Sudan University of Science and Technology
College of Science
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Extraction of Fixed Oil from Seeds of ammomum Sublatum (Black Cardamom)

A thesis Submitted in partial Fulfillment Requirement for the B.Sc Degree in Scientific Laboratories Chemistry

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الآية

قال تعالى:

بسم الله الرحمن الرحيم

﴿أَلَمْ تَرَ أَنَّ اللَّهَ أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَسَلَكَهُ يَنَابِيعَ فِي الْأَرْضِ ثُمَّ يُخْرِجُ بِهِ زَرْعًا مُخْتَلِفًا أَلْوَانُهُ ثُمَّ يَهِيجُ فَتَرَاهُ مُصْفَرًّا ثُمَّ يَجْعَلُهُ حُطَامًا ۚ إِنَّ فِي ذَٰلِكَ لَذِكْرَىٰ لِأُولِي الْأَلْبَابِ﴾

صدق الله العظيم

سورة الزمر، الآية (21)

Dedication

We dedicate this study to my wonderful family for their kindness and care particularly to my mother the candle that lightens the way for us.

To those such great men and women who teach us how to be better and have confident in ourselves and our abilities.

To our brothers, sisters who always help us do best.

To our colleagues and wonderful friends.

Acknowledgment

Thanks to Allah the Almighty for His blessing. We would like to express our gratitude and thanks to the most wonder full supervisor in the University prof. Mohammed Abdalkareem for his patience, help, advice, encouragement and kindness.

Our thanks are also extended to all our friends.

Abstract

The aim of study is to extract fixed oil from black cardamom by normal hexane.

The study also aimed to determine antibacterial and antifungal activities.

The gas chromatography – mass spectrometry techniques were used.

The result of analysis shown that 74 compounds.

ملخص البحث

تهدف هذه الدراسة الى استخلاص زيت ثابت من بذور نبات Amomunsublitum و اجراء عدة اختبارات على الزيت المستخلص وهذه الاختبارات تتمثل فى: gas chromatography – Mass spectrometry (GC-MS) و اختبار antimicrobial

اظهرت نتائج هذا البحث احتواء الزيت على 74 مركب كيميائى.

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Chapter One

Introduction

1-Introduction

1.1-Essential oils

Essential oil or "ethereal oil" is the volatile oil obtained by the steam distillation of plants or by other means than by direct stea distillation.Table (1) illustrates difference between volatile oil and fixed oils.

Table 1 :Points of differentiation exist between volatile oil and fixed oils:

Volatile Oils	Fixed Oils
(Capable of volatilization) can be distilled from their natural sources.	Cannot be distilled from their natural sources.
Do not leave a permanent grease spot on paper.	Leave a permanent grease spot on paper.
Do not consist of glyceryl esters of fatty acids.	Consist of glyceryl esters of fatty acids.
Can not be saponified with alkalies.	Can be saponified with alkalies.
Do not become rancid upon long storage. Instead on exposure to light and air, they become oxidized and resinified.	Become rancid upon long storage.

1.1.1-Effects and side effects of essential oils

Volatile oils have been used in therapy and as spices since the ancient times.Very complex mixtures mono-and sesquiter penes hydrocarbons, oxygen-containing compounds and phenylpropane derivative . So they exert different effects.

Table 2: Effects of volatile oils

Internal application	External application
-Expectorating - Appetite stimulating - Cholertic, chole kinetic, carminative - Antiseptic/disinfectant - sedative - Circulation stimulating	-Hyperaemic -Antiinflammatory - Antiseptic/disinfectant -Granulation stimulating - Deodorizing - Insecticidal/insect repellent

1.1.2-Methods of extraction of volatile oils

1. Hydrodistillation. 2. Scarification and expression.
3. Extraction with solvents. 4. Enzymatic hydrolysis.

5.Supercritical methods.

1.1.2.1-Hydrodistillation

This method is based on the fact that when water is mixed with another liquid which is immiscible with it, and the two liquids are boiled. They will boil at a temperature lower than that of the high boiling point component, and nearly equal or slightly lower than the boiling point of water. At this temperature the vapour pressure of the two components will equal to the vapour pressure of the atmosphere

Example: The boiling point of turpentine oil is about 160°C, but on mixing with water and heating, it is found that the mixture boils at about 95.6°C.

At this temperature: vapour pressure of water =647 mmHg and vapour pressure of turpentine oil = 113 mmHg. The sum of these vapour pressures is 760 mm/Hg which is the same as normal atmospheric pressure. and consequently the mixture boils.

