from the below graphs. The retention time is observed and peak area is calculated. X-axis denotes the retention time and Y-axis denotes the intensity.

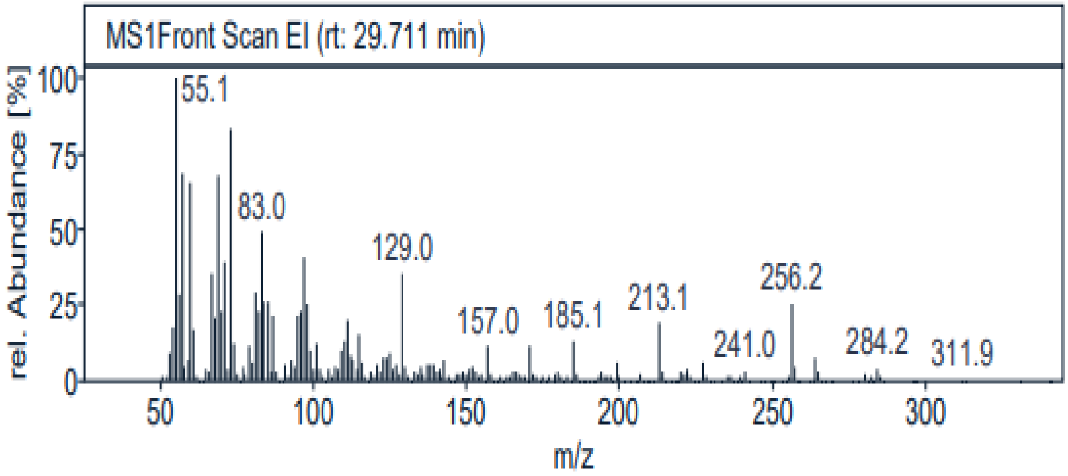
Peak @ 29.708 **Area** 36838952.277 **Area %** 6.62



Ion Table

55.0 999 • 41.0 607 • 69.0 560 • 57.0 471 • 43.0 356 • 73.0 332

Compound Name	**Score**	**Rev. Score**	**Prob. %**	**Library Name**	**CAS #**	**Library Id**
Oleic Acid	836	880	45.62	replib	112-80-1	5489



Ion Table

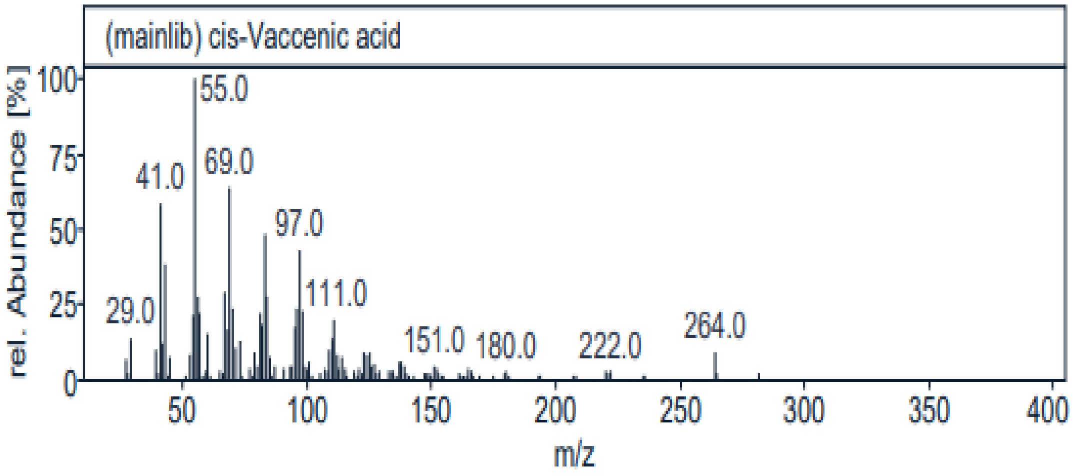
55.1 999 • 73.0 828 • 57.1 683 • 69.0 677 • 60.0 657 • 83.0 494

Summary Hit Table

Compound Name	**Score**	**Rev. Score**	**Prob. %**	**Library Name**	**CAS #**	**Library Id**
Oleic Acid	836	880	45.62	replib	112-80-1	5489

Fig.5.3. Represents Oleic acid

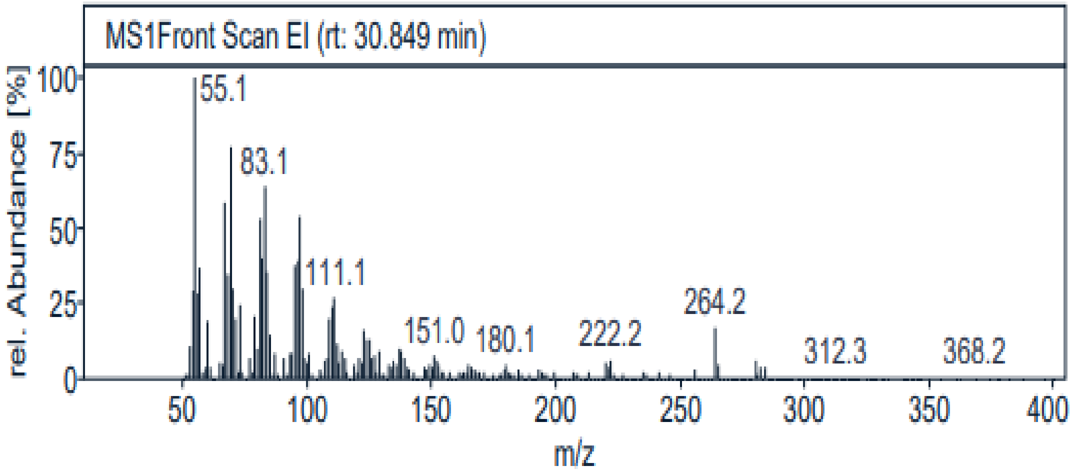
Peak @ 30.847 **Area** 414949073.619 **Area %** 74.61



Ion Table

55.0 999 • 69.0 638 • 41.0 583 • 83.0 487 • 97.0 426 • 43.0 386

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS #	Library Id
cis-Vaccenic acid	896	903	13.49	mainlib	506-17-2	21723



Ion Table

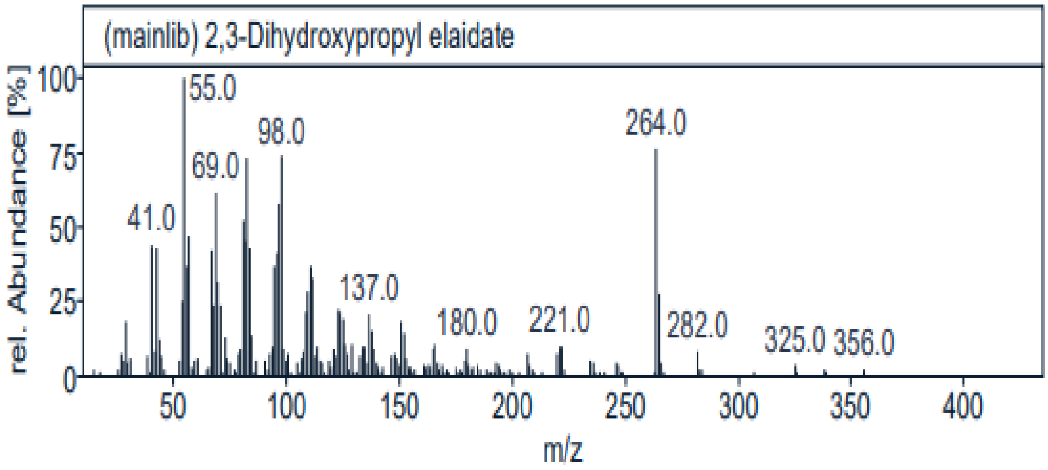
55.1 999 • 69.1 770 • 83.1 641 • 67.1 584 • 97.1 540 • 81.0 527

