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COMPARATIVE IN-VITRO ANTIBACTERIAL ACTIVITY OF VOLATILE OILS INDIGENEOUS TO CHHATTISGARH REGION

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ABSTRACT

In the present pharmaceutical are the science which treat in detail medicinal, pharmacognosy and related product of drug obtained from plant, animal and mineral origins. In short, it is an study crude drugs from natural sources treated scientifically and it encompasses the knowledge of the history, distribution, cultivation, collection, processing for market and preservation, the study of sensory, physical, chemical, and structural characters and the uses of crud drugs. The aim to study of some crude drug in Chhattisgarh region like Ginger, Ajwain, and Black paper.

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INTRODUCTION

Definition: Volatile oils are the odorous and volatile products of various plant and animal species. As they have a tendency to undergo evaporation on being exposed to the air even at an ambient temperature, they are invariably termed as volatile oils, essential oil or ethereal oils**.** They mostly contribute to the odorferous constituents or *'essences'* of the aromatic plants that are used abundantly in enhancing the aroma by seasoning of eatables. A large number of herb materials Volatile oils with extensive bioactivities. Acknowledging the importance of plant and its medicinal value, extraction of volatile oil has been done using steam plant. The distillation was conducted in Clevenger apparatus in which condensing and distillation. In this project Steam Distillation was used to extract oil from different plant material like Ginger, Ajwain, black pepper etc. volatile oil are so termed as they are believed to represent the very essence of odor and flavor. There is variety of method for obtaining volatile oils from plants. Steam Distillation method was found to be one of the promising techniques for the extraction of volatile oil from plant as reputable distiller will preserve the original qualities of the decantation was done. Infections due to bacterial species also remain a serious clinical problem. Emerging resistance of bacterial species is seriously decreasing the number of effective antimicrobials. Because of increasing pressure of consumers and legal authorities, the food industry has tended to reduce the use of chemical preservatives in their products to either completely nil or to adopt more natural alternatives for the maintenance or extension of product shelf life (Seenivasan Prabuseenivasan *et al*., 2006). Plants and their essential oils are potentially useful sources of antimicrobial compounds. Numerous studies have been published on the antimicrobial activities of plant compounds against many different types of microbes, including food-borne pathogens (Baratta *et al*., 1998; Nazia Masood Ahmed Chaudhry and Perween Tariq, 2006; Omidbeygi *et al*., 2007; Abadias *et al*., 2008 and Bari *et al*., 2010). The main constituents of essential oils – mono- and sesquiterpenes including carbohydrates, phenols, alcohols, ethers, aldehydes and ketones – are responsible for the biological activity of aromatic and medicinal plants as well as for their fragrance. Due to these properties, spices and herbs have been added to food since ancient time, not only as flavouring agents but also as preservatives (Burt, 2004)

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Pharmacology

Although medicinal use of essential oils is seen as pseudoscience in the healthcare community, essential oils retain considerable popular use among advocates of alternative medicine. Therefore, it is difficult to obtain reliable references concerning their pharmacological merits. Studies have shown that certain essential oils may have the ability to prevent the transmission of some drug-resistant strains of pathogen, specifically Staphylococcus, Streptococcus and Candida. Taken by mouth, many essential oils can be dangerous in high concentrations. Typical effects begin with a burning feeling, followed by salivation. In the stomach, the effect is carminative, relaxing the gastric sphincter and encouraging eructation (belching). Further down the gut, the effect typically is antispasmodic. Typical ingredients for such applications include eucalyptus oils, menthol, capsaicin, anise and camphor. Other essential oils work well in these applications, but it is notable that others offer no significant benefit. This illustrates the fact that different essential oils may have drastically different pharmacology. Those that do work well for upper respiratory tract and bronchial problems act variously as mild expectorants and decongestants. Some act as locally anaesthetic counterirritants and, thereby, exert an antitussive effect.

Some essential oils, such as those of juniper and agathosma, are valued for their diuretic effects. With relatively recent concerns about the overuse of antibacterial agents, many essential oils have seen resurgence in off-label use for such properties and are being examined for this use clinically. Many essential oils affect the skin and mucous membranes in ways that are valuable or harmful. Many essential oils, particularly tea tree oil, may cause a contact dermatitis. They are used in antiseptics and liniments in particular. Typically, they produce rubefacient irritation at first and then counterirritant numbness. Oil and camphor are two typical examples of oils that cause such effects. Menthol and some others produce a feeling of cold followed by a sense of burning. This is caused by its effect on heat-sensing nerve endings. Some essential oils, such as clove oil or eugenol, were popular for many hundred years in dentistry as antiseptics and local anaesthetics. Thymol is well known for its antiseptic effects.

Use in aromatherapy

Aromatherapy is a form of alternative medicine in which healing effects are ascribed to the aromatic compounds in essential oils and other plant extracts. Some essential oils are claimed to have an uplifting effect on the mind. Such claims, if meaningful, are not necessarily false but are difficult to quantify in the light of the sheer variability of materials used in the practice.

- **Essential oils are convenient, quick and easy to use:** You can wear them during the day, diffuse them in your home or work place, or simply keep them in your pocket. Essential oils can be used in massage, and to enhance meditation and concentration. Read through the Use Section for more ideas!
- **Essential oils are organic substances from the volatile liquid of plants:** The essential oils support healthy body functions such as healthy immune system function.
- **Essential oils benefits include that they can penetrate the skin and affect the emotional center:** Oils cross the brain-blood barrier and reach the amygdala and other limbic parts of the brain that control our mood, emotions and beliefs. So they can help us with our ability to handle stress, anger or any other emotion.
- **Essential oils soothe muscle discomfort after exercise**.
- **Essential oils benefits include animals**: orses and dogs in particular respond very well to essential oils. While there are some limitations with cats, they can be used for cats as well.
- **Essential oils support healthy digestive system:** Peppermint (*Mentha piperita*)is one of the oldest and most highly regarded herbs for soothing digestion.
- **Essential oil benefits include d-limonene**
- **Essential oil benefits include green household products**: Oils are non-toxic and promote wellness.

MATERIALS AND METHODS

Selection of Plant Material

Due to ease in availability and on the basis of literature survey some drug was selected for the present study

1. Ginger
2. Ajwain
3. Black pepper

Collection

The selected plant material like ginger, garlic, ajwain and black pepper were collected from local market of Ambikapur (C.G.) drug were clean and dried.

Extraction Method

- Traditional Steam Distillation
- Simple Distillation
- Hydro Diffusion
- Hydro Distillation
- Combination Water and Steam Distillation.

Distillation

- A master distiller must the entire process by harvesting at just the right time for the plant maturity and distilling with the proper temperature, pressure, and time which varies with different material.
- Distillation converts the volatile liquid (the essential oils) into a vapor and then condenses the vapor back into a liquid. This method is more commonly used in essential oil extraction than vapor back into a liquid.

Traditional Steam Distillation

- Steam distillation is a separation process for materials that are temperature sensitive like essential oils.
- Today, the traditional method of steam distillation is still used around the world. Plant material is loaded into the extraction chamber and tightly compacted. As the boiler heats the water, steam is released into the bottom of the chamber and starts to travel upward, saturating the material.

- The steam impregnates the plant fiber, causing it to release the oil molecule as a gas from the molecule pocked or channel. Then the steam carries the gas to the condenser where it goes through a phase-change condensation as it passes through the cooling process in the swan neck and liquefies into water and oil.
- The water and oil mixture then flows into the separator where the oil can rise to the top of the water to be poured off into containers.
- In each of these processes as the steam rises, it carries the released oil vapor into the condenser where the water and oil vapor convert to a liquid and flow into the separator so that the oil can rise to the top of the water and be drained off.
- There are many variables in steam distillation. Subtle differences in equipment design and processing conditions can translate into huge differences in essential oil quality. The size and material of the extraction chamber, the type of condenser and separator, and the temperature and pressure can all have a huge impact on the oil quality.
- Distillation is as much a science as it is an art. If the pressure or temperatures is too high, or if the cooking chambers are constructed from reactive materials, all may not be therapeutic or genuine essential oils.
- Vertical steam distillation offers the greatest potential for protecting the therapeutic benefits and quality of essential oils. In ancient distillation, low pressure (5 pounds or lower) and low temperature were extremely important to produce the therapeutic benefits. The late Marcel Espieu, who was president of the Lavender Growers Association in Southern France for 20 years, maintained that the best oil quality can only be produced when the pressure is zero pounds during distillation.
- Temperature also has a distinct effect. At certain temperatures, the oil fragrance and chemical constituents become altered. High pressures and high temperatures seem to cause harshness in the oil. Even the oil pH and the polarity are greatly affected.
- For example, cypress requires a minimum of 24 hours of distillation at 265 degrees and 5 pounds of pressure to extract most of the therapeutically-active constituents. If distillation time is cut by only two hours, 18-20 constituents will be missing from the resulting oil.
- The most commercial cypress oil is distilled for only 2 hours and 15 minutes! This short distillation time allows the producer to cut costs and produces cheaper oil, since money is saved on the fuel needed to generate.

Water Distillation/Simple Distillation

- The plant material is loaded into the extraction chamber filed with water, which is heated to soften the plant fiber so that the oil molecules can be released.
- As steam begins to rise, the oil molecules are released as vapors, which are carried with the steam into the condenser. The cooling water in the condenser converts the steam to water and the vapors to oil.
- The oil and water mixture continues to flow into the separator where the oil rises to the top of the water so that it can be drained off into containers. Clove and nutmeg are distilled this way.

Hydro Diffusion

- When essential oils are extracted using hydro diffusion it is a type of steam distillation, and only varies in the actual way in which the steam is introduced into the still. The steam is fed in from the top onto the botanical material instead of from the bottom as in normal steam distillation.
- At the bottom of the still is a grill that holds the botanical material in place. The condensation of the oil containing steam mixture occurs below the grill. The main advantage of this method is that less steam is used, shorter processing time and a higher oil yield.

Hydro Distillation

- Resinous material like frankincense and myrrh are extracted through this method of hydro-distillation.
- The resin is immersed in boiling water that is in constant motion while steam is injected into the chamber.
- The resinous gas is then released into the steam, which carries it to the condenser where the steam and vapor are gradually cooled to a liquefied form.
- The water and oil mixture travel into the separator so the oil can flow to the top of the water and be poured off into containers. Resinous materials such as frankincense and myrrh are distilled in this manner.

Combination Water and Steam Distillation

- This process is a combination of normal water distillation and steam distillation.
- The botanical material is immersed in water in a still, which has a heat source, plus live steam is fed into the water and botanical material mixture

Expression

Most citrus peel oils are expressed mechanically or cold-pressed (similar to olive oil extraction). Due to the relatively large quantities of oil in citrus peel and low cost to grow and harvest the raw materials, citrus-fruit oils are cheaper than most other essential oils. Lemon or sweet orange oils that are obtained as byproducts of the citrus industry are even cheaper. Before the discovery of distillation, all essential oils were extracted by pressing.

Solvent extraction

Most flowers contain too little volatile oil to undergo expression; their chemical components are too delicate and easily denatured by the high heat used in steam distillation. Instead, a solvent such as hexane or supercritical carbon dioxide is used to extract the oils. Extracts from hexane and other hydrophobic solvents are called *concretes*, which are a mixture of essential oil, waxes, resins, and other lipophilic (oil-soluble) plant material. Although highly fragrant, concretes contain large quantities of non-fragrant waxes and resins. Often, another solvent, such as ethyl alcohol, which is more polar in nature, is used to extract the fragrant oil from the concrete. The alcohol solution is chilled to −18 °C (0 °F) for

more than 48 hour which causes the waxes and lipids to precipitate out. The precipitates are then filtered out and the ethanol is removed from the remaining solution by evaporation, vacuum purge, or both, leaving behind the absolute. Supercritical carbon dioxide is used as a solvent in supercritical fluid extraction. This method has many benefits including avoiding petrochemical residues in the product and the loss of some "top notes" when steam distillation is used. It does not yield an absolute directly. The supercritical carbon dioxide will extract both the waxes and the essential oils that make up the concrete. Subsequent processing with liquid carbon dioxide, achieved in the same extractor by merely lowering the extraction temperature, will separate the waxes from the essential oils. This lower temperature process prevents the decomposition and denaturing of compounds. When the extraction is complete, the pressure is reduced to ambient and the carbon dioxide reverts to a gas, leaving no residue. Supercritical carbon dioxide is also used for making decaffeinated coffee. Although it uses the same basic principles, it is a different process because of the difference in scale.

Chemical Constituents of Volatile Oil

Terpenes Hydrocarbons:

Monoterpene

- These monoterpene compounds are found in nearly all essential oils and have a structure of 10 carbon atoms and at least one double bond. The 10 carbon atoms are derived from two isoprene units.
- They react readily to air and heat sources. For this reason citrus oils do not last well, since they are high in monoterpene hydrocarbons and have a quick reaction to air, and are readily oxidized.
- Although some quarters may simply state that these components have anti-inflammatory, antiseptic, antiviral and antibacterial therapeutic properties while some can be analgesic or stimulating with a tonic effect, it could be seen as a very broad generalization, since this large group of chemicals vary greatly. Since some have a stimulating effect on the mucus membranes they are also often used as decongestants.

Sesquiterpenes

- These sesquiterpenes consist of 15 carbon atoms and have complex pharmacological actions and here we can look at chamazulene, which is found in German chamomile.
- It has anti-inflammatory and anti-allergy properties. Another sesquiterpene often found in chamomile and rose, as well as other floral oils is farnesene.
- History highlight of terpene research
- The 1910 Nobel Prize winner for Chemistry was Professor Otto Wallach for his work on terpenes which influenced the essential oil industry. Click here to read more.

Oxygenated Compounds

Phenols

- The phenols found in essential oils normally have a carbon side chain and here we can look at compounds such as thymol, eugenol and carvacrol. These components have great antiseptic, anti-bacterial and disinfectant qualities and also have greatly stimulating therapeutic properties.
- Due to the nature of phenols, essential oils that are high in them should be used in low concentrations and for short periods of time, since they can lead to toxicity if used over long periods of time, as the liver will be required to work harder to excrete them.
- Phenols are also classified as skin and mucus membrane irritants and although they have great antiseptic qualities, like cinnamon and clove oil, they can cause severe skin reactions.

Alcohols

Monoterpene alcohols

- These oils have good antiseptic, anti-viral and anti-fungal properties with very few side effects such as skin irritation or toxicity and have an uplifting energizing effect.
- Examples of these alcohols are linalool, citronellol and terpineol found respectively in lavender, rose and geranium, and in juniper and tea tree oil.

Sesquiterpene alcohols

- These alcohols are not commonly found in essential oils, but when found, like bisabolol in German chamomile, have great properties, which include liver and glandular stimulant, anti-allergen and anti-inflammatory.
- Other oils that contain sesquiterpene alcohols are sandalwood (a-santalol) as well as ginger, patchouli, vetiver, carrot seed, everlasting and valerian.