Vap. press. of water + vap. press, of turpentine oil = Atmospheric pressure

(647 mmHg) + (113 mmHg) = (760 mmHg).

There are three types of hydrodistillation:

1. Water distillation.
2. Water and steam distillation.
3. Direct steam distillation.

The characteristic feature of these methods lie in the boiling water or steam peristic enetrate the plant tissue and vaporizes all volatile substances.

Before distillation the plant material must be disintegrated or subjected to size reduction to some extent.The disintegration or size reduction process, commonly termed "comminution".Flowers, leaves and other thin and nonfibrous parts of the plant can be distilled without comminution. Roots, stalks and all woody materials should be cut into short length in order to expose a great number of oil glands. The comminuted material should be distilled at once to avoid secondary transformations of oil constituents by oxidations, and loss of oil by evaporation.

a)Water distillation

The material to be distilled comes in direct contact with boiling water. It may float on the water or be completely immersed, depending upon its specific gravity and the quantity of material handled per charge. The water is boiled by application of heat by any of the usual methods.

Some plant materials (e.g., powdered almonds, rose petals, and orange blossoms) must be distilled while fully immersed and moving freely in boiling water, because on distillation with injection live steam (direct steam distillation) these materials agglutinate and form large compact lumps, through which the steam cannot penetrate.

b)Water and steam distillation

The plant material is supported on a perforated grid screen inserted some distance above the bottom of the still. The lower part of the still is filled with water, to a level somewhat below this grid. The water may be heated by any usual methods. The water is boiled by application of heat by any of the usual methods. Saturated, wet, steam of low pressure rises through the plant material .The typical features of this methods are:

First… the stream is always fully saturated, wet and never superheated**.**
Second.. the plant material is in contact with steam only, and not with boiling water.

c)Direct steam distillation

This method resembles the preceding one except that no water is kept in

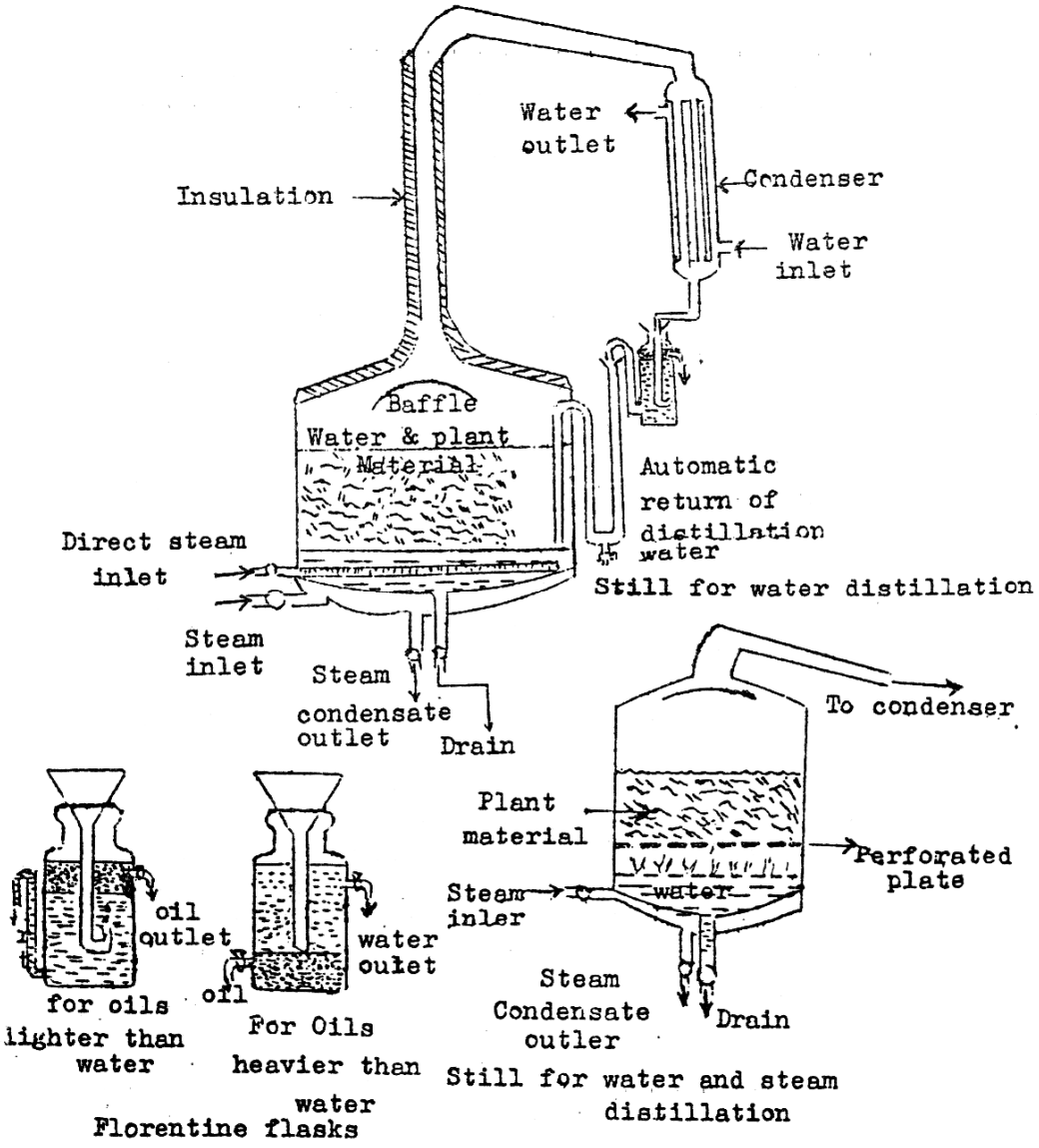
the bottom of the still. Live steam, saturated or superheated, and frequently at pressure higher than atmospheric, is introduced through open or perforated steam coil, below the charge, and proceed upward through the plant material above the supporting grid.