Summary Hit Table

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS #	Library Id
cis-Vaccenic acid	896	903	13.49	mainlib	506-17-2	21723

Fig.5.4. Represents cis-Vaccenic acid

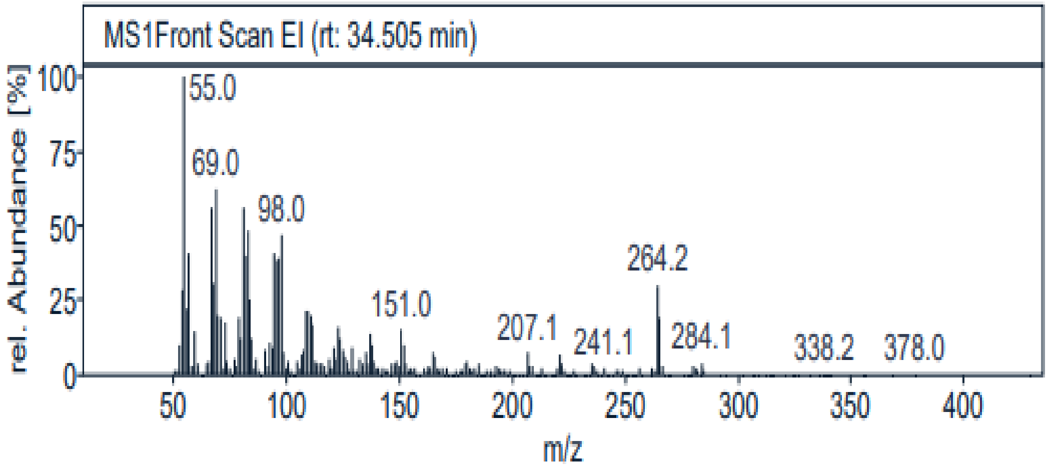
Peak @ 34.506 **Area** 2430839.269 **Area %** 0.44



Ion Table

55.0 999 • 264.0 761 • 98.0 738 • 83.0 728 • 69.0 611 • 97.0 573

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS #	Library Id
2,3-Dihydroxypropyl elaidate	868	872	12.35	mainlib	2716-53-2	23542



Ion Table

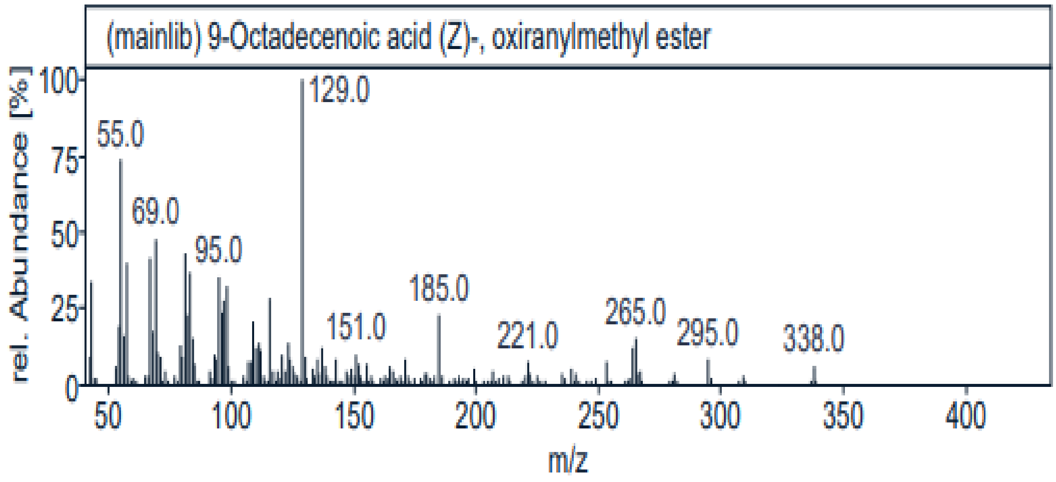
55.0 999 • 69.0 626 • 67.1 560 • 81.0 559 • 83.1 480 • 98.0 471

Summary Hit Table

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS #	Library Id
2,3-Dihydroxypropyl elaidate	868	872	12.35	mainlib	2716-53-2	23542

Fig.5.5. Represents 2,3-Dihydroxypropyl elaidate

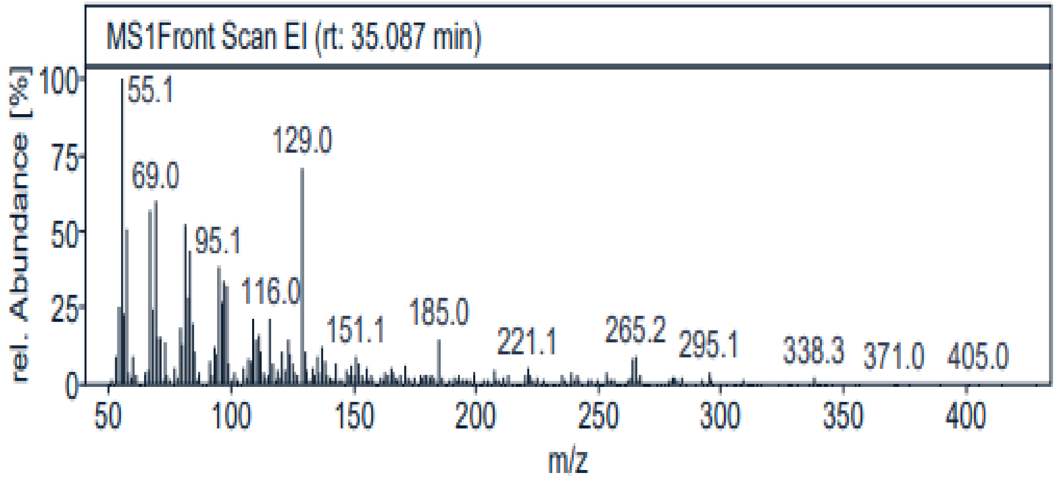
Peak @ 35.091 **Area** 6420576.454 **Area %** 1.15



Ion Table

129.0 999 • 55.0 741 • 69.0 473 • 81.0 426 • 67.0 414 • 57.0 399

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS #	Library Id
9-Octadecenoic acid (Z)-, oxiranylmethyl ester	912	932	70.68	mainlib	5431-33-4	123490



Ion Table

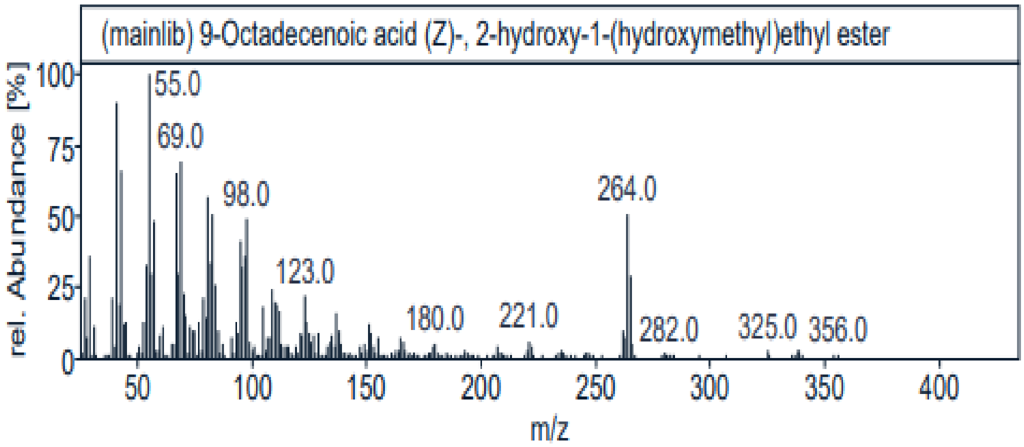
55.1 999 • 129.0 705 • 69.0 596 • 67.0 566 • 81.0 524 • 57.0 510