Aldehydes

- These aldehydes have anti-fungal, anti-inflammatory, disinfectant, sedative yet uplifting therapeutic qualities and are the component that imparts the citrus-like fragrance in melissa, lemongrass and citronella. These properties are best used in aromatherapy when the essential oil is used in low dilutions - around 1%.
- Should oils high in this component be used, it could cause skin irritation and sensitivity as for instance lemongrass oil. Aldehydes are also unstable and will easily oxidize in the presence of oxygen and even low heat.

Ketones

- Although ketones can be toxic, as in the case of thujone found in thuja and wormwood oil as well as pinocamphone found in others, they also have some great therapeutic benefits - especially in the field of easing the secretion of mucus as well as cell and tissue regeneration.
- Other oils, such as hyssop, eucalyptus and rosemary have moderate amounts of ketones, and when used properly in aromatherapy can be greatly beneficial to the body.
- The ketone italidone found in everlasting, not only has the mucolytic (mucus easing) properties, but is also

useful in skin regeneration, wound healing and reducing old scar tissue such as in wounds, stretch marks and adhesions.
- Essential oils high in ketones need to be used with care in pregnancy.

Esters

- Esters are formed from alcohols and acids, and are named after both their original molecules with the alcohols dropping the "ol" and gaining an "yl" and the acids dropping the "ic" and gaining an "ate".
- The esters found in essential oils are normally very fragrant and tend to be fruity and their therapeutic effects include being sedative and antispasmodic. Some esters also have anti-fungal and anti-microbial properties - like the anti-fungal properties in geranium oil.
- The most well known ester must be linalyl acetate, which is found in lavender, clary sage as well as petitgrain.
- These components are normally gentle in their actions and can be used with great ease.

Lactones and coumarins

- Lactones contain an ester group integrated into a carbon ring system and coumarins are also types of lactones. There are similarities between the actions of lactones, coumarins and ketones since they also have some neurotoxic effects and can cause skin sensitizing and irritation.
- Yet the sesquiterpene lactone, called helenalin found in arnica oil, seems to be responsible for the anti-inflammatory action of arnica oil.
- The amount of lactones and coumarins normally found in essential oils is very low, and does not pose a huge problem. Lactones also have great mucus moving and expectorant properties and for this reason elecampane is often used in the treatment of bronchitis and chest complaints.
- Some coumarins, like furocoumarin - bergaptene - found in bergamot oil are severely skin UV sensitive and should be used with great care should you be exposed to sunlight. For more info onsun sensitizing (phototoxic) oils, please click here.

Ethers

- Phenolic ethers are the most widely found ethers in essential oils with anethol found in aniseed, the only real ether of importance together with methyl chavicol found in basil and tarragon.

Oxides

- The main therapeutic effect of oxides are that of expectorant, with 1,8-cineole - commonly known as eucalyptol being the most well known. The above is a brief overview of the chemical components found in essential oils, and should not be seen as a lesson in organic chemistry, but was merely included for the sake of interest. If you are interested to view

Plant Profile

Ginger

- Ginger consist of whole or cut, dried scrapped or un scrapped rhizomes of *zingiber officinale* Roscoe
- It consist 1 - 4% of volatile oil

Drug Profile

Kingdom - Plantae
Odour - Agreeble and aromatic
Genus - Zingiber
Family - Zingiberaceae
Clade - Angiosperm
Species - Z. officinale

Fig. 1. Ginger Rhizome with Buds

Numerous active ingredients are present in ginger including terpenes and oleoresin which called ginger oil. Ginger also constitutes volatile oils approximately 1% to 3% and non-volatile pungent components oleoresin. The major identified components from terpene are sesquiterpene hydrocarbons and phenolic compounds which are gingerol and shogaol (8) and lipophilic rhizome extracts, yielded potentially active gingerols, which can be converted to shogaols, zingerone, and paradol. An glabrous or minutely pubescent, branched annual, up to 90 cm., tall, cultivated almost throughout India. Stems striate; leaves rather distant, 2-3 pinnately divided, segments linear, ultimate segments 1.0-2.5 cm. long; flowers in terminal or seemingly-lateral pedunculate, compounds umbels, white, small; fruits ovoid, muricate, aromatic cremocrps, 2-3 mm. long, grayish brown; mericarp compressed, with distinct ridges and tubercular surface, 1-seeded. Flowers and fruits from January-April.

Ajwain

- It consists of dried ripe fruits of the plant *Trychyspermum ammi* Sprague
- It contains 2-4 % of volatile oil.

Drug Profile

Kingdom - Plantae
Clade - Angiosperm
Odur - Agreeble
Family - Apiaceae
Genus - Trychyspermum
Species - T. ammi

Medicinal plants are used to maintain and promote healthy life, prevent disease and cure ailments.

Fig. 2. Ajwain fruits

It has been estimated that even today, 80% of the world population rely on herbal traditional medicine for their primary health care, *Trachyspermumammi* which is commonly known as Ajowan. Among traditional potential herbs used as spice in day to day life, ajwain (*Trachyspermum ammi* L.) belonging to family Apiaceae, is widely used for curing various diseases in both humans and animals. *Trachyspermum ammi* were analyzed for the presence of various phytoconstituents like carbohydrates, reducing sugars, monosaccharide, Tannin Saponnins, Flavonoid, Terpenes,/ steroids, Alkaloids, Anthraquinones, cardiac glucosides proteins.

Black Pepper

- Pepper is the dried unripe fruit of perennial climbing vine *Piper nigrum* Linn.
- It contain 1 – 2.5% volatile oil

Drug Profile

Kingdom – Plantae
Clade – Fruits
Family – Piperaceae
Odour – Piperels
Genus – Piper
Species – P. nigrum

Black pepper: (*Piper nigrum*) is a flowering vine in the family *Piperaceae*, cultivated for its fruit, which is usually dried and used as a spice and seasoning. When dried, the fruit is known as a peppercorn. When fresh and fully mature, it is approximately 5 mm (0.20 in) in diameter, dark red, and, like all drupes, contains a single seed. Peppercorns, and the ground pepper derived from them, may be described simply as pepper, or more precisely as black pepper (cooked and dried unripe fruit), green pepper (dried unripe fruit) and white pepper (ripe fruit seeds).

Phytochemical Analysis

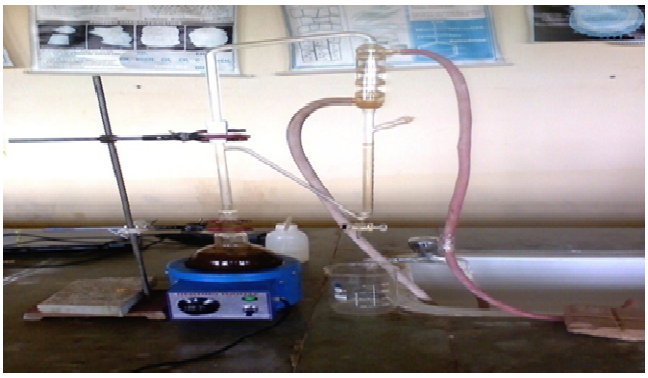
- **Test for Saponins:** Take 2 gm of the powered sample was boiled in 20 ml of distilled water in a water bath. Add 10 ml of the filterable was mixed with 5 ml of distilled water shaken vigorously for a stable persistent froth. Then mixed with 3 drops of Olive oil and shaken vigorously. Then observed for the formation of emulsion.
- **Test for Tannins:** A quantity of 0.5 gm of the dried powdered sample was boiled in 20 ml of water and filtered. A few drops of 0.1%Ferric chloride was added and observed for brownish or bluish black color.
- **Test for Alkaloids (Meyer's test):** A quantity of 0.5 g of the dried powdered sample was boiled in 20 ml of water and filtered. To a few drops of the filtrate, a drop of Meyer's reagent was added by t1 side of the test tube. A creamy or white precipitate indicates the test is positive.
- **Test for Flavonoids:** A portion of the powdered sample was heated with 10 ml of Ethyl acetate over a steam bath for 3 minutes and then mixture was filtered. 4ml of the filtrate was shaken with diluted Ammonia. Yellow coloration indicates the presence of flavonoid.
- **Test for Cardiac Glycosides:** Take 0.5 g of the extract was dissolved in 2 ml of glacial acetic acid containing 1 drop of 1% FeCl3. This was under laid with conc. H2SO4. A brown ring obtained at the interface indicated the presence of a deoxy sugar, characteristic of cardiac glycosides. A violet ring may appear below the ring while in the acetic acid layer; a greenish ring may form just above ring and gradually spreads throughout this layer.

Preparation of plant extract by soxhlet apparatus

The plant material were cleaned and the dust particle were remove and crushed with the help of electronic blinder, grainded plant material was subject to soxhlet apparatus employing ethanol 95% as a solvent and temperature was maintained.after completion of extraction .it was filtered solvent was remove residue was obtained and it was store in a desiccators.

Separation of volatile oil by Clevenger Apparatus

Take 100gm of powdered or crushed form drug with 250ml. of water in distillation flask. Add a few pieces of porcelain to it (to avoid bumping during distillation.) For heating the flask heating mantle can be used. The flask at intervals and shake the contents until the liquid are boiling stability. Continue heating till no more oil collects.



(This requires two hours or more) Turn out the gas and allow the liquid in the condenser to drain for 5 or 10 minutes then read the volume of oil. Express the result as a volume in weight percentage. The crude oil, thus produced is rectified again after treatment with sodium hydroxide. The filtered and filled in suitable container.

Antimicrobial Activity

Sterilization: First step to sterilize the glass apparatus & other equipments. Glass apparatus are sterilized using hot air oven while gloves are sterilized by using moist air sterilization in autoclave.

Test Organisms

In this project were used references strains of food borne microorganisms involved in food toxi-infections. The selected test organisms used to evaluate antimicrobial activity of the essential oil were as follows: Pathogen bacteria: Gram positive (*staphylococcus aureus, bacillus subtilis),* Gram negative (*klebsiella oytoca)* was brought from Department of Biotechnalogy Guru Ghasidas University, koni, bilaspur, (C.G.)

Preparation of culture medium

The culture medium is prepared and medium used was nutrient agar medium. Composition of nutrient agar as is follows:

Table

S.no.	Chemical	Quantitity
1.	Beef extract	10.00gm
2.	Peptone	10.00gm
3.	Sodium chloride	05.00gm
4.	Agar	15.00gm
5.	Distilled water	1000.00ml (q.s.)

Weigh accurately the ingredient and dissolve them with the aid of heat till a homogeneous solution is obtained .Adjust to pH 8.0 to 8.5 using 5M sodium hydroxide. Filter if necessary in some amount of hot water agar is added with continuous stirring .Then add this to the nutrient both and mixed properly. Then sterilize it by autoclaving at 115°C for 30 minutes.

In antimicrobial activity testing vitro

Because it appears that no standardized test has been developed for evaluating the antibacterial activity of possible preservatives against food-related bacteria, the CLSI (Clinical and Laboratory Standard Institute) method for antibacterial susceptibility testing has been modified for testing essential oils. We used preliminary methods agar well diffusion method for detecting the most efficient oils against test organism.

Agar well diffusion method

Test solution of each sample were prepare at the concentration of (100mg/ml). Azithromycin was taken as standard for antibacterial activity at a concentration of (10mg /ml). Specified media were prepared and sterilized by an autoclave. For the antimicrobial activity, the media was inoculated with the test organism (one day old sub culture) by uniform mixing and was poured into a sterile Petridis to a uniform depth and them allowed to cool & solidified at room temperature in an aseptic room. This provided a uniform surface for the growth of bacterium and was used antibacterial activity studies. Then the wells were made in solidified media with the help of sterile glass borer of 8mm diameter in such a way that there was no overlapping of zone of inhibition Plate were kept at room temperature for ½ an hour's for diffusion of the sample into agar media. The organism inoculated Petridis were incubated in specified temperature for specified time. After the incubation period of over, the zone of inhibition produced by the sample in different plates were measured and recorded immediately.

RESULT AND DISCUSSION

Comparative in-vitro antibacterial activity of volatile oils was performed by various methods such as photochemical screening, GC-MS Analysis, antibacterial activity has been done.

A. Phytochemical Screenin

Ginger

Sr. No.	Test	Phytochemical Compound	Result
1	Molish's test	Carbohydrate	Present
2	Iodine test	Starch	Present
3	Brontranger' test	Antroquinone Glycoside	present
4	Salkowoski test	Terpenoids	Present
5	Liebermann batchad	Steroids	Present
6	Mayar's test	Alkaloids	Present
7	Shinoda's test	Flavonide	Present

Ajwain

Sr No.	Phytochemical Compound	Test	Result
1	Carbohydrate	Molish's test	Present
2	Reducing sugar	Fehling test	Present
3	Tannins	Ferric chloride	Present
4	Saponnins	Frothing test	Absent
5	Flavonide	Shinoda's test	Present
6	Amino acid	Ninhydrin test	Absent

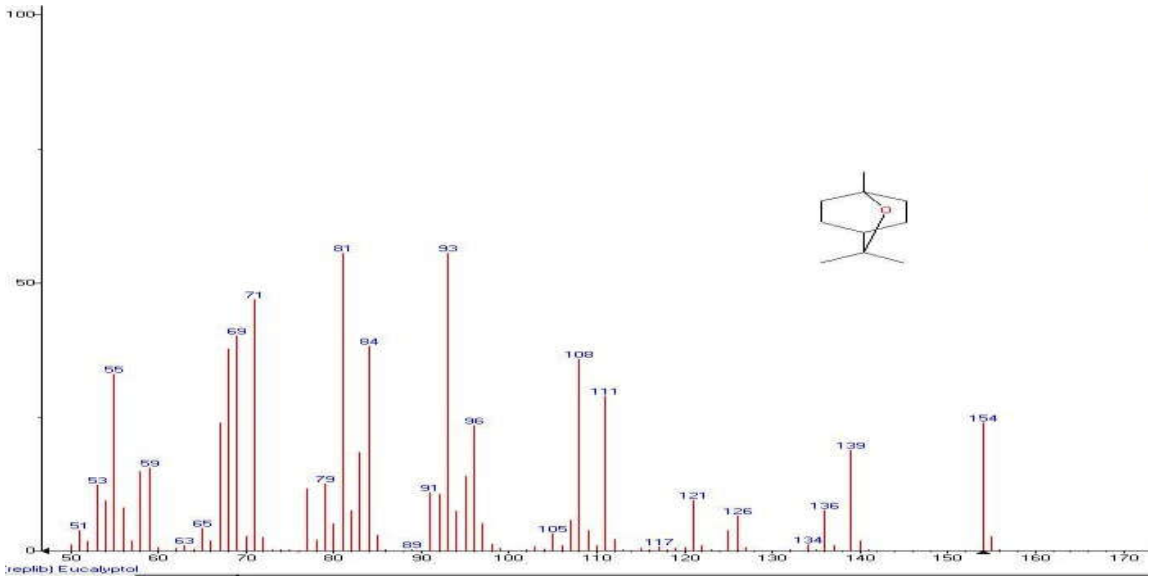
Black pepper

Sr. No.	Phytochemical Compound	Test	Result
1	Saponnins	No Frothing	Absent
2	Tannins	Brownish green colour was appeared	Present
3	Alkaloids	Creamy white pept was apeared	Present
4	Flavonide	No yellow colour	Absent
5	Cardiac glycoside	Greenish and white ring apeared	Present
6	Phenalic compound	No dark green colour	Absent