The high temperature in the presence of water during steam distillation induce some effect upon certain constituents of the oil and other plant components.

Volatile amines and degradation products of carbohydrates (furfural and similar products) pass over during distillation.

Hydrolysis of esters and loss of water from resulting tertiary alcohols may give hydrocarbons. These volatile products formed by heat decomposition of plant substances will often contaminate the oil. In some cases the essential oil serves as a source of a specific constituent and is then fractionated by distillation under vacuum.

When the mixture of the oil and water is passed through condenser, the oil, being only slightly soluble in water, separates from the aqueous layer and can be isolated, usually by use of a Florentine receiver. The distillation water contains small amounts of dissolved volatile oil and is often returned to the distillation still in order to recover the dissolved oil. This process is called Cohobation.


Water outlet
Condenser
Insulation
Water inlet
Baffle
Water & plant Material
Automatic return of distillation water
Direct steam inlet
Still for water distillation
Steam inlet
Steam condensate outlet
Drain
To condenser
Plant material
Perforated plate
Steam inlet
water
Steam Condensate outlet
Drain
Still for water and steam distillation
oil outlet
water outlet
oil
for oils lighter than water
For Oils heavier than water
Florentine flasks

1.1.2.2-Scarification and expression methods

The volatile oils of the citrus family (orange, lemon, and bergamot) cannot be distilled without decomposition and are generally obtained by expressing the rind of the fresh fruit. These are usually known as "hand pressed".

i)The sponge method

The citrus fruit is washed, cut into halves. and the juicy part is removed. The rind is turned inside out (squeezed) when secretion gland rupture and the oil collected by means of the sponge, until the sponge becomes saturated and periodically squeezed in a vessel. The upper oily layer in the vessel is separated. Methods have now been developed in which machines scratch the surface of the fruit and the oil is absorbed by a sponge.

ii)The ecuelle a piquer method

The entire fruits are rotated in a saucer-shaped metal pins or sharp projections just long enough to penetrate the epidermis. The pins rupture the oil cells, the exuding oil collecting in a long narrow depression in the bottom of the saucer. The liquid is poured off at intervals into a large vessel, where it is allowed to stand until the oil can be decanted and filtered.

Machines are employed for extracting oils from bergamots and lemons, sometimes whole fruits are pressed and juice and oil which run out together are collected and centrifuged to separate the oil. A large part of the lemon oil of commerce is obtained as a by-product in the preparation of lime juice.

1.1.2.3-Extraction with solvents

The relatively long action of boiling water or steam on the plant material affects some of the more delicate constituents of the oil (hydrolysis, polymerization and resinification).

Also a few types of flowers, yield no direct oil at all on distillation. The oil is either destroyed by the action of steam or the minute quantities of oil actually distilling over are "lost" in the large volume of distillate from which the oil cannot be recovered. This applies to jasmine, tuberose, violet, gardenia and few others.

i)- Extraction with volatile solvents

The material containing the volatile oil (fresh flowers) is extracted with volatile solvent usually light petroleum by percolation or by continuous extraction. The solvent penetrates the flowers and dissolves the natural flower perfume together with some waxes and albuminous and colouring matter, then concentrated at low temperature.