Summary Hit Table

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS #	Library Id
9-Octadecenoic acid (Z)-, oxiranylmethyl ester	912	932	70.68	mainlib	5431-33-4	123490

Fig.5.6. Represents 9-Octadecenoic acid (Z)-, oxiranylmethyl ester

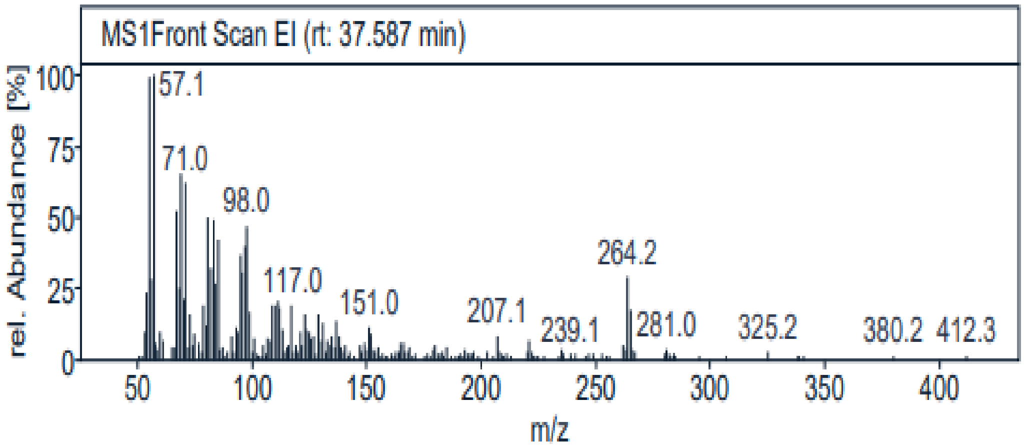
Peak @ 37.590 **Area** 5293264.658 **Area %** 0.95



Ion Table

55.0 999 • 41.0 900 • 69.0 690 • 43.0 660 • 67.0 650 • 81.0 570

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS #	Library Id
9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl	849	886	32.89	mainlib	3443-84-3	20887



Ion Table

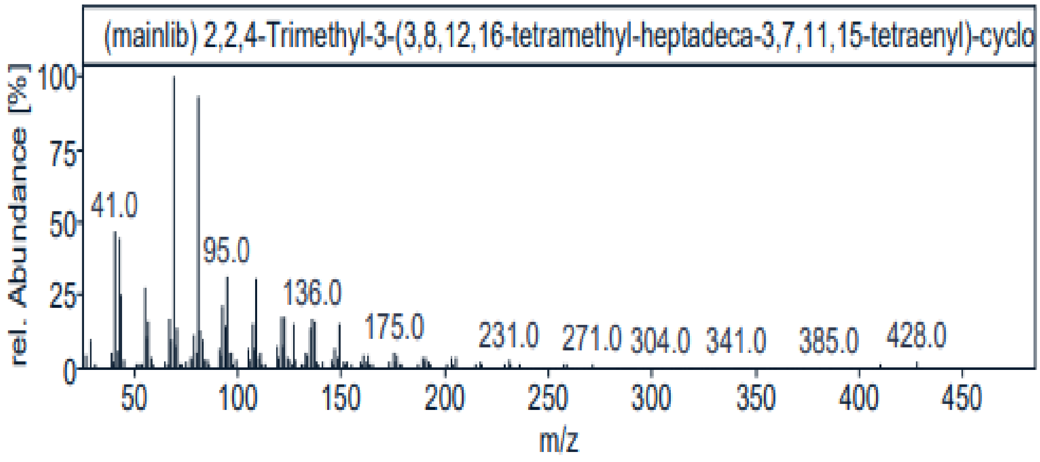
57.1 999 • 55.1 992 • 69.0 657 • 71.0 623 • 67.0 522 • 81.0 496

Summary Hit Table

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS #	Library Id
9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl	849	886	32.89	mainlib	3443-84-3	20887

Fig.5.7. Represents 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester

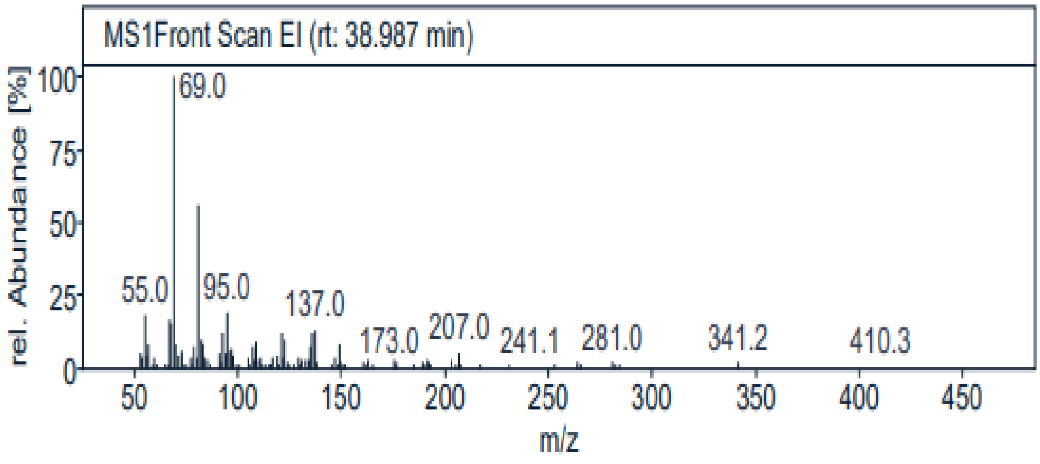
Peak @ 38.986 **Area** 1573684.888 **Area %** 0.28



Ion Table

69.0 999 • 81.0 933 • 41.0 470 • 43.0 447 • 95.0 313 • 109.0 302

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS #	Library Id
2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,1	792	814	17.9	mainlib		38116



Ion Table

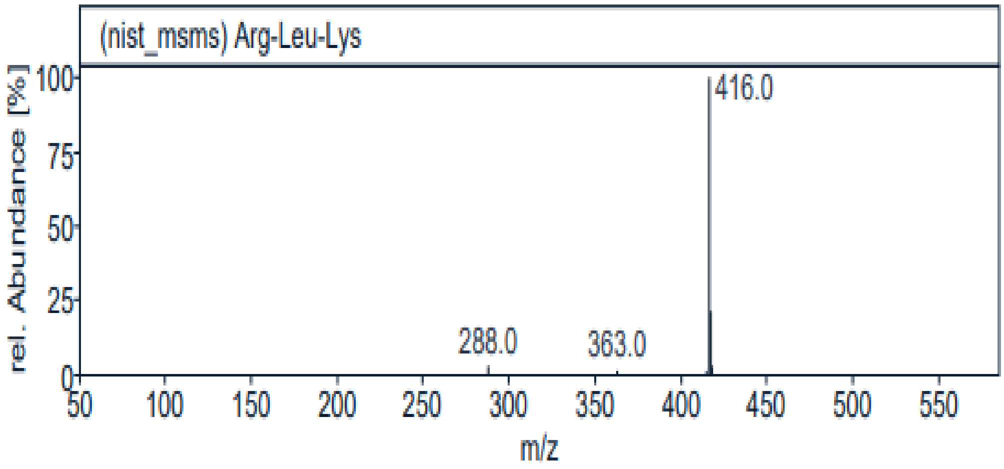
69.0 999 • 81.0 558 • 95.0 187 • 55.0 184 • 67.1 163 • 68.1 153

Summary Hit Table

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS #	Library Id
2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,1	792	814	17.9	mainlib		38116