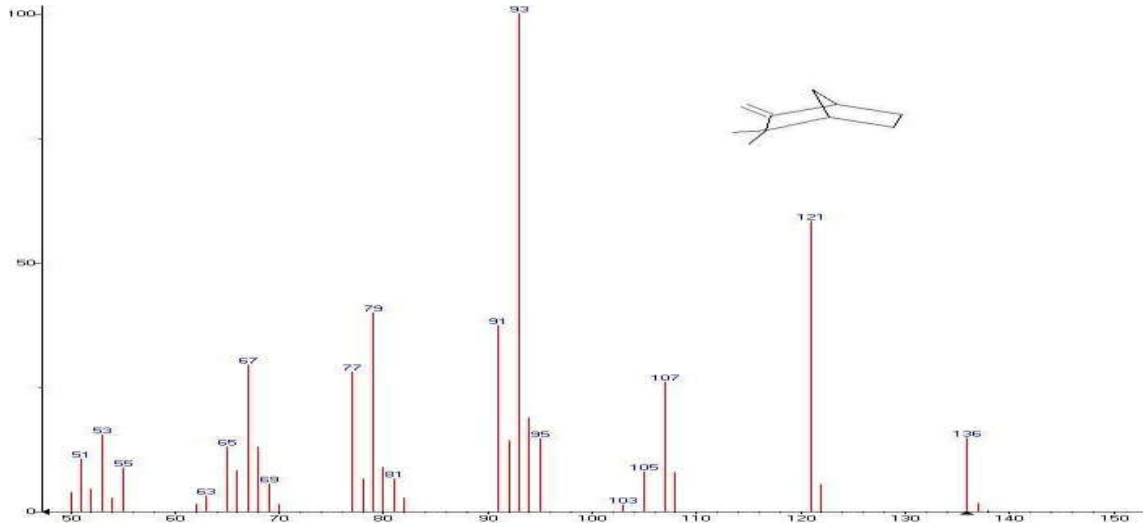
B. GC-MS Study OF Ginger

GC/MS study was carried out for the volatile oil of Ginger, ajwain and black pepper and the various compound present in the volatile oils was found show in graph:-

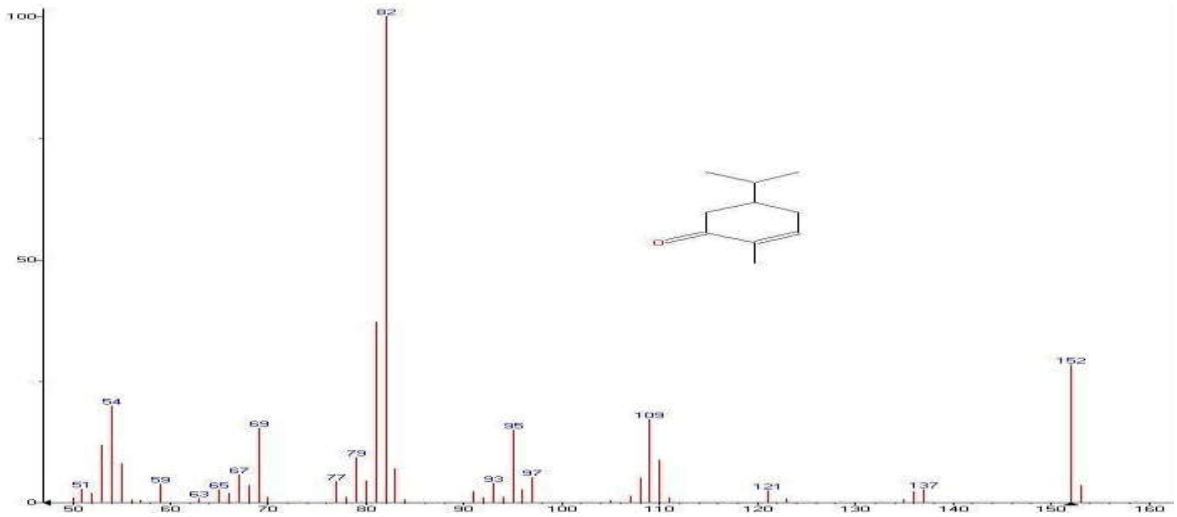
1. **NAME-Eucalyptol ($C_{10}H_{18}O$)**



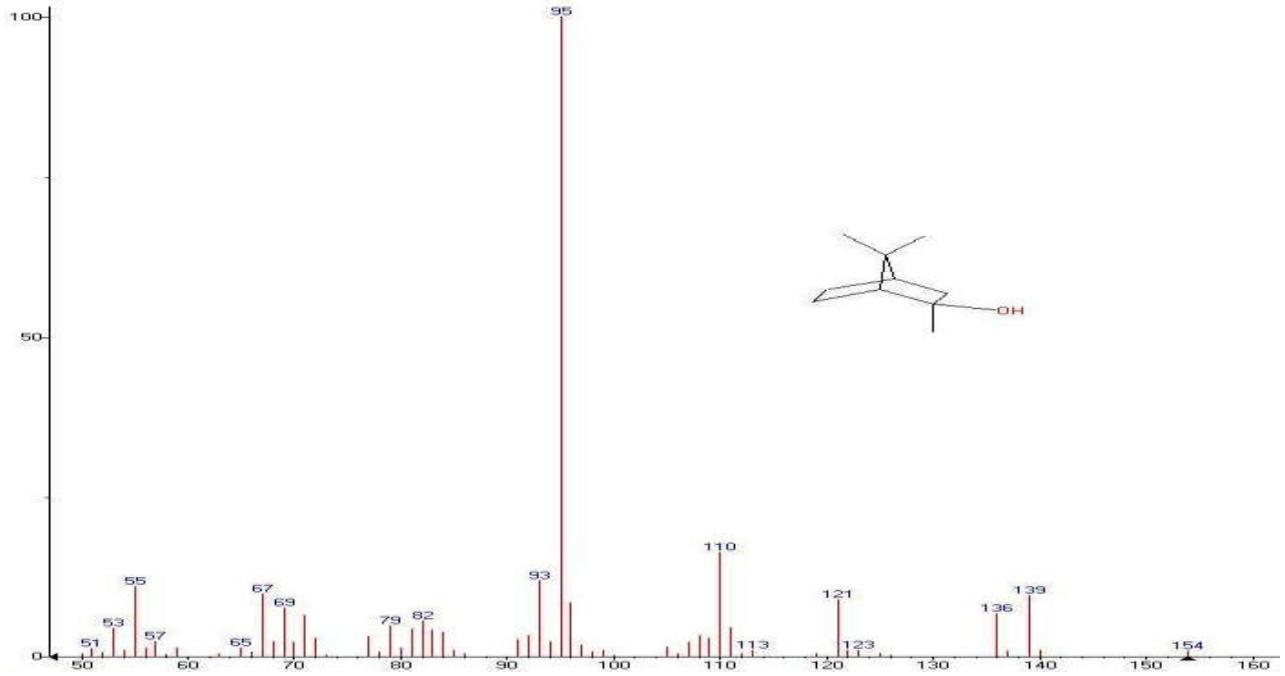
2. NAME-Camphene ($C_{10}H_{16}$)



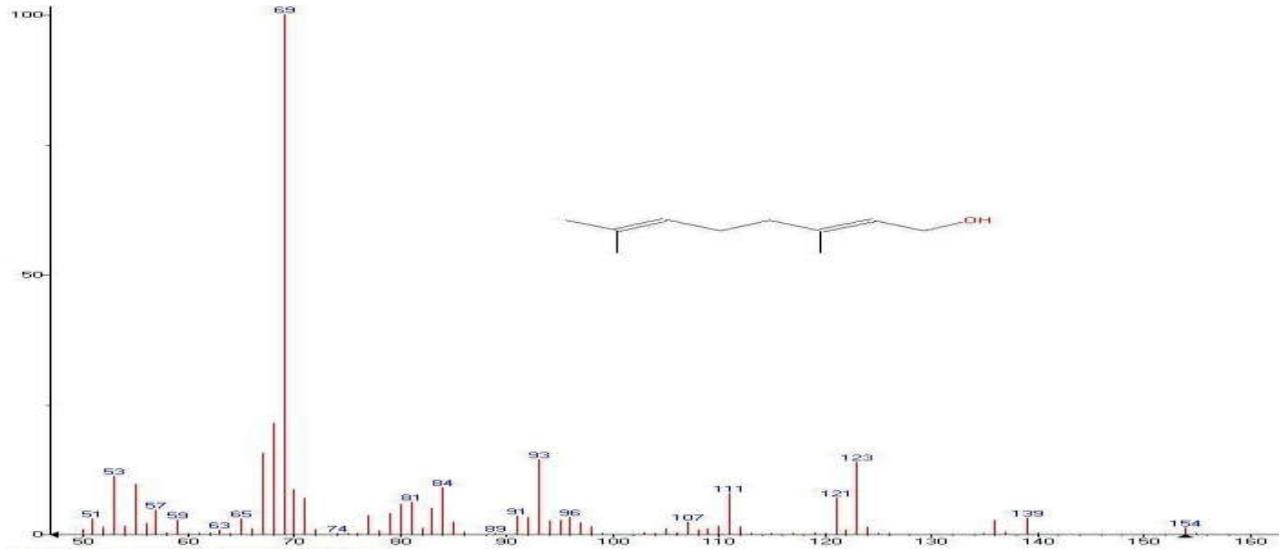
3. NAME -Borneol($C_{10}H_{18}O$)



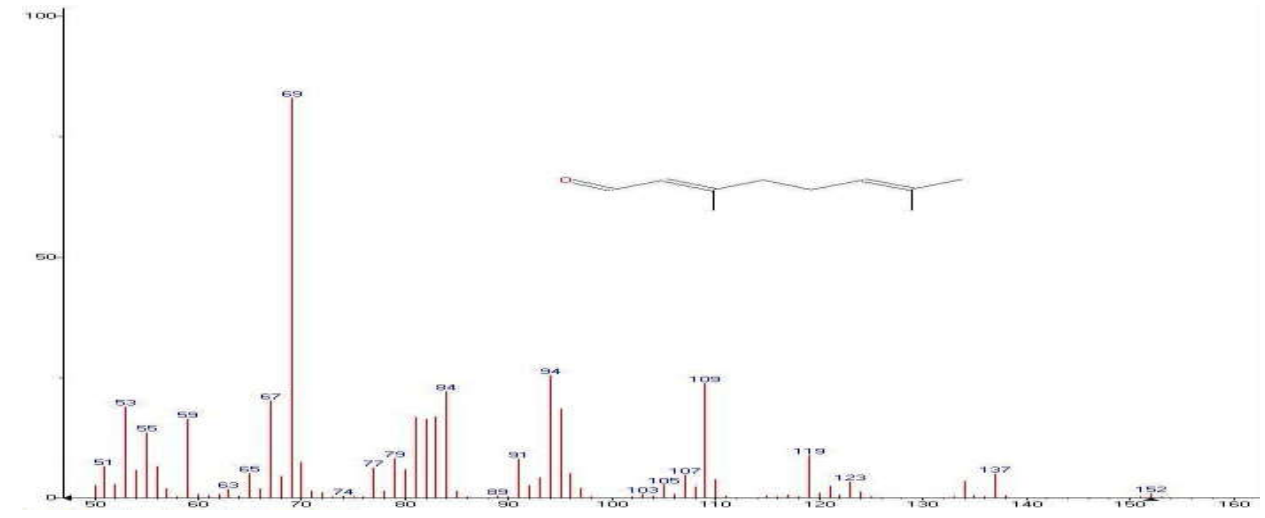
4. NAME – Guaniol ($C_{10}H_{18}O$)



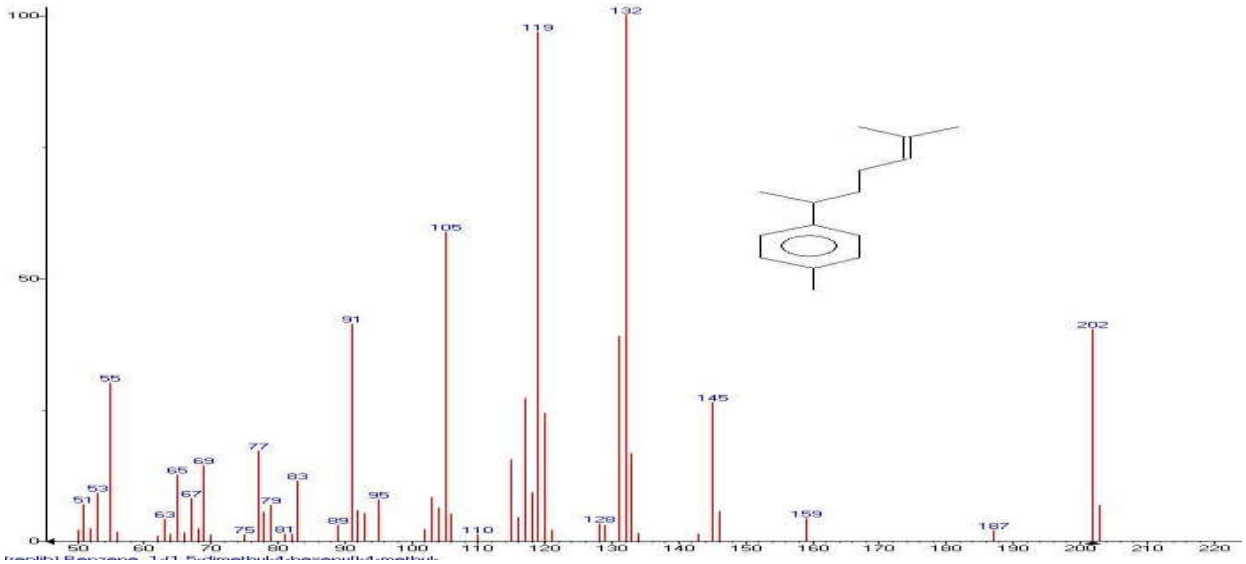
5. NAME- β-CITRAL($C_{10}H_{16}O$)