The solvent used must be:

1.Completely and quickly dissolve all the odourferous principles.

2.Low boiling point.
3.Immiscible with water.
4.Chemically inert.
5.Uniform boiling point.
6.Low priced.

ii) Extraction with a non-volatile solvent

a) Enfleurage or extraction with cold fat

The principles of enfleurage are simple. Certain flowers (e.g., tuberose and jasmine) continue the physiological activities of developing and giving off perfume even after picking. Fat possesses a high power of absorption and if brought in contact with fragrant flowers readily absorbs the perfume emitted.

In the enfleurage process, batches of fresh flowers are lightly layered over a specially prepared fat base (refined lard, tallow or fatty acids of high molecular weight), known as "corps". The fat is spread in a thin layer on both sides of a glass plate supported on a rectangular wooden frame or "Chassis". Several chassis are placed one above the other so that the flowers get sandwiched between two layers of fat which absorbs the perfume as it is given off.

Exhausted flowers are removed (De flèurage) and replaced by the fresh flowers until the fat is relatively saturated with flower oil and possesses

their typical fragrance. The perfumed fat must then be removed from the glass plates , the final product is called , Pomade.

The most highly saturated pomade being Pomade No. 36 means the chassis has been treated with fresh flowers 36 times during the whole process of enfleurage. The pomade is extracted three times with alcohol 95% (Triple extraction), the clear solution known as"extracts". The extract contains a small quantity of alcohol soluble fat (about 1%), which can not be eliminated even by cooling. The extract is concentrated in a vaccum still at low temperature till free from alcohol, the concentrated flower oil is called absolute of enfleurage, absolute of Pomade or liquid concretes.

b)Maceration method or extraction with hot fat

The physiological activities of some flowers e.g. roses, orange blossoms and acacia, for instance are stopped by picking.

In the maceration process, the flowers are extracted by immersion in hot fat or fixed oil. In other words, the same batch of hot fat is systematically treated with several batches of fresh flowers until the fat becomes quite saturated with flower perfume. The exhausted flowers are removed and the fragrant fat, called "Pomade". The Pomade may be treated further by washing it with alcohol 95%, exactly as cold enfleurage.

c)Digestion method

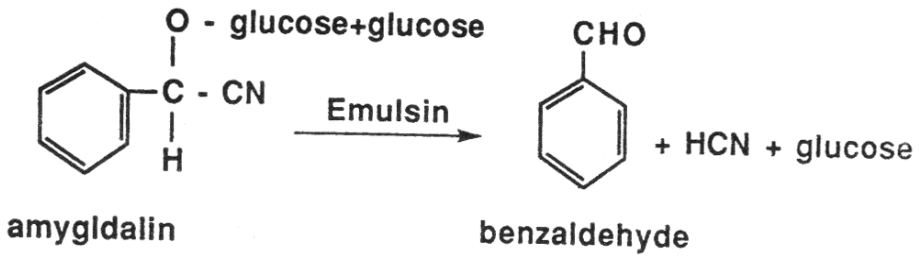
This process is similar to maceration except that a moderate heat is employed, by the use of a salt bath, to aid in the extraction.

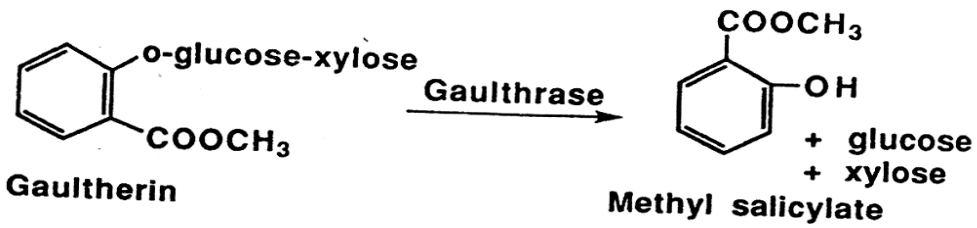
d)Pneumatic or spraying method

The pneumatic method, which is similar in principle to the enfleurage process, involves the passage of a current of warm air through the flowers. The air, laden with suspended volatile oil, is then passed through a spray of melted fat in which the volatile oil is absorbed. The volatile oil is obtained from the perfumed fat by three successive extraction with alcohol (triple extraction).

1.1.2.4-Enzymatic hydrolysis method

Glycosidic volatile oils (e.g. bitter almond oil, mustard oil and