Fig.5.8. **Represents 2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol**

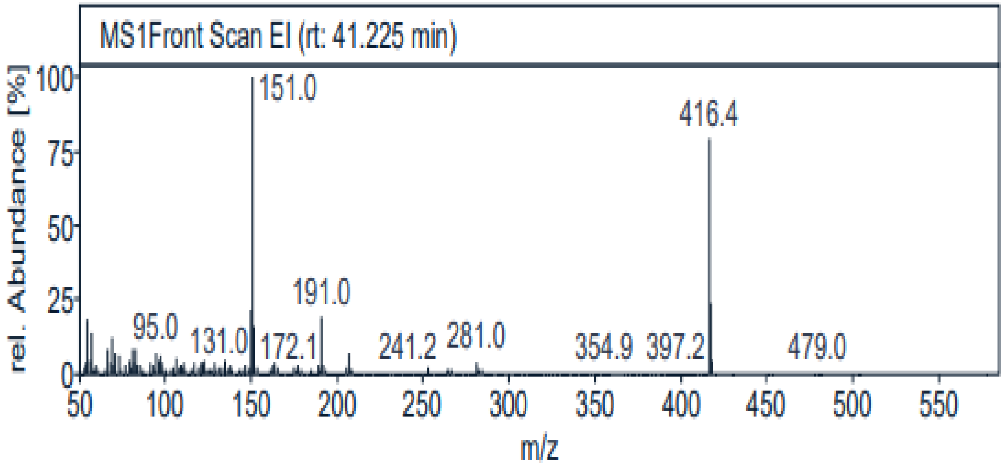
Peak @ 41.224 **Area** 3042845.286 **Area %** 0.55



Ion Table

416.0 999 • 417.0 209 • 288.0 29 • 418.0 24 • 415.0 11 • 363.0 10

Compound Name	**Score**	**Rev. Score**	**Prob. %**	**Library Name**	**CAS #**	**Library Id**
Arg-Leu-Lys	861	916	19.6	nist_msms		55844



Ion Table

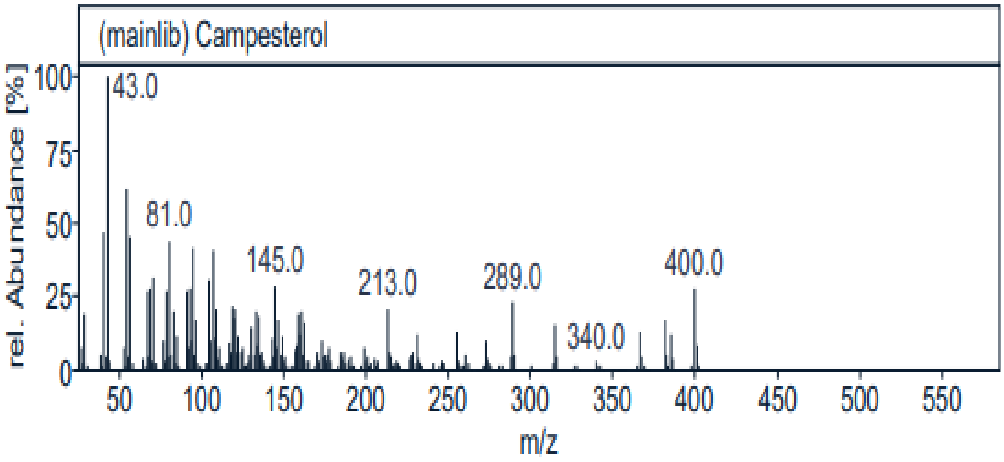
151.0 999 • 416.4 795 • 417.3 235 • 150.0 211 • 191.0 189 • 55.0 183

Summary Hit Table

Compound Name	**Score**	**Rev. Score**	**Prob. %**	**Library Name**	**CAS #**	**Library Id**
Arg-Leu-Lys	861	916	19.6	nist_msms		55844

Fig.5.9. Represents Arg-Leu-Lys

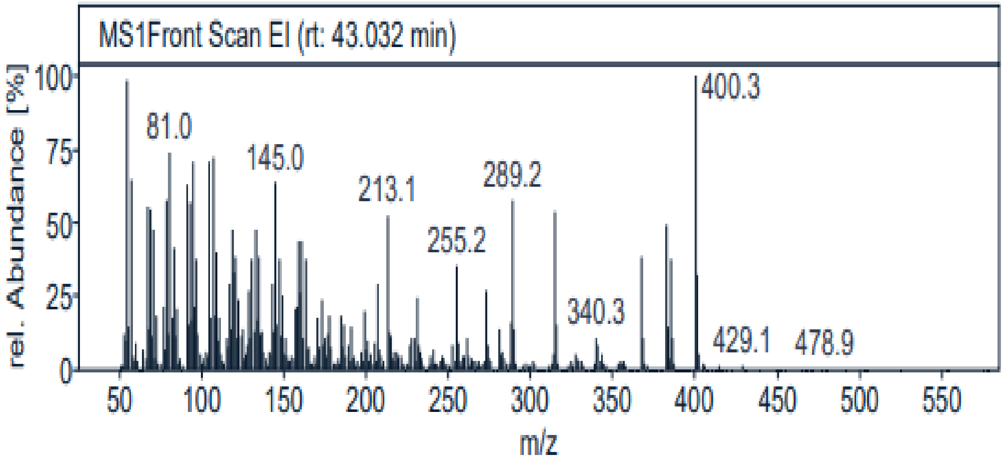
Peak @ 43.028 **Area** 13480674.265 **Area %** 2.42



Ion Table

43.0 999 • 55.0 617 • 41.0 470 • 57.0 452 • 81.0 436 • 95.0 414

Compound Name	**Score**	**Rev. Score**	**Prob. %**	**Library Name**	**CAS #**	**Library Id**
Campesterol	900	916	62.87	mainlib	474-62-4	7617



Ion Table

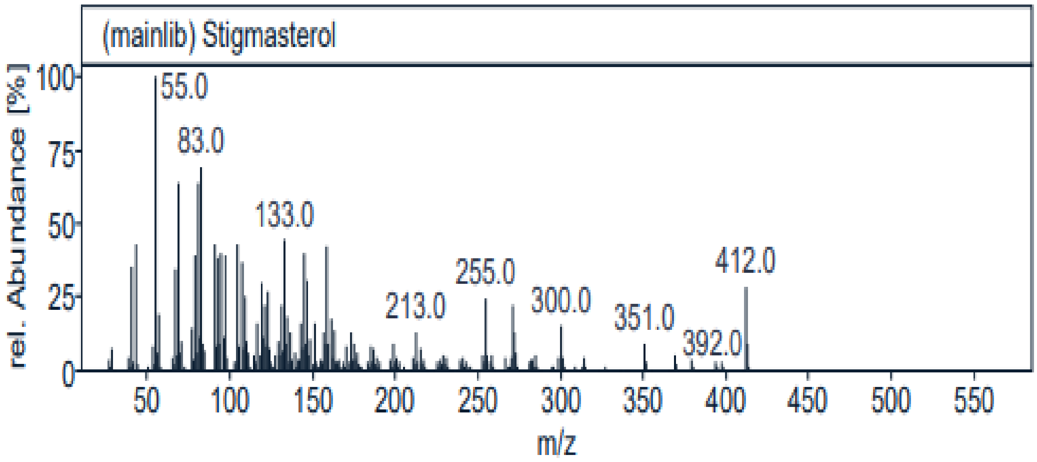
400.3 999 • 55.0 983 • 81.0 742 • 107.0 720 • 95.0 710 • 105.0 704

Summary Hit Table

Compound Name	**Score**	**Rev. Score**	**Prob. %**	**Library Name**	**CAS #**	**Library Id**
Campesterol	900	916	62.87	mainlib	474-62-4	7617