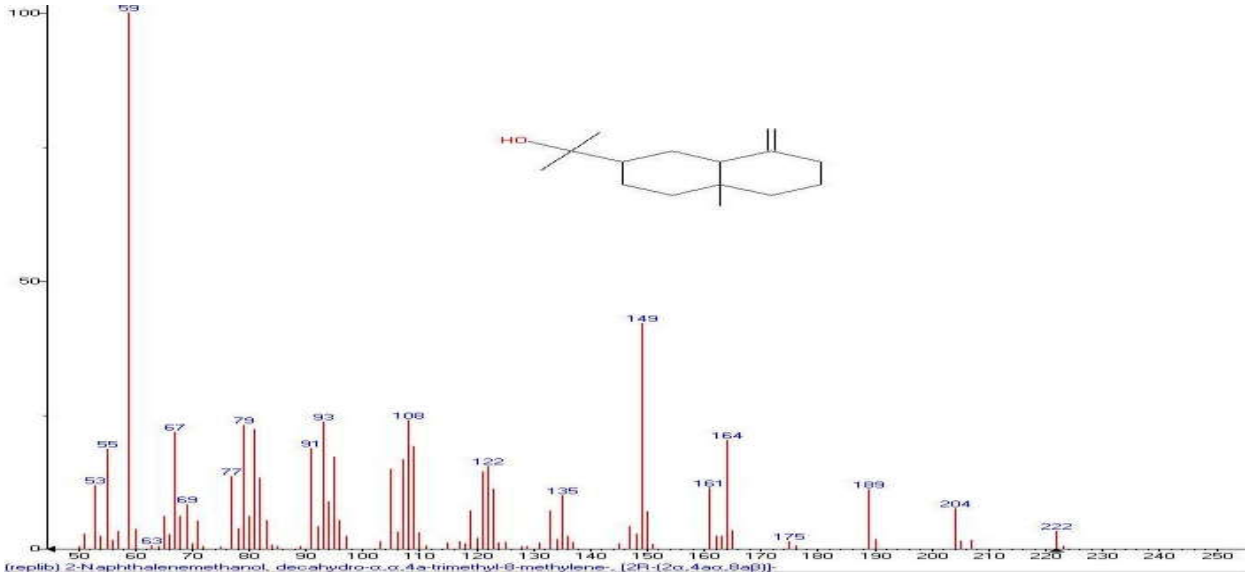
6. NAME - CURCUMENE($C_{15}H_{22}$)



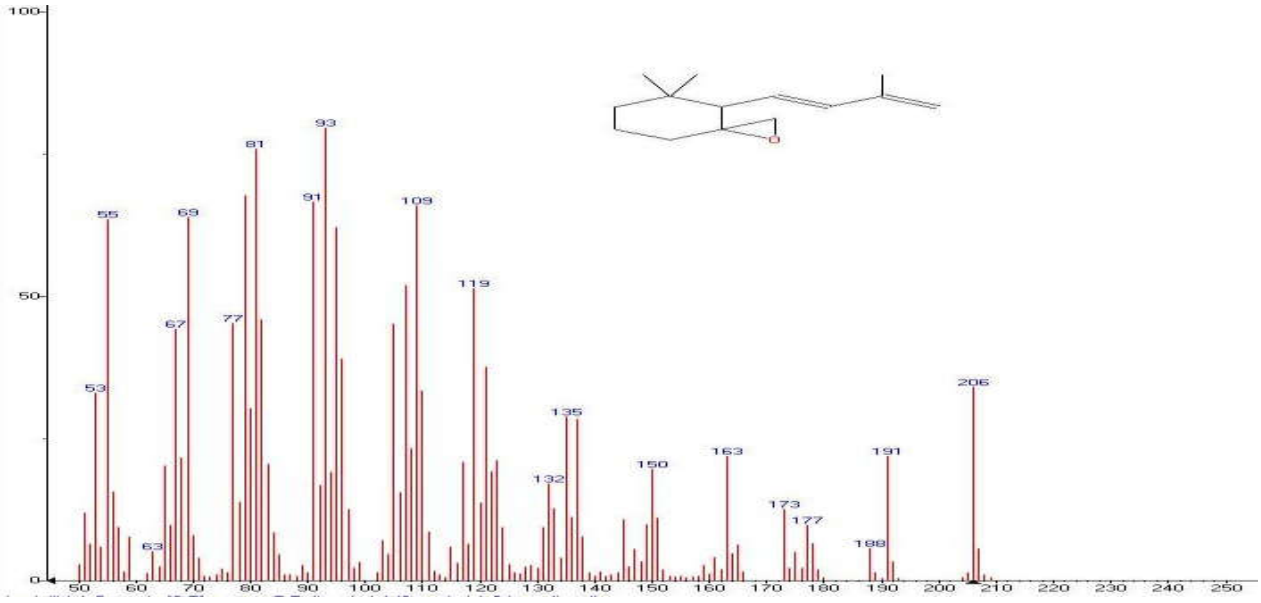
7. NAME –EUDESM-4 (14)-en-11-ol ($C_{15}H_{26}O$)



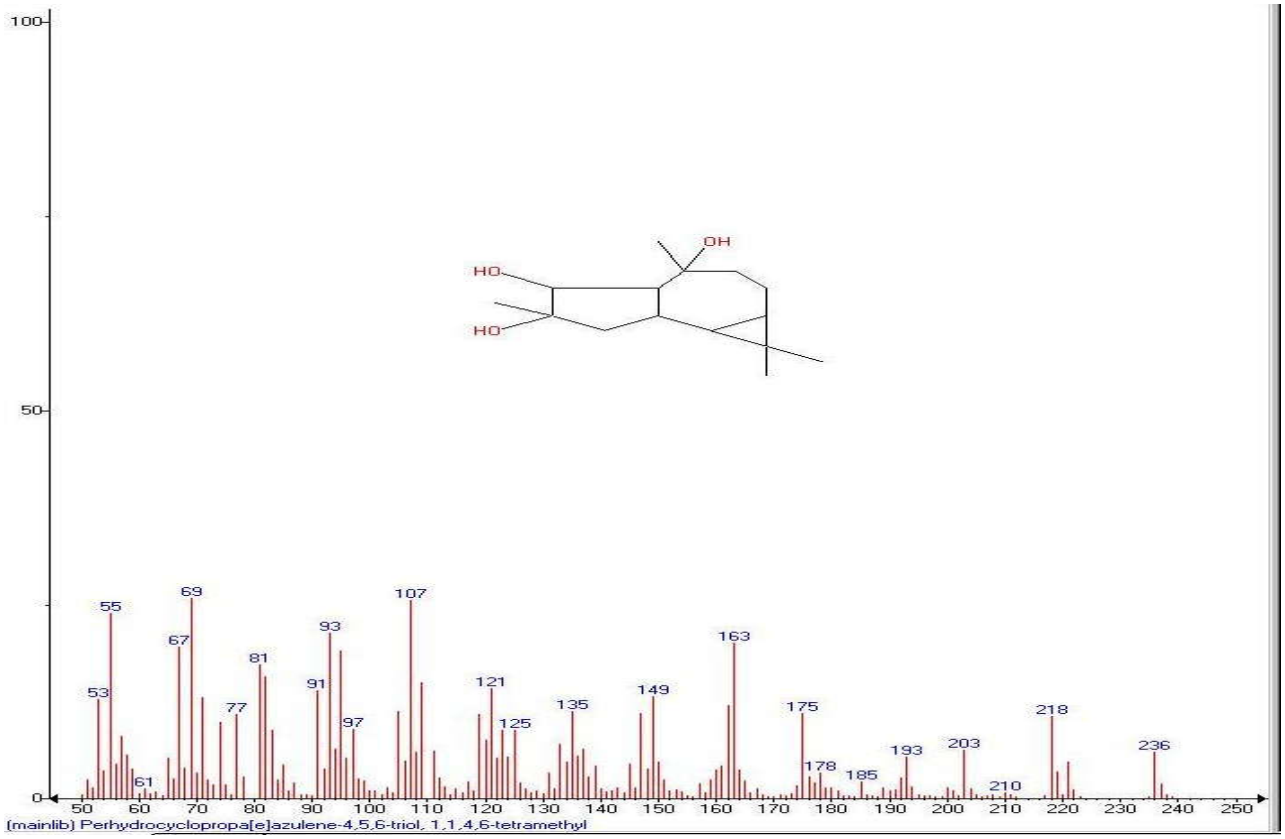
8. NAME -1oxapiro(2,5)octane,5,5-dimethyl-4-(3-methyl-1,3 butadienyl)($C_{14}H_{22}O$)



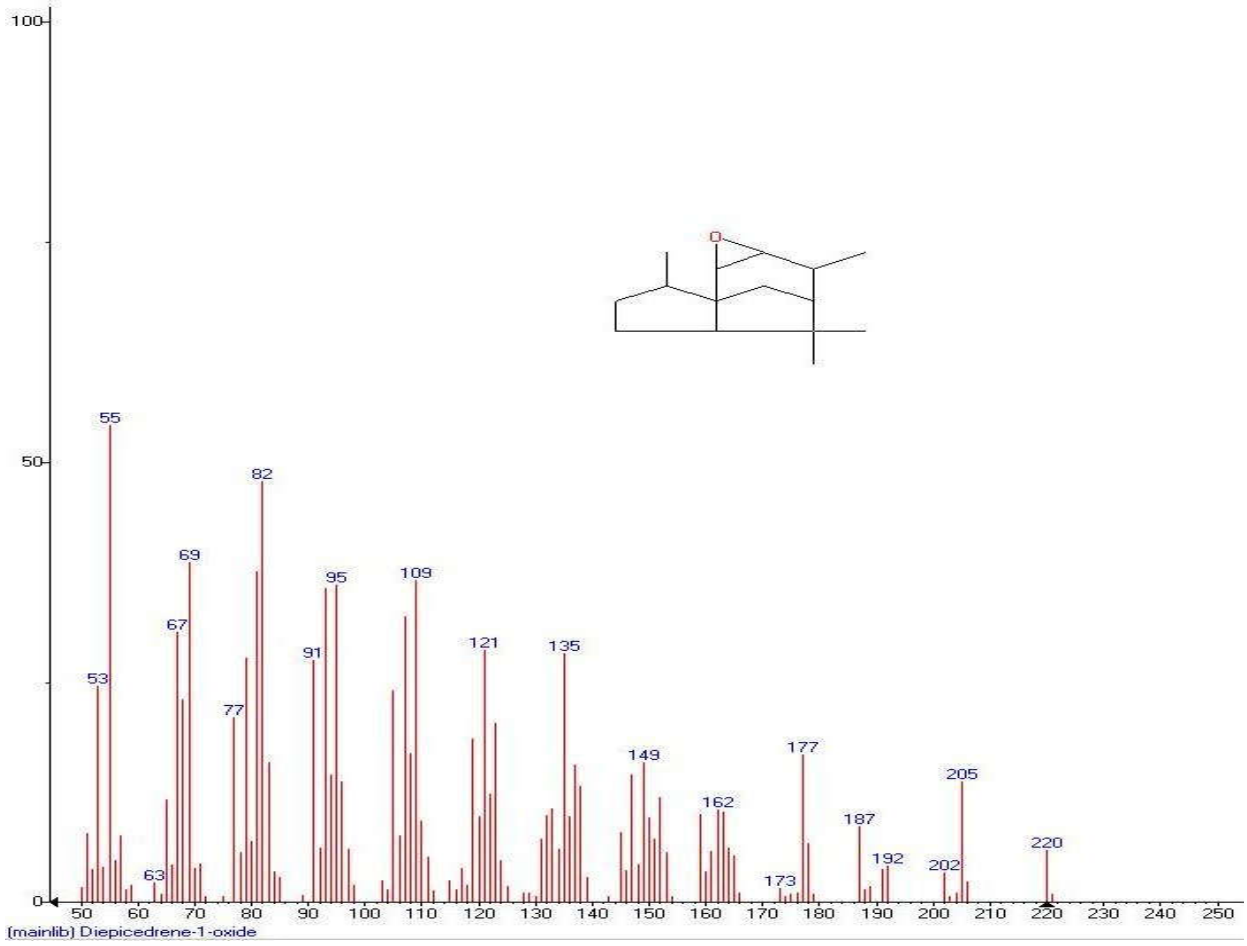
9. NAME –Perhydrocycloprope(e)azulene4,5,6-trid,1,1,4,6 tetramethyl.($C_{15}H_{26}O_3$)



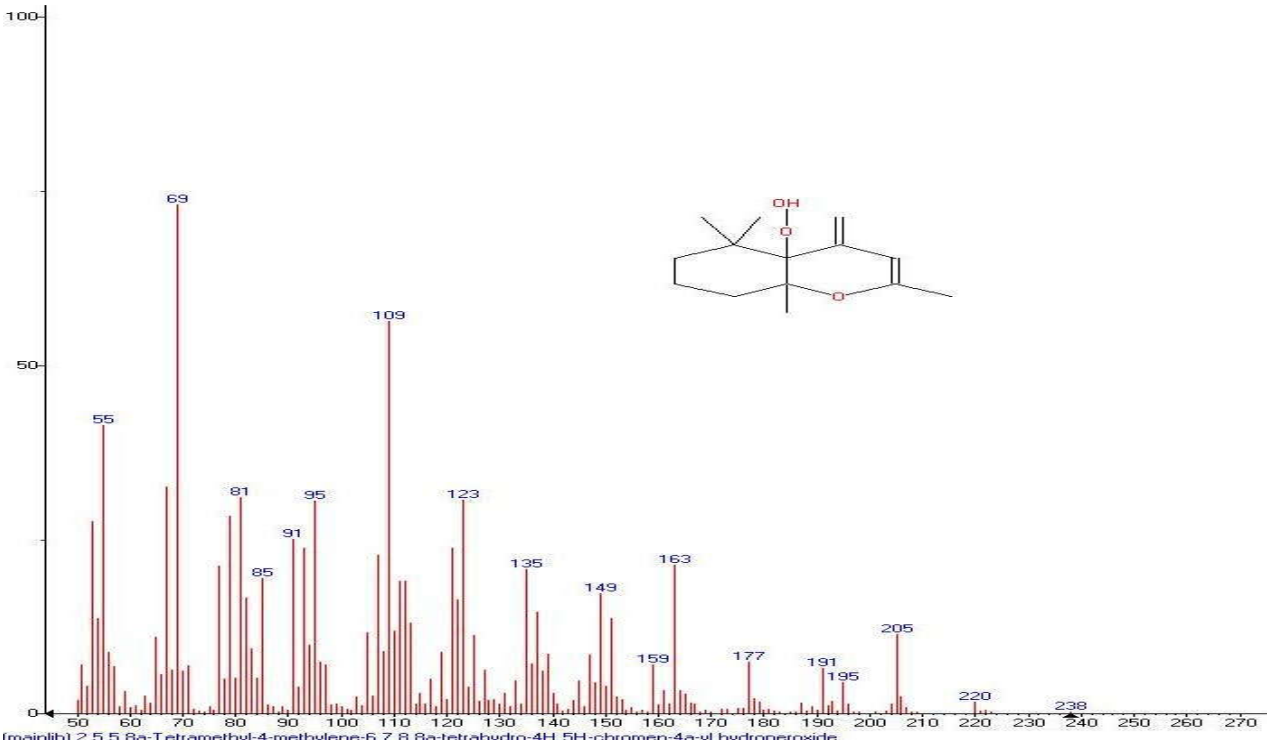
10. NAME –Diepicedrene-1-0xide ($C_{15}H_{24}O$)