Fig.5.10. Represents Campesterol

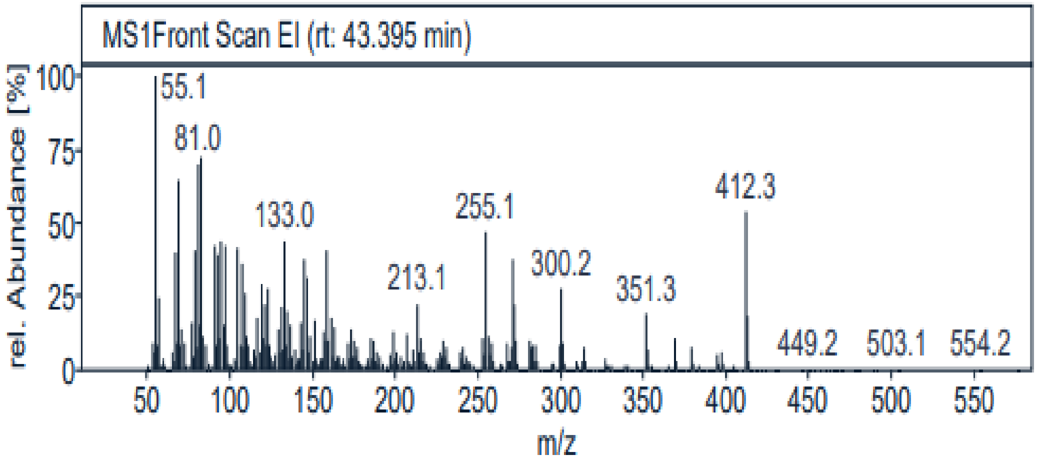
Peak @ 43.395 **Area** 16748353.420 **Area %** 3.01



Ion Table

55.0 999 • 83.0 692 • 81.0 638 • 69.0 636 • 133.0 444 • 43.0 431

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS #	Library Id
Stigmasterol	921	937	77.47	mainlib	83-48-7	22487



Ion Table

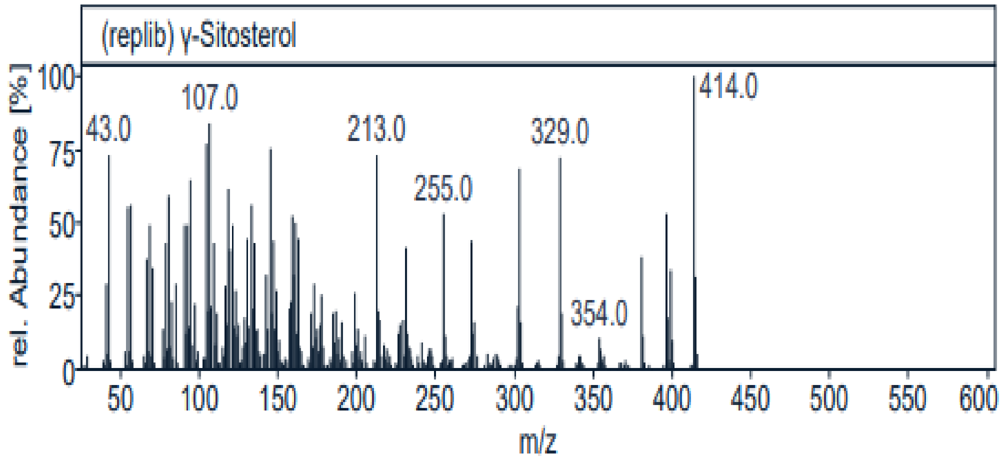
55.1 999 • 83.0 725 • 81.0 698 • 69.1 642 • 412.3 537 • 255.1 466

Summary Hit Table

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS #	Library Id
Stigmasterol	921	937	77.47	mainlib	83-48-7	22487

Fig.5.11. Represents Stigmasterol

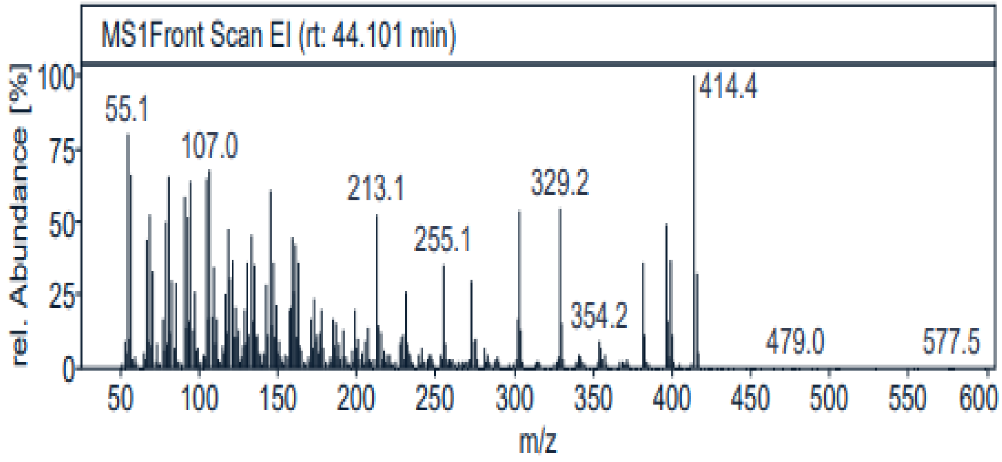
Peak @ 44.099 **Area** 27080183.990 **Area %** 4.87



Ion Table

414.0 999 • 107.0 836 • 105.0 770 • 145.0 757 • 213.0 730 • 43.0 728

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS #	Library Id
γ-Sitosterol	944	956	79.68	replib	83-47-6	39080



Ion Table

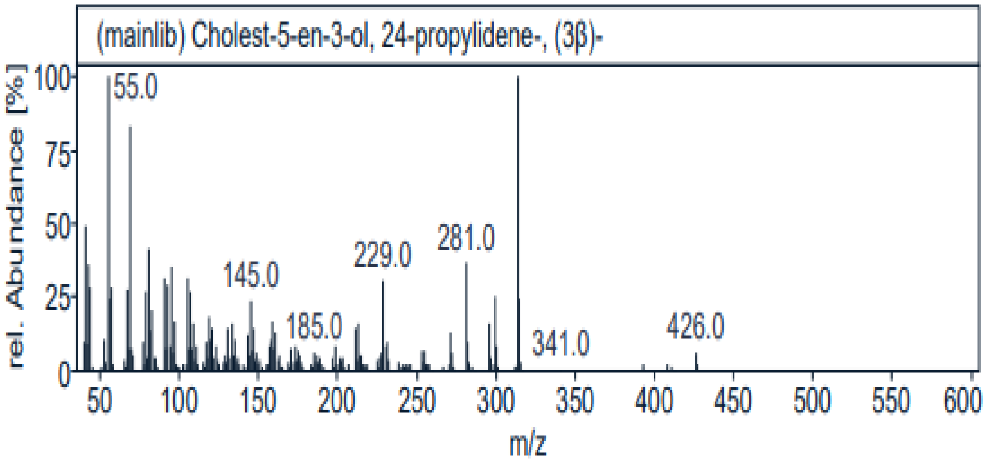
414.4 999 • 55.1 799 • 107.0 673 • 57.1 658 • 81.0 656 • 105.0 648

Summary Hit Table

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS #	Library Id
γ-Sitosterol	944	956	79.68	replib	83-47-6	39080

Fig.5.12. Represents γ-Sitosterol

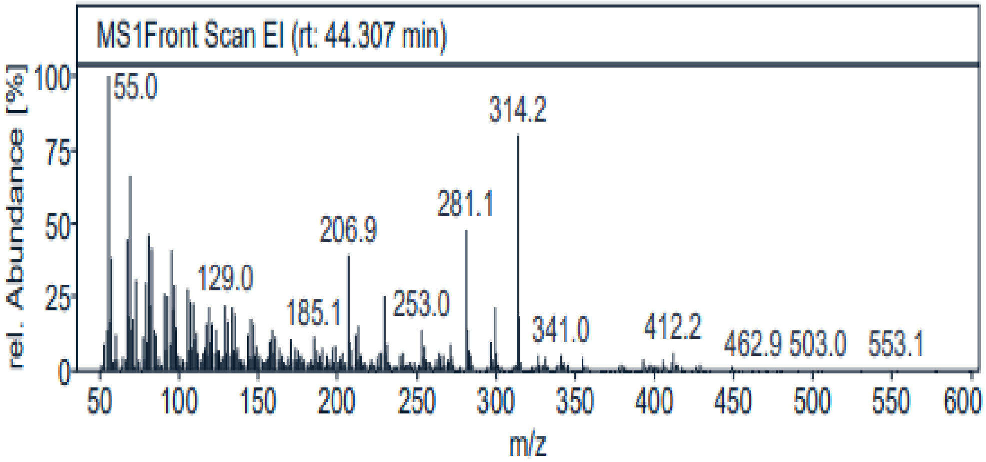
Peak @ 44.302 **Area** 2867427.431 **Area %** 0.52