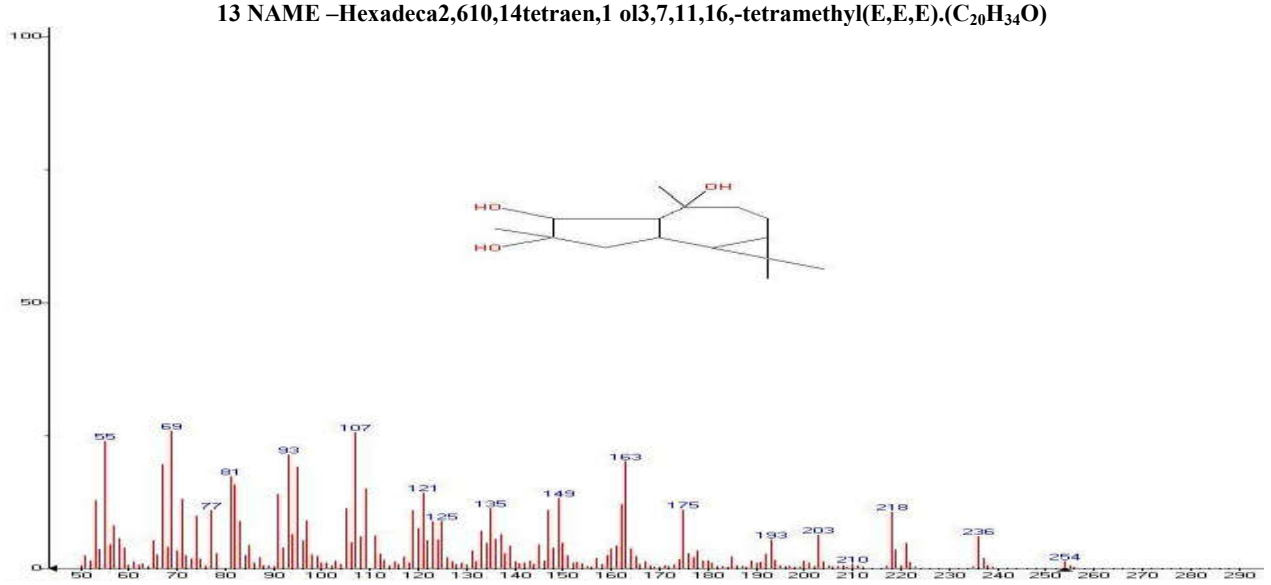
11. NAME –2,5,5,8a-tetramethyl-4methylene6,7,8,8a-tetrahydro-4H,5H-Chronen-4a-yl ($C_{14}H_{22}O_3$)



12. NAME –Perhydrocycloprope(e)azulene-4,5,6-triol,1,1,4,6-tetramethyl($C_{15}H_{26}O3$)

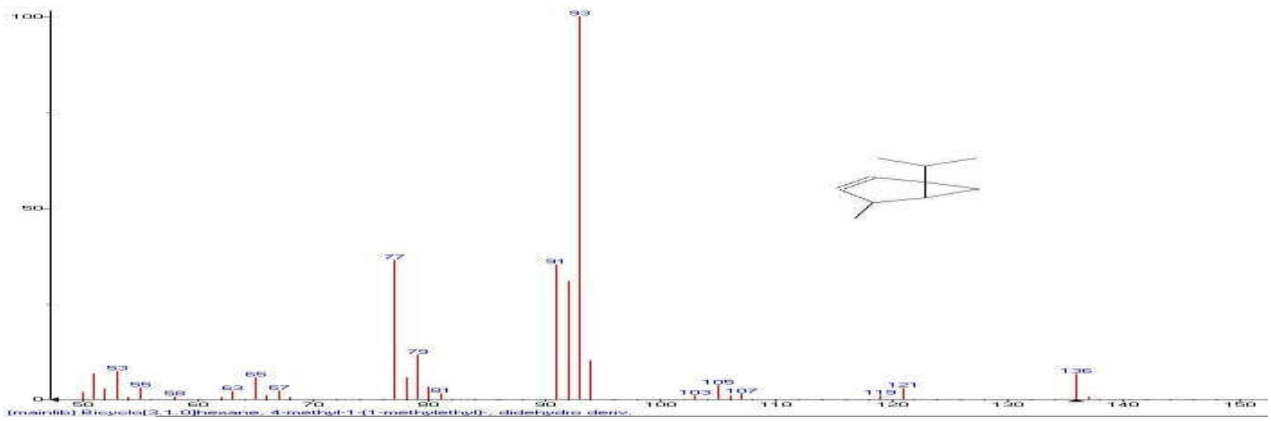


13 NAME –Hexadeca2,610,14tetraen,1 ol3,7,11,16,-tetramethyl(E,E,E).($C_{20}H_{34}O$)

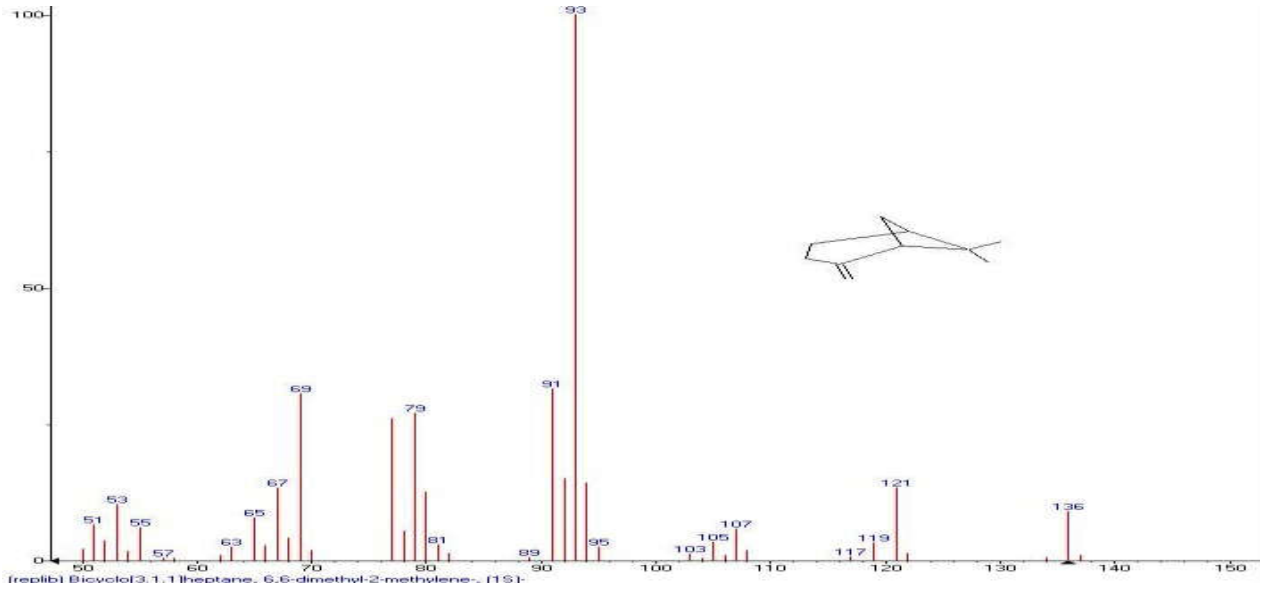


GC-MS STUDY OF AJWAIN

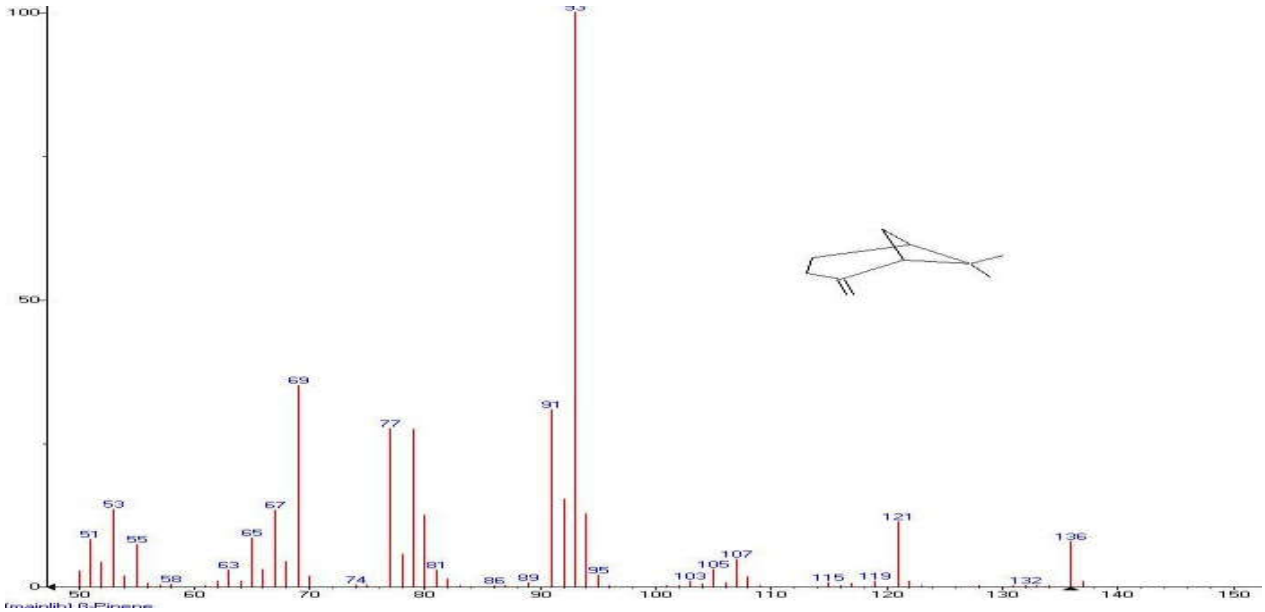
1. 1-Isopropyl- 4methylbicyclo 3, 1, 0 hexane-($C_{10}H_{16}$)



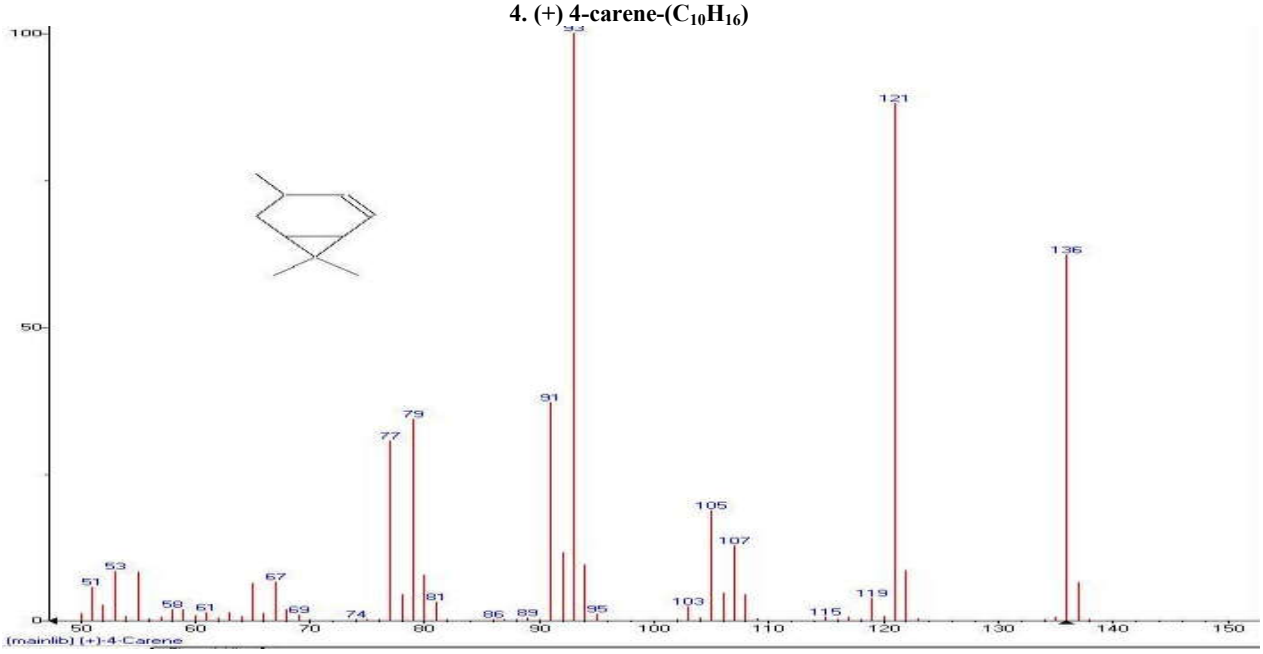
2. β- pinene -($C_{10}H_{16}$)



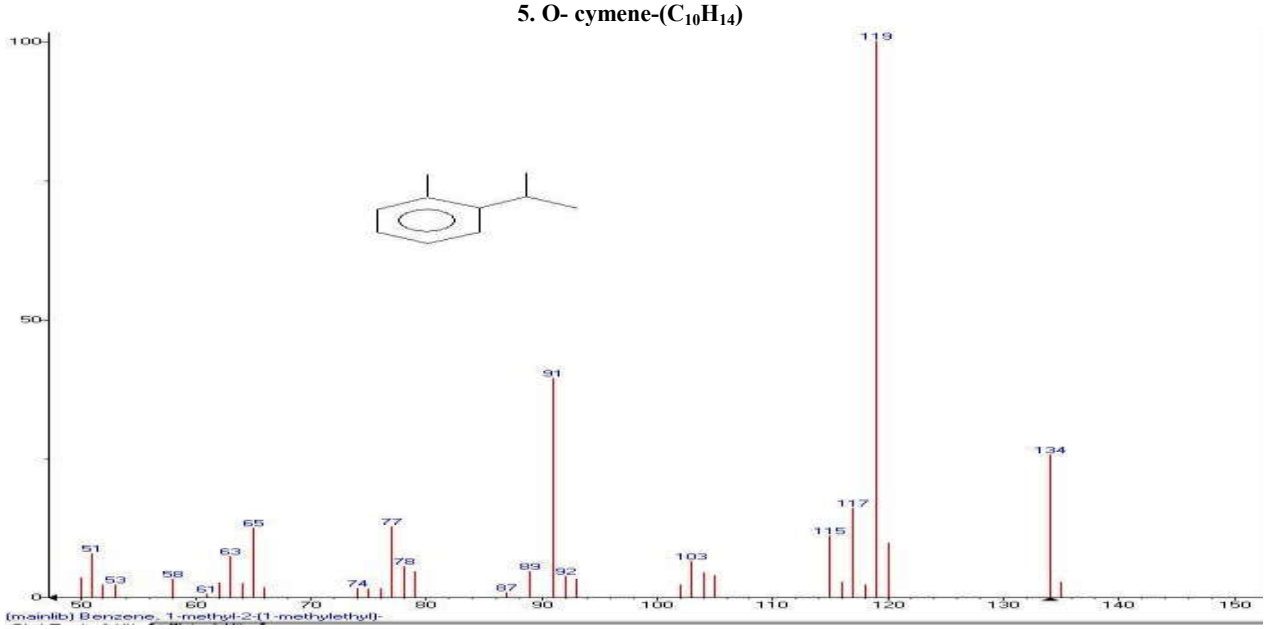
3. Noeinen -($C_{10}H_{16}$)



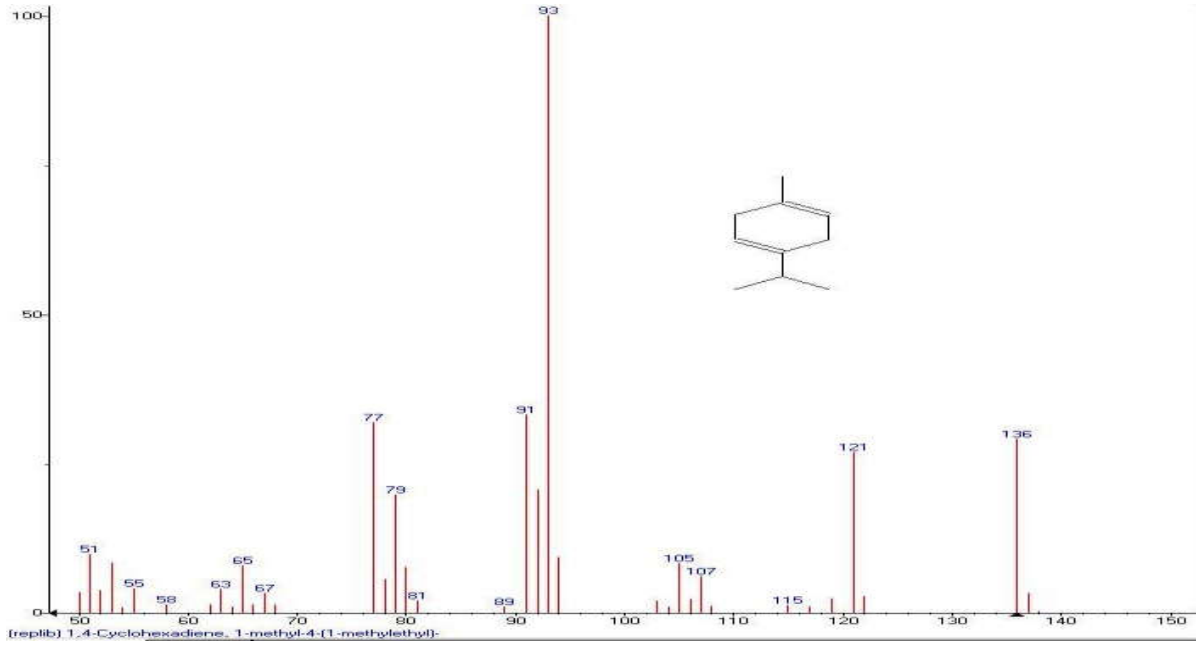
4. (+) 4-carene-($C_{10}H_{16}$)



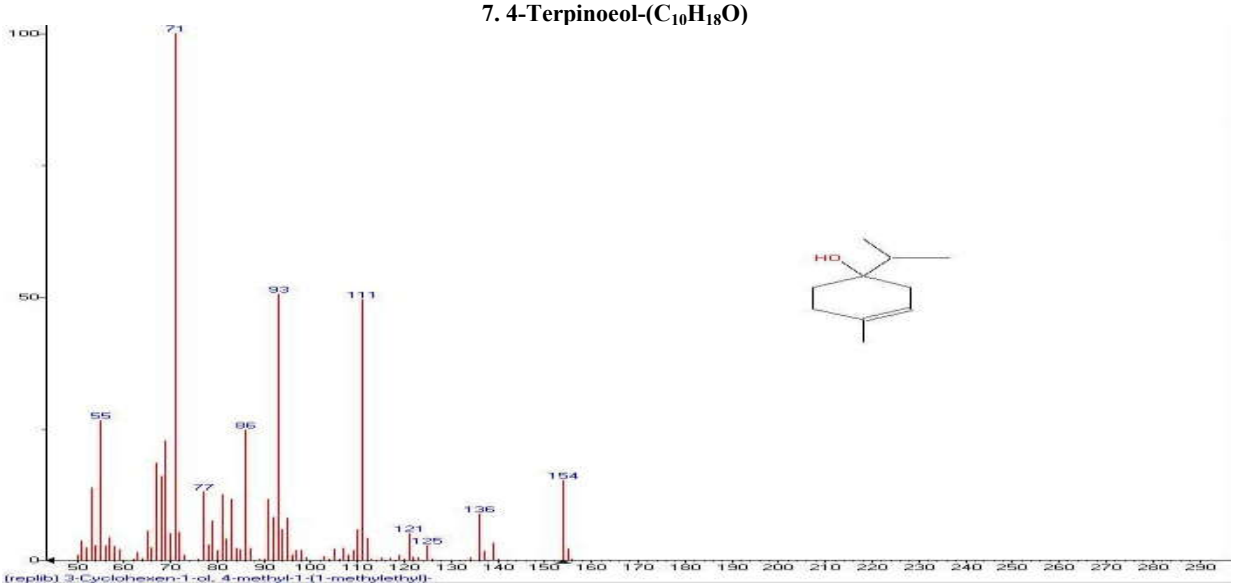
5. O- cymene-($C_{10}H_{14}$)



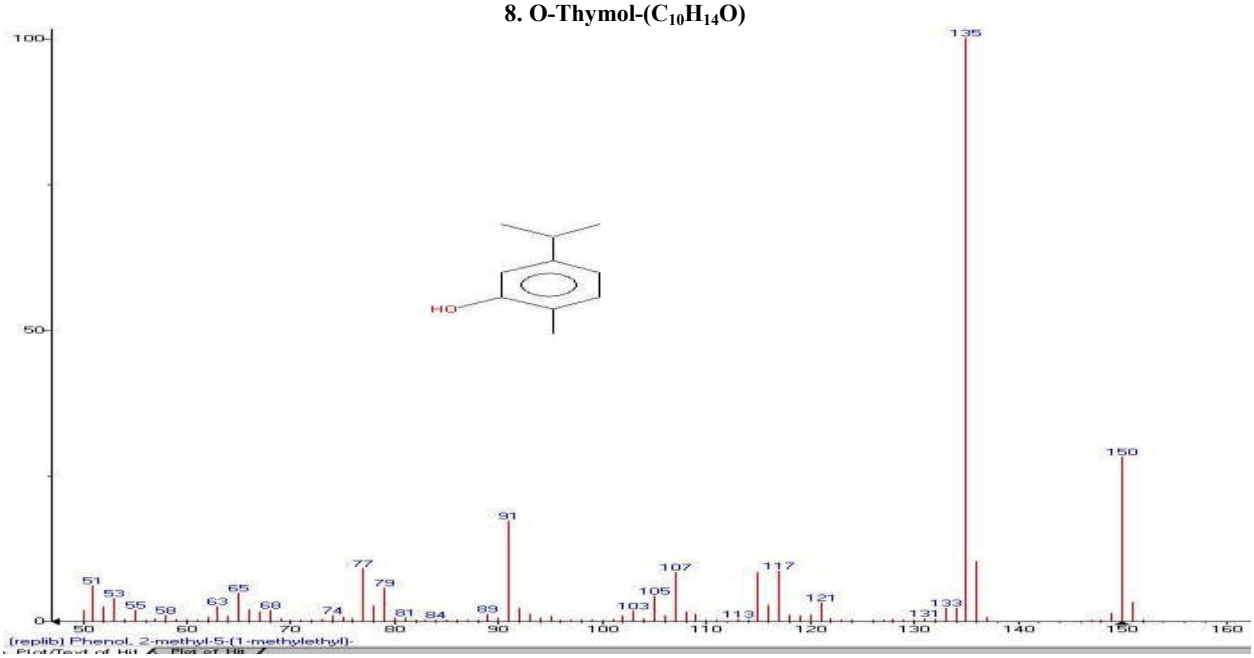
6. Moslene-($C_{10}H_{16}$)



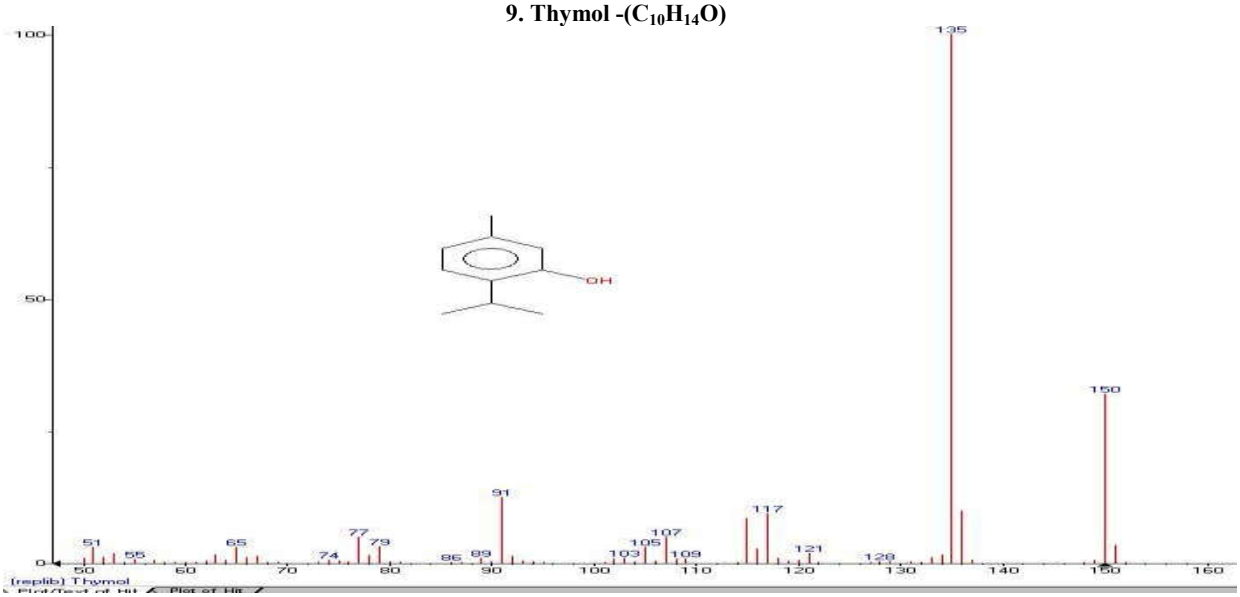
7. 4-Terpinoeol-($C_{10}H_{18}O$)



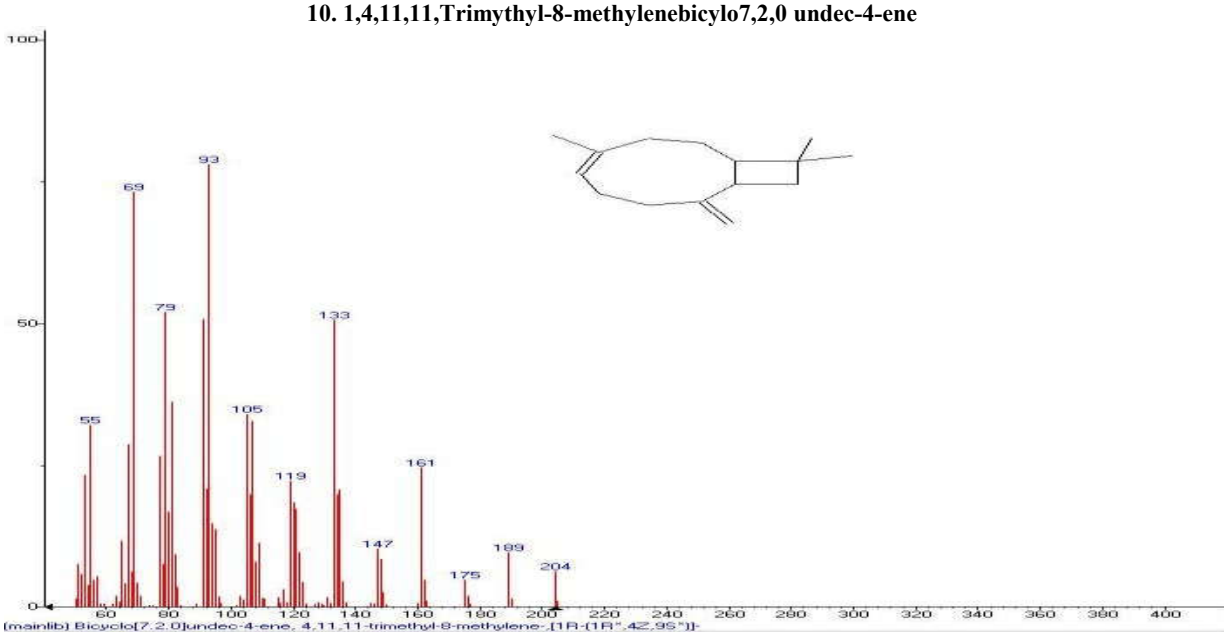
8. O-Thymol-($C_{10}H_{14}O$)



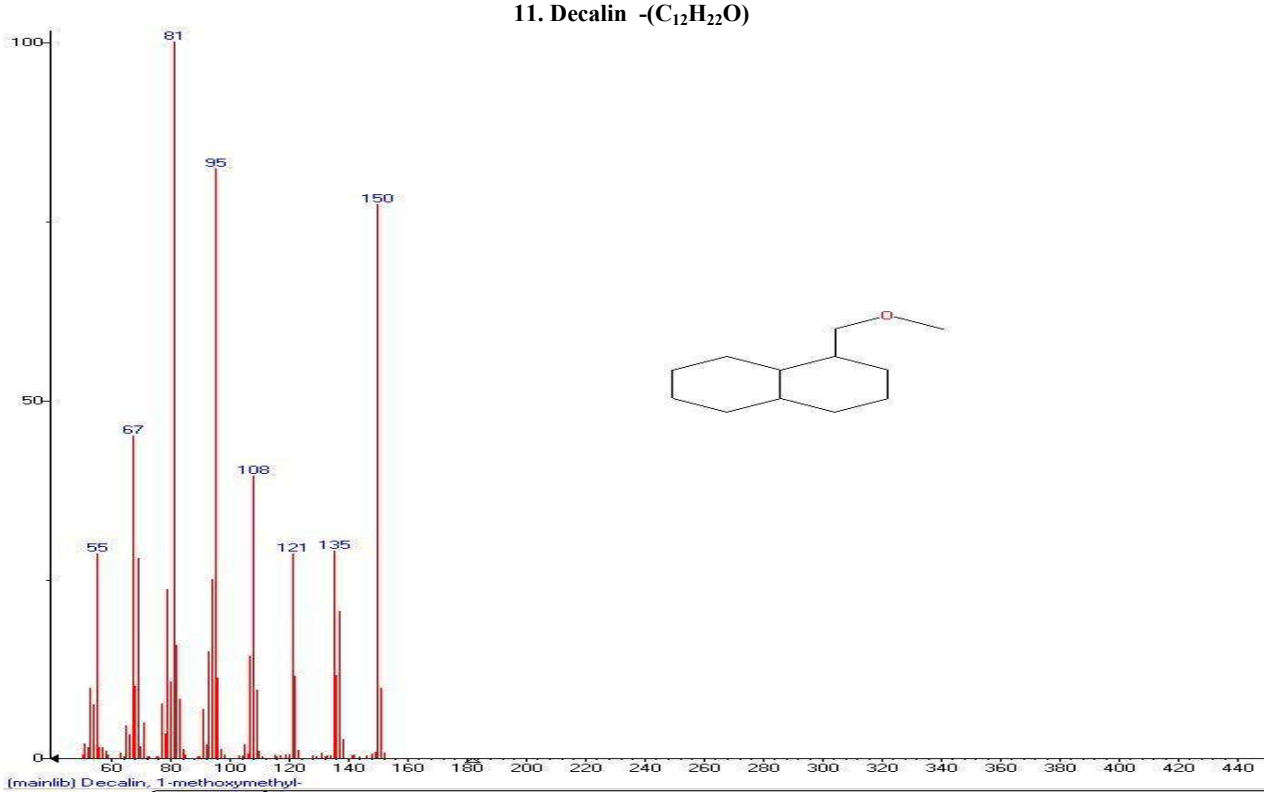
9. Thymol -($C_{10}H_{14}O$)