Ion Table

55.0 999 • 314.0 999 • 69.0 830 • 41.0 494 • 81.0 412 • 281.0 364

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS #	Library Id
Cholest-5-en-3-ol, 24-propylidene-, (3β)-	786	853	20.73	mainlib	56362-45-9	23581



Ion Table

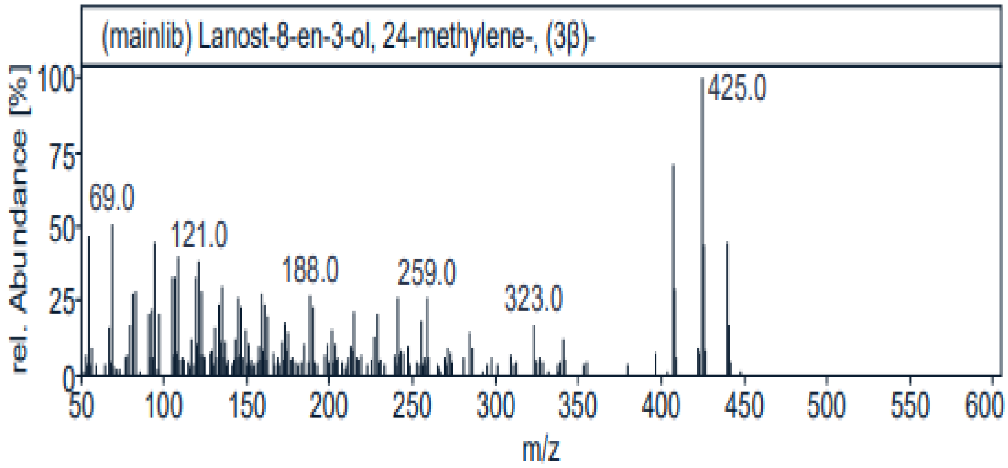
55.0 999 • 314.2 799 • 69.1 665 • 281.1 477 • 81.0 461 • 67.0 442

Summary Hit Table

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS #	Library Id
Cholest-5-en-3-ol, 24-propylidene-, (3β)-	786	853	20.73	mainlib	56362-45-9	23581

Fig.5.13. Represents Cholest-5-en-3-ol, 24-propylidene-, (3β)-

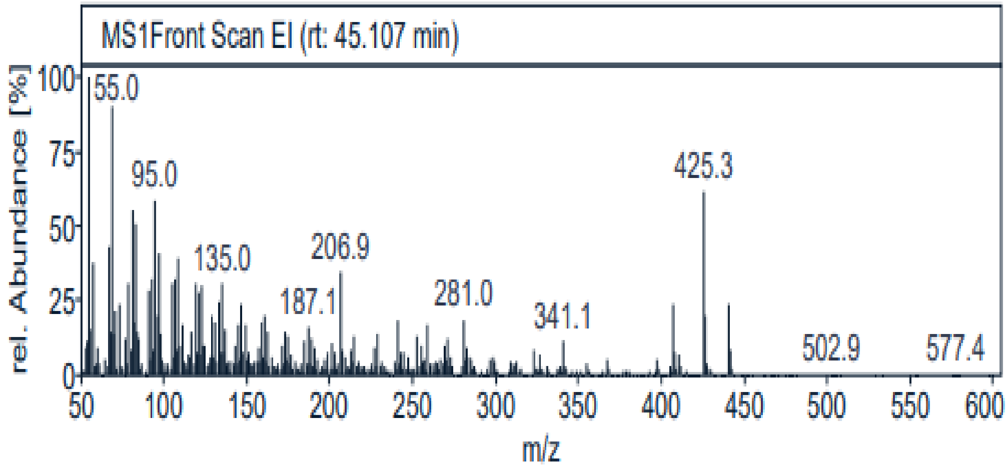
Peak @ 45.108 **Area** 6259647.298 **Area %** 1.13



Ion Table

425.0 999 • 407.0 710 • 69.0 510 • 55.0 469 • 95.0 448 • 440.0 448

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS #	Library Id
Lanost-8-en-3-ol, 24-methylene-, (3β)-	748	865	35.98	mainlib	6890-88-6	264116



Ion Table

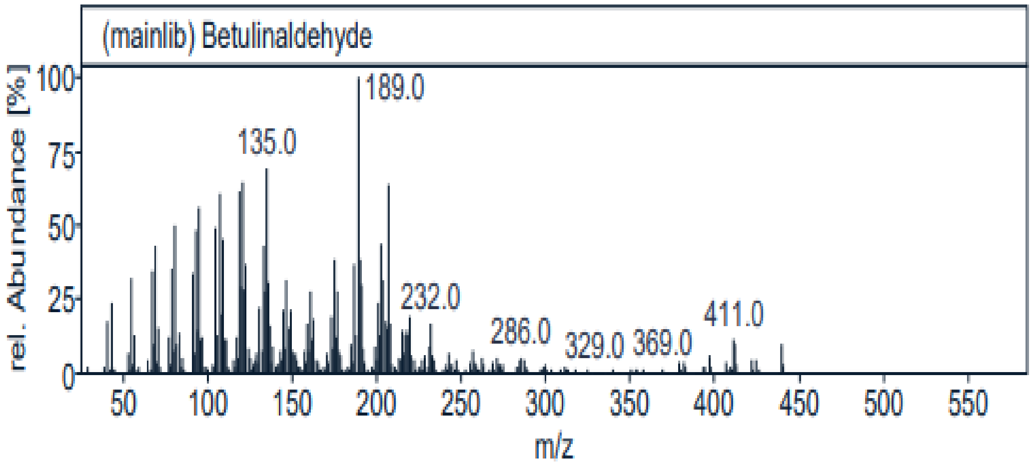
55.0 999 • 69.0 904 • 425.3 611 • 95.0 587 • 81.0 555 • 83.0 507

Summary Hit Table

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS #	Library Id
Lanost-8-en-3-ol, 24-methylene-, (3β)-	748	865	35.98	mainlib	6890-88-6	264116

Fig.5.14. Represents Lanost-8-en-3-ol, 24-methylene-, (3β)-

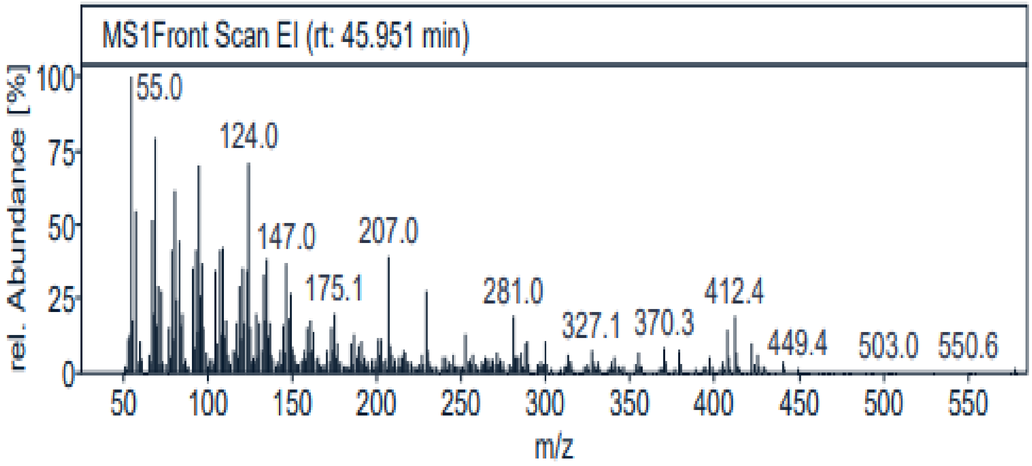
Peak @ 45.952 **Area** 8707671.660 **Area %** 1.57