10. 1,4,11,11,Trimythyl-8-methylenebicylo7,2,0 undec-4-ene

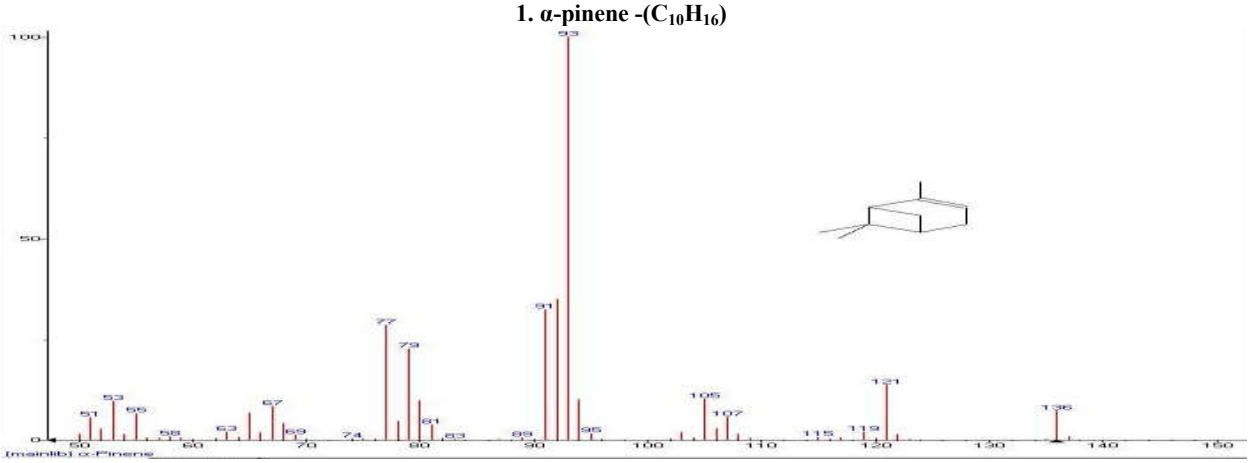


11. Decalin -($C_{12}H_{22}O$)

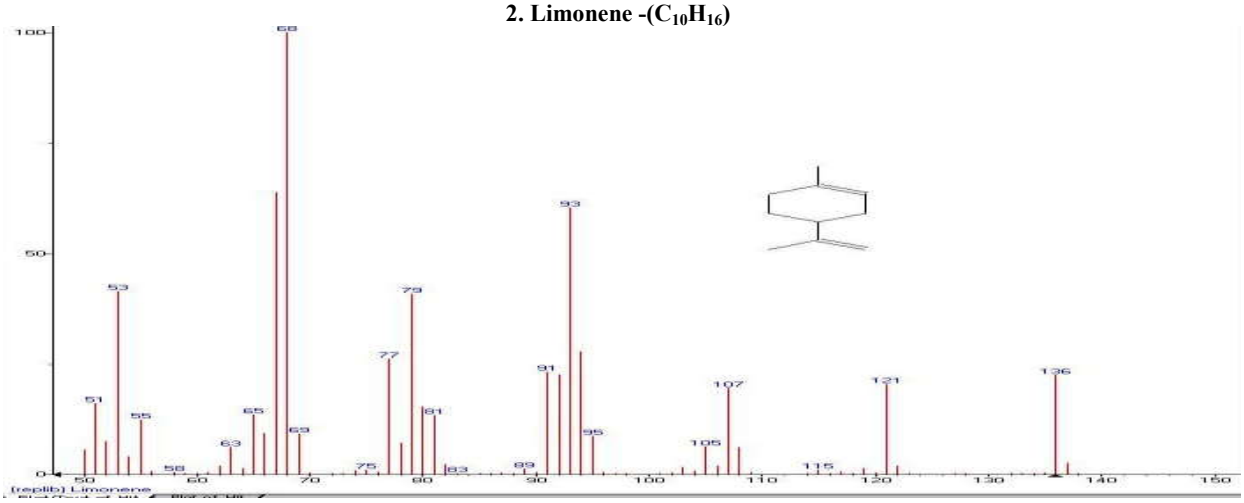


GC-MS STUDY OF BLACK PEPPER

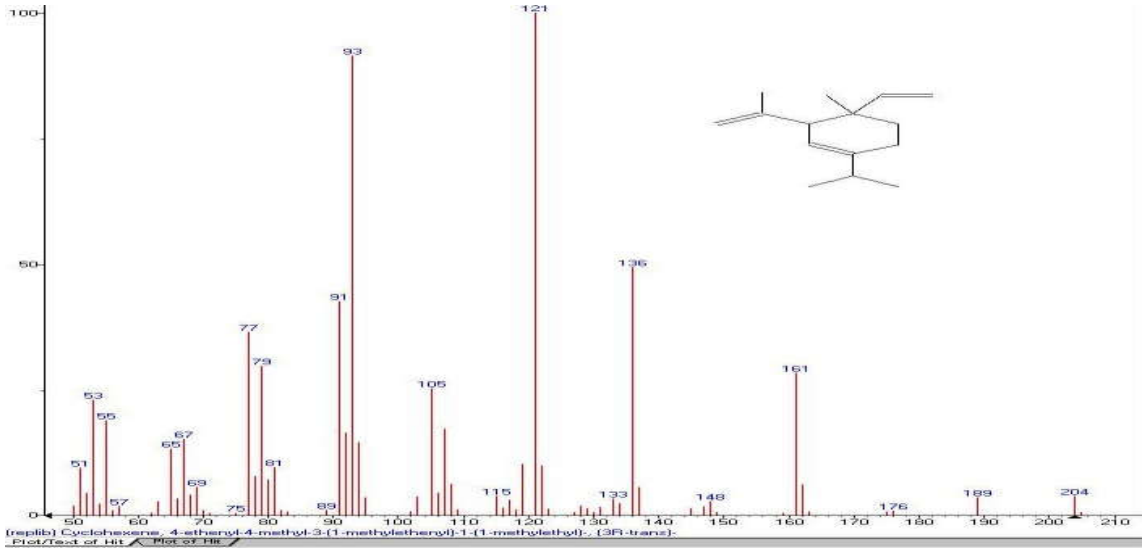
1. α-pinene -($C_{10}H_{16}$)



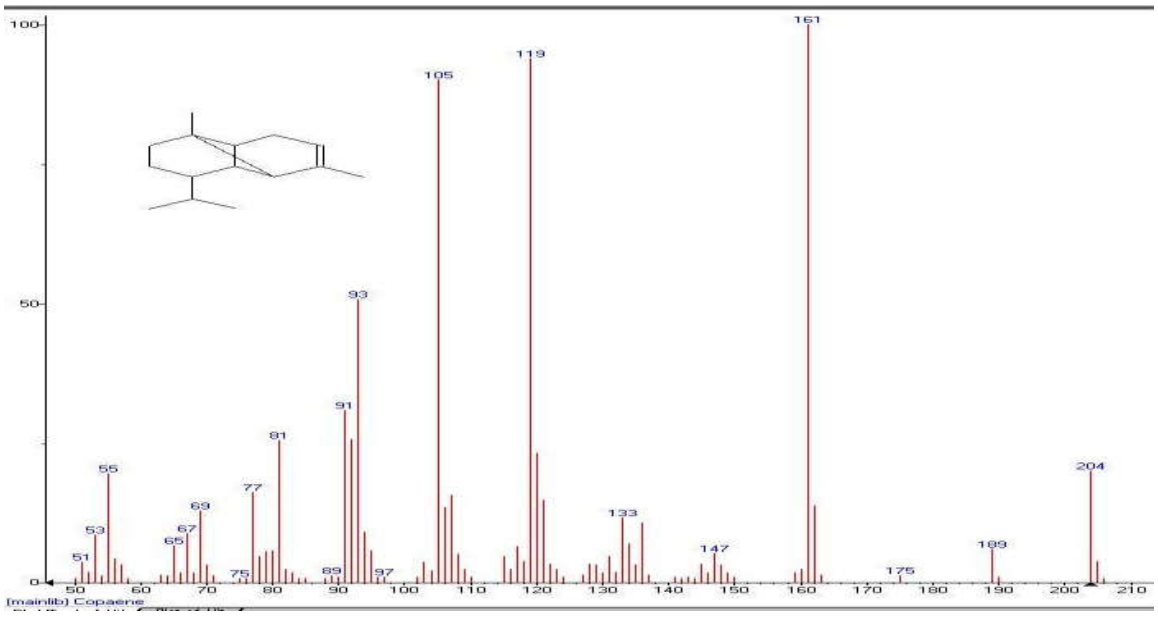
2. Limonene -($C_{10}H_{16}$)



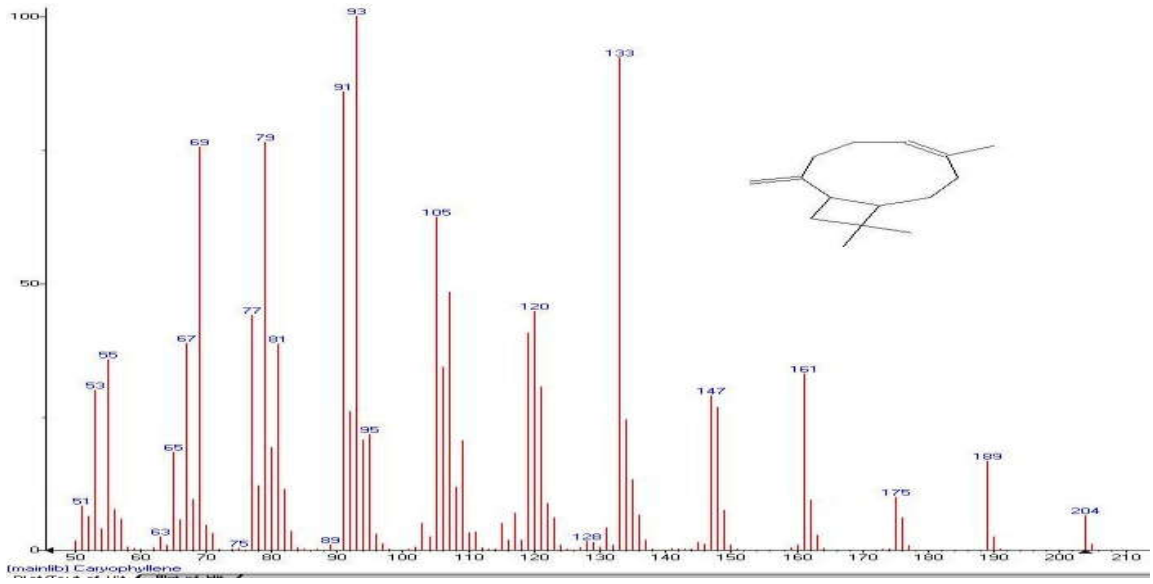
3. δ-Elemene -($C_{15}H_{24}$)



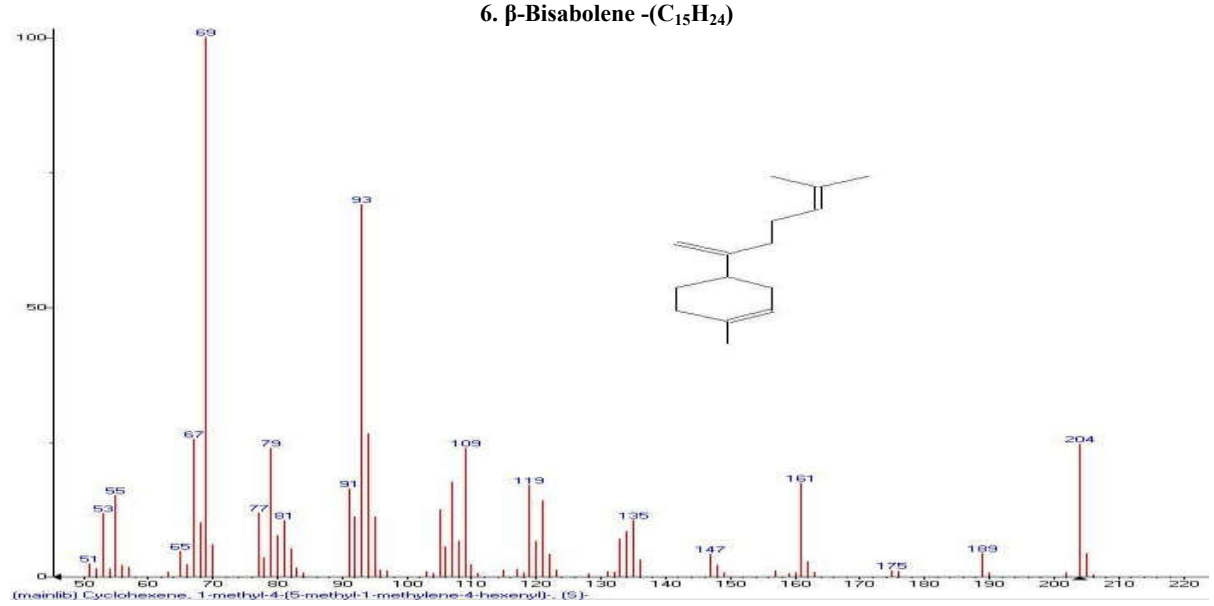
4. Copaene -($C_{15}H_{24}$)