Ion Table

189.0 999 • 135.0 694 • 121.0 643 • 207.0 636 • 119.0 618 • 107.0 610

Compound Name	**Score**	**Rev. Score**	**Prob. %**	**Library Name**	**CAS #**	**Library Id**
Betulinaldehyde	744	776	11.34	mainlib	13159-28-9	193372



Ion Table

55.0 999 • 69.0 789 • 124.0 705 • 95.0 700 • 81.1 612 • 57.1 545

Summary Hit Table

Compound Name	**Score**	**Rev. Score**	**Prob. %**	**Library Name**	**CAS #**	**Library Id**
Betulinaldehyde	744	776	11.34	mainlib	13159-28-9	193372

Fig.5.15. Represents Betulinaldehyde

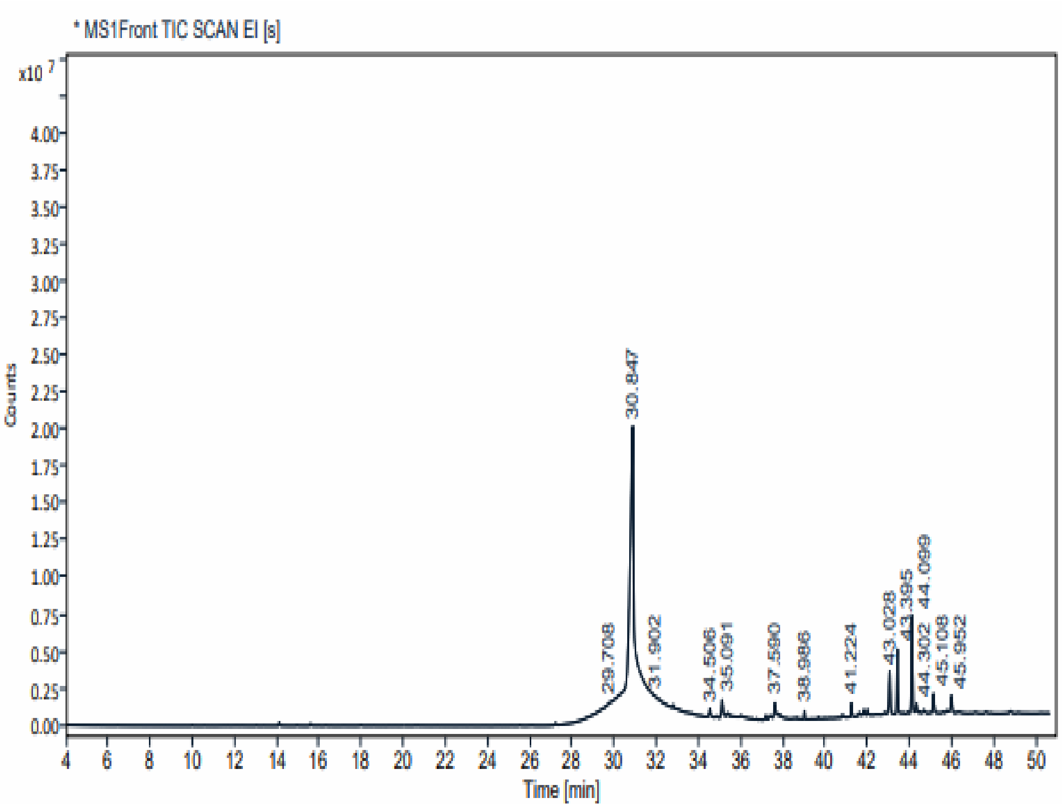


Fig. 5.16. GC-MS analysis of Annona squamosa

S:No.	Retention time	Name of the compound	Molecular weight	Molecular formula	Peak area	%
01	29.711	Oleic Acid	282.47	$C_{18}H_{34}O_2$	36838952	6.62
02	30.849	cis-Vaccenic acid	282.5	$C_{18}H_{34}O_2$	414949073	74.61
03	34.505	2,3-Dihydroxypropyl elaidate	356.54	$C_{21}H_{40}O_4$	2430839	0.44
04	35.087	9-Octadecenoic acid(Z)-, oxiranyl methyl ester	338.5	$C_{21}H_{38}O_3$	6420576	1.15
05	37.587	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	356.5	$C_{21}H_{40}O_4$	5293264	0.95

06	38.987	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	482.7	$C_{30}H_{52}O$	1573684	0.28
07	41.225	Arg-Leu-Lys	415.5	$C_{18}H_{37}N_7O_4$	3042845	0.55
08	43.032	Campesterol	400.7	$C_{28}H_{48}O$	13480674	2.42
09	43.395	Stigmasterol	412.7	$C_{29}H_{48}O$	16748353	3.01
10	44.101	γ-Sitosterol	414.7	$C_{29}H_{50}O$	27080183	4.87
11	44.307	Cholest-5-en-3-ol, 24-propylidene-, (3β)-	426.7	$C_{30}H_{50}O$	2867427	0.52
12	45.107	Lanost-8-en-3-ol, 24-methylene-, (3β)-	440.8	$C_{31}H_{52}0$	6259647	1.13
13	45.951	Betulinaldehyde	440.7	$C_{30}H_{48}O_2$	8707671	1.57

Table 5.4. List of fatty acids present in the given sample

From the Table 5.4. The retention time, molecular weight, molecular formula, peak area and its percentage was observed. Since the percentage of the oleic acid was absorbed more this has been used as an application for mineral processing as a reagent.

5.4. FTIR RESULTS

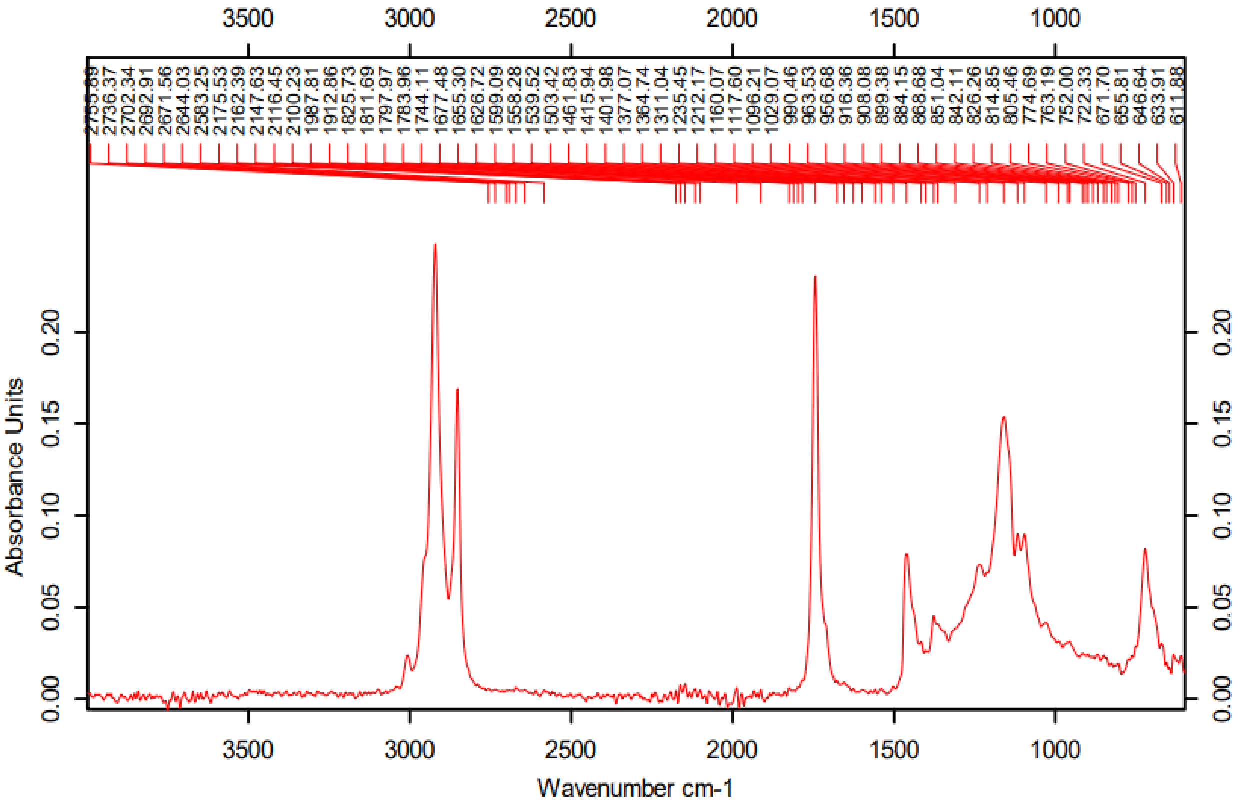


Fig.5.17. Graphical representation of FTIR

The extracted oil sample was subjected for Fourier Transform Infrared Spectroscopy (FTIR) spectrum ranging from 4000 cm^{-1} to 500 cm-^{1} to find the functional groups presents in it. The results of the study are given in the figure. It may be seen that the broad band at 2921cm-^{1} is due to the stretching mode of the sp3-C-H groups in hydrocarbon. The peak at 2852 cm-^{1} is due to the stretching mode of sp^{2}-C-H groups in hydrocarbon. The peak 1461cm^{-1}denotes methylene group of C-H bending and the peak 1377 cm $^{-}$1 denotes C-H bending of methyl group. The peaks at 1744cm^{-1} denotes stretching mode of C=O in amino acids. The peak 1160cm^{-1} denotes C-O stretching vibration. The peak 722 cm^{-1} denotes methylene group of CH_2 rocking vibration. These are the compound present in the given oil. X-axis denotes the wavenumber (cm-^{1}) and Y-axis denotes the absorbance unit.

5.5. NMR RESULTS

From the 1H NMR spectra, it can be concluded that the chemical shift value at 0.8 is due to the primary proton and the chemical shift value at 1.3 is due to the secondary proton and the chemical shift value at 1.5 is due to the 3º proton. The chemical shift value at 2.5 is due to the carboxyl group and the chemical shift value at 3.5 is due to ester/ether linkage and the chemical shift value at 5.35 is due to vinylic carbon (C=C-H).

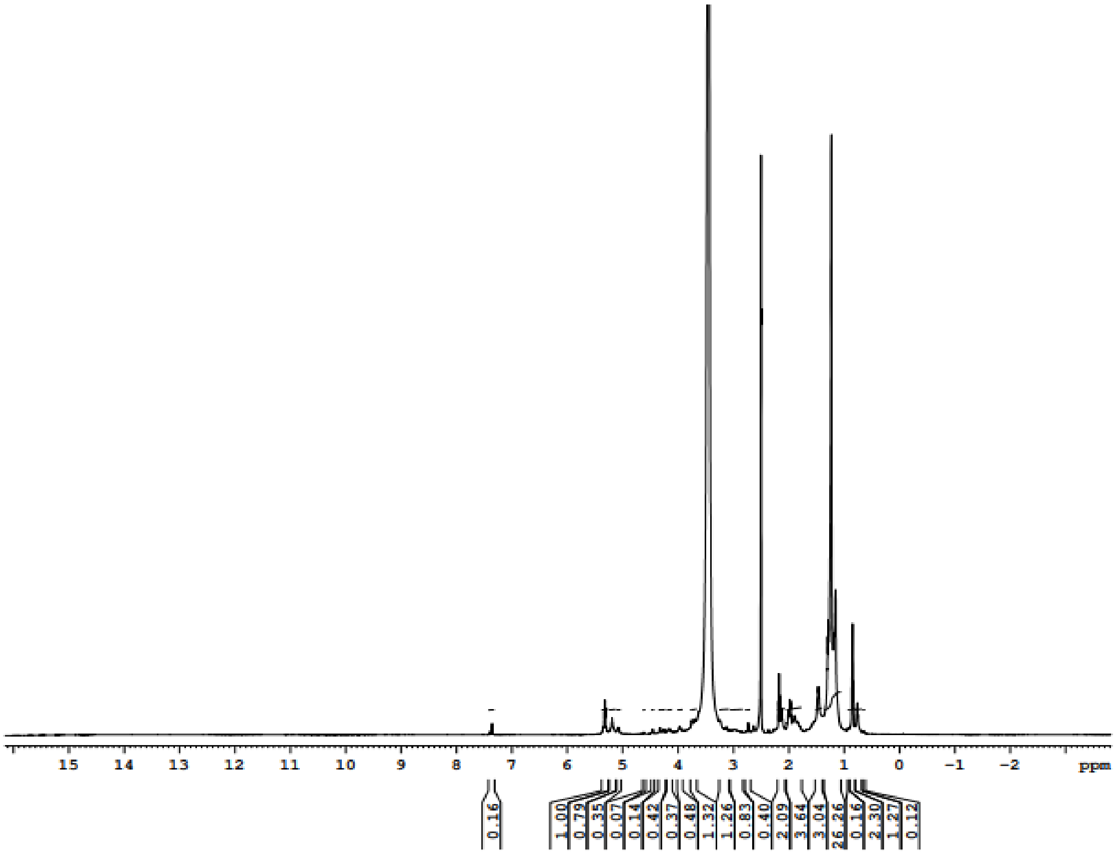


Fig.5.18. Graphical representation of NMR

S.no	Product	While grinding		While flotation		Weight %	Ash %	Ash distribution%
		Time (mins)	Sodium silicate (1%)	Dispersant (Sodium silicate 1%)	Collector (extracted oil)			
1	F	3	37.5	37.5	0.5	51.36	17.17	26.32
2	NF	3	37.5	37.5	0.5	48.64	50.75	73.63
						Head calculated		33.50

Table 5.5. Results for coal flotation

CHAPTER – 6

SUMMARY AND CONCLUSION

The results obtained from the extraction and characterization of the essential oil from aromatic plant have shown the maximum yield of essential oil using Soxhlet extraction method by varying the parameters such as temperature, time and particle size. Soxhlet extraction gave the maximum yield that was achieved at a lesser temperature (75°C) which was capable of giving oil of better quality. Not only the temperature but also the particle size played an important role in exhibiting the maximum yield. At same temperature (75°C), the particle size at -800 µm it gave the highest yield of about 28.6%.

The experimental design and data analysis carried out on Soxhlet extraction method was to represent the extraction process with significance and a reasonable value.

It was also shown by the analysis of variance of the developed model that temperature, time and particle size are the very significant factors on the yield of the oil extraction. The physical and chemical properties values that are obtained from the characterization of the oil revealed that it could be used in different industrial process for the production of other valuable products.

As the large amount of the raw material, which was not used frequently would be required for the large-scale extraction of the oil, thus making this process an advantage for the environmental and waste management sectors of the community.

In the effect of particle size, since the highest yield was at 75°C, in this case the size of the particle was varied by maintaining at 75°C constant. From this the highest yield (28.6%) was at -800 µm. This is because as the size is decreased the surface area becomes high, resulting in the highest oil yield.

As the percentage of the oleic acid was absorbed more in GC-MS, this has been used as an application for mineral processing as a reagent. The extracted oil was used as a collector for low grade graphite ore flotation. From GC-MS and FTIR studies the peaks that contains hydrocarbons, fatty acid and other compounds are well resolved and well aggregated.

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GEO TAGGED IMAGE

This project was done at CSIR-NML and the Geo tagged location is attached above.