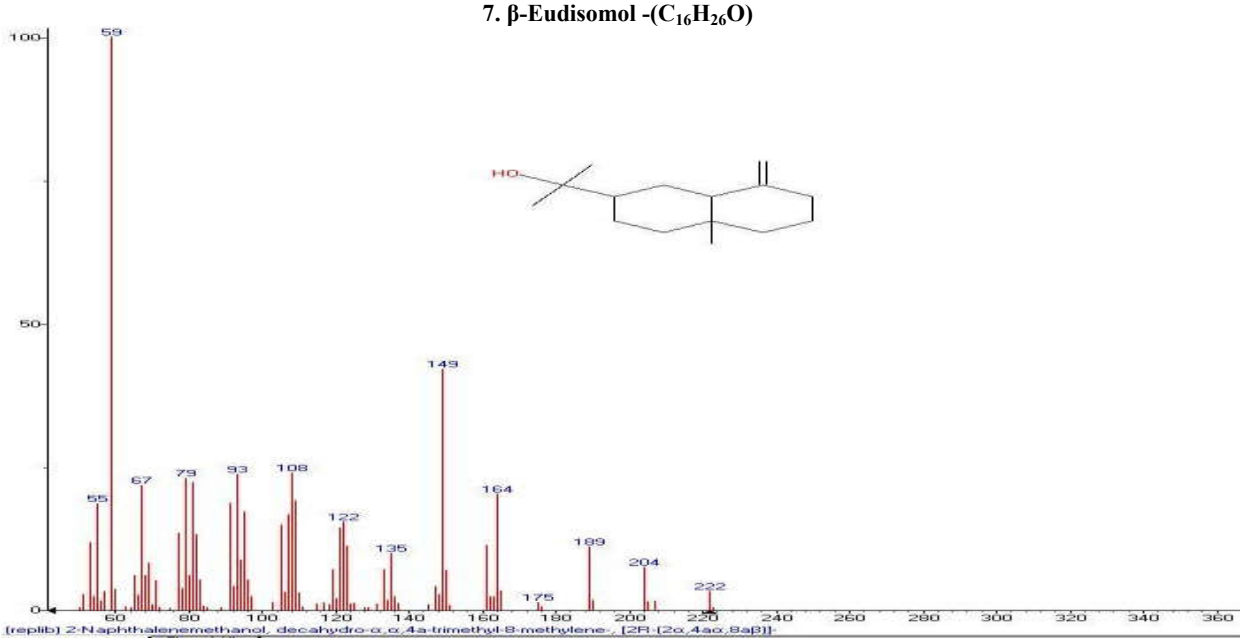
5. Caryophyllene -($C_{15}H_{24}$)



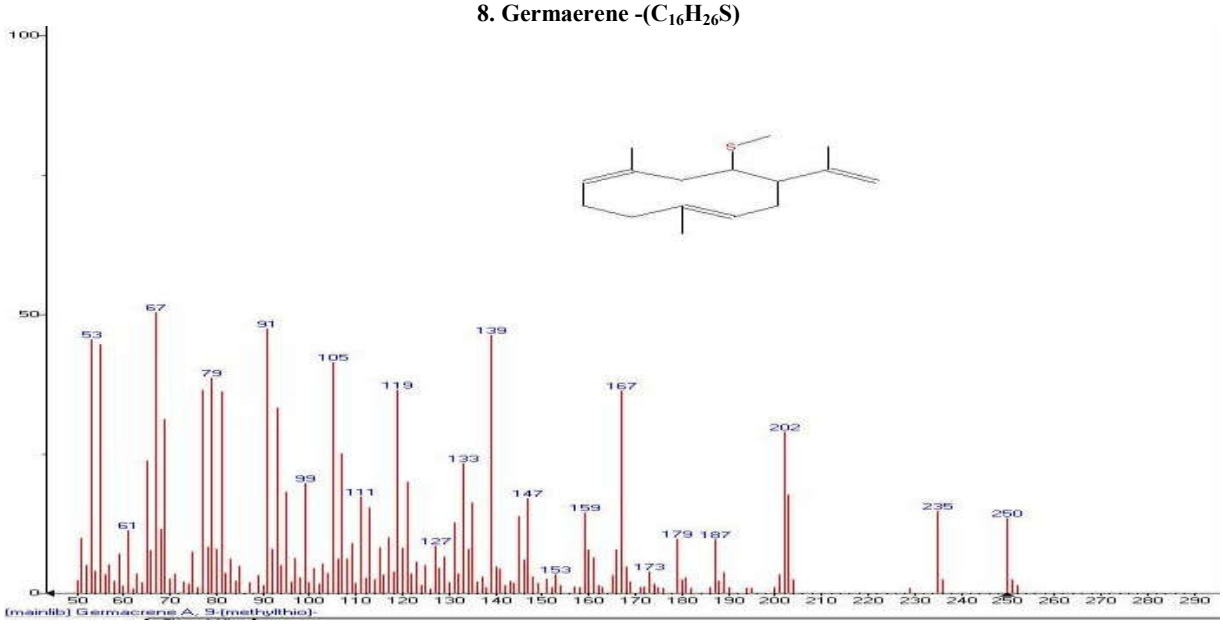
6. β-Bisabolene -($C_{15}H_{24}$)



7. β-Eudisomol -($C_{16}H_{26}O$)



8. Germaerene -($C_{16}H_{26}S$)



C. Antibacterial Test

Agar Well Diffusion Method

The antibacterial activity of the selected volatile oils by agar well diffusion method against four bacterial species was qualitatively assessed by presence or absence of the inhibition zone. The result revealed that the volatile oils showed antibacterial activity with a higher activity in Agar well diffusion method. Among the volatile oils, Ginger oil, ajwain oil, and black pepper exhibited the most effective antibacterial activity in diffusion method in particular against *Staphylococcus aureus, Bcillus subtilis* and *Klebsiell oxytoca* with inhibition zones of……

The bacterial activity is summarized in table

Volatile oil	Gram positive		Gram negative
	Staphylococcus aureus MTCC 3160	*Bcillus subtilis* BAB 2437	*Klebsiell oxytoca* ATCC 13182
Ginger	21.8	21	19
Ajwain	25.5	24.7	22
Black pepper	19	19.2	18.8

Antibacterial activity of gentamycin

Drug	Gram positive		Gram negative
Azithromycin (50mg/ml)	*Staphylococcus aureus* MTCC 3160	*Bcillus subtilis* BAB 2437	*Klebsiell oxytoca* ATCC 13182
	33±0.1	30	28

The result revealed that the selected essential oil showed antibacterial activity with the higher activity in agar well diffusion method. Its having a impressive inhibition diameter then the control sample (azethromycin 50mg /ml). The essential oil with the widest spectrum of activity was found to be followed by ajwain oil > Ginger oil >Black pepper. Which enable them to partition the lipid of the bacterial cell membrane and mitochondria.

Summary and Conclusion

Pharmaceutical cognosy as branch of bioscience which treat in detail medicinal and related product of crude or primary type obtained from plant, animal and mineral origins. In short, it is an objective study crude drugs from natural sources treated scientifically and it encompasses the knowledge of the history, distribution, cultivation, collection, processing for market and preservation, the study of sensory , physical, chemical and structural characters and the uses of crud drugs. Pharmacognosy is an important link between pharmacology and meditional chemistry. As a result of rapid development of phytochemistry and pharmacological testing methods. Chemically volatile oils are derived from terpenes and their oxygenated compounds. They are made made up of isoprene unit (C_6H_8) and are usually mono- sesqui and diterpenes. Volatile oil has wide range of antibacterial activity against different microorganism this are possible for kill or suppress the bacterial activity beside they are used as medicinal agent such as anthelmintics , antiseptic etc. they are also used as flavoring and perfuming agents in pharmaceutical formulation.

REFERENCES

Abadias, M., Usall, J., Anguuera, M., Solsona, C. and Viñas, I. 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *Int. J. Food Microbiol.,* 123: 121–129

Abbasi, BH., Ahmad, N,, Fazal, H. and Mahmood, T. 2010. Conventional and modern propagation techniques in Piper nigrum. *J. Med. Plant Res.,* 4: 007-012.

Adams, R. 2001. Identification of Essential Oil Components by Gas Chromatography/ Mass Spectrometry. Quadrupole. Allured Publishing Co., Carol Stream, Illinois.

Ahmad, N., Fazal, H., Abbasi, BH., Rashid, M., Mahmood, T. and Fatima, N. 2010. Efficient regeneration and antioxidant potential in regenerated-tissues of Piper nigrum L. Plant Cell, *Tissue and Organ Culture,*102: 129-134.

Al-Sheddi, E.S 2009. Pharmacognostical Study of Salvia merjamieForsk growing in Saudi Arabia, PhD thesis. King Saud University.

Altanlar, N., Soner, O. and Tanker, M. 1999. Antimicrobial activity of some volatile oils. *Journal of Turkish Microbiological Society,* 29: 169□172.

Aneesh, TP., Mohamed Hisham, Sonal Sekhar, M. and Manjusree Madhu Deepa, TV. 2009. International Market Scenario of Traditional Indian Herbal Drugs. *Int. J. Green Pharm.,* 3 suppl 3: 184-190.

Ayurvedic medicinal plants http:// ayurvedicmedicinalplants .com/index.php?option=com_zoom&Itemid=26&page= view&catid=14&key=2 .

Baratta, M.T., Dorman, H.J.D., Deans, S.G., Figueiredo, A.C., Barroso, J.G. and Ruberto, G. 1998. Antimicrobial andantioxidant properties of some commercial essential oils. Flavour and Fragrance Journal 13, 235–244.

Bari, M.A., Islam, W., Khan, A.R. and Abdul Mandal, 2010. Antibacterial and antifungal activity of Salonum torvum (solanaceae). *Int. J. Agric. Biol.,* 12: 386–390

Bhandari, U., Shamsher, A.A., Pillai, K. and Khan, M. 2003. Antihepatotoxic activity of ginger ethanol extract in rats. *Pharmaceutical biology*, 41(1), 68-71. [DOI]

Bhosale, K.S. and Shinde, B.P. 2011. Influence of arbuscular mycorrhizal fungi on proline and chlorophyll content in Zingiber officinale rosc grown under water stress. *Indian Journal of Fundamental and Applied Life Sciences*, 1 (3): 172-176.

Bliddal, H., Rosetzsky, A., Schlichting, P., Weidner, M.S., Andersen, L.A., Ibfelt, H.H., Christensen, K., Jensen, O.N. and Barslev, J. 2000. A randomized, placebo-controlled, cross-over study of ginger extracts and ibuprofen in osteoarthritis. *Osteoarthritis Cartilage*, 8(1), 9-12.

Bolanle, A.O. 2011. Effect of ginger powder (Zingiber officinale) on plasma lipid profile and liver enzyme activities of hypercholesterolemic rats. JLS, 5, 712-716.

Boyd, I.W. 1999. Angiotensin receptor antagonists. Aust Prescr, 22(3), 53-54. Bryer, E., (2005). A literature review of the effectiveness of ginger in alleviating mild□to□moderate nausea and vomiting of pregnancy. *Journal of Midwifery & Women's Health*, 50(1), e1-e3. [DOI]

Burt, S. 2004. Essential oils: their antibacterial properties and potential application in food a review. *Int J Food Microbiol.,* 94: 223–253.

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods - a review. *Int. J. Food Microbiol.,* 94: 223–253

Cerruti, P. and Alzamora, S.M. 1996. Inhibitory effects of vanillin on some food spoilage yeasts in laboratory media and fruit purées. *Int. J. Food Microbiol.,* 29: 379–386

Chang, C., Griffith, G. and Maino, G., 2011. The feasibility of fresh ginger exports from Papua New Guinea to New Zealand. *Australasian Agribusiness Review*, 19, 75-97.

Chen, C.C. and Ho, C.T. 1988. Gas chromatographic analysis of volatile components of ginger oil (Zingiber officinale Roscoe) extracted with liquid carbon dioxide. *J. Agric. Food Chem.,* 36: 322–328

Delaquis, P.J., Stanich, K., Girard, B. and Mazza, G. 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *Int. J. Food Microbiol.,* 74: 101–109

Fonseca, J.M. 2006. Postharvest handling and processing: sources of microorganisms and impact of sanitizing procedures, In: Matthews, K.R. (ed.), Microbiology of Fresh Produce. ASM Press, Washington DC

Home page on the internet, World Health Organization, available from url http:// www.who.int / medicines/ areas / traditional/ definitions /en/.

Isman, M. B. 2000. Plant essential oils for pest and disease management. *Crop Protect.,*19: 603–608.

Janssen, A., Sheffer, J. and Baerheim□Svendsen, A. 1987. Antimicrobial activity of essential oils: Aspects of the test methods. *Planta Med.,* 1987; 53: 395□ 398.

Khan, S., Mirza, KJ., Anwar, F. and Zainul Abdin, M. 2010. Development of RAPD markers for authentication of Piper nigrum (L.). Environ.We *Int. J. Sci. Tech.,* 5: 47-56.

Kirtikar, KR. and Basu, BD. 1918. Indian Medicinal Plants. Indian Press. p. 34-44.

Kurita, N., Miyaji, M., Kurane, R. and Takahara, Y. 1981. Antifungal activity of components of essential oils. *Agric. Biol Chem.,* 45: 945□ 952.

Miresmailli, S., Bradbury, R. and Isman, M. 2006. Comparative toxicity ofRosmarinus officinalis L. essential oil and blends of its major constituents against Tetranychus urticae Koch (Acari: Tetranychidae) on two different host plants. *Pest Manag Sci.,* 62: 366–371.

Nair, RR. and Gupta, SD. 2003. Somatic embryogenesis and plant regeneration in black pepper (Piper nigrum L.). Direct somatic embryogenesis from tissue of germinating seeds and ontogeny of somatic embryos. *J. Hortic. Sci. Biotchnol.,* 78: 416-421.

Nazia Masood Ahmed Chaudhry and Perween Tariq, 2006. Antimicrobial Activity of Cinnamonum cassia against diverse microbial flora with its nutritional and medicinal impacts, *Pak. J. Bot.,* 38(1), 169-174.

Nychas, G. 1995. Natural Antimicrobials from plants, In New Methods of Food Preservation, ed Gould, G.W; pp. 58□89, London: Blackie Academic.

Oka, Y., Nacar, S. and Putievsky, E. 2000. Nematicidal activity of essentials oils and their components against the root□knot nematode, *Phytopathology,* 90(7): 710□715.

Omidbeygi, M., Barzegar, M., Hamidi, Z. and Naghdibadi, H. 2007. Antifungal activity of thyme, summer savory and clove essential oils against Aspergillusflavus in liquid medium and tomato paste. *Food Control*, 18, (2007), 1518–1523.

Palevitch, P. and Yaniv, Z. 1991. Medicinal plants of Holy land. Vol. 1 and 2. Tel□Aviv: Tammuz Publisher Ltd.

Ronald Hites, A. 1997. Gas Chromatography Mass Spectroscopy: Handbook of Instrumental Techniques for *Analytical Chemistry,* p. 609-611.

Seenivasan Prabuseenivasan, Manickkam Jayakumar and Savarimuthu Ignacimuthu. 2006. In vitro antibacterial activity of some plant essential oils, BMC Complementary and Alternative Medicine 2006, 1186/1472-6882-6-39, This article is available from: http://www.biomedcentral. com/1472-6882/6/39 .

Shelef, L. 1983. Antimicrobial effects of spices. *J. Food Safety,* 6:29□44.

Srinivasan K. 2007. Black pepper and its pungent principle-piperine. A review of diverse physiological effects. *Crit. Rev. Food Sci. Nutr.,* 47: 735-748.

Zargari, A. 1995. Medical Plants, 5th Edition, Tehran University Press.

Zaveri, M., Khandhar, A., Patel, S. and Patel, A. 2010. Chemistry and pharmacology of Piper longum L. *Inter. J. Pharma. Sci. Rev.Res.,* 5: 67-76.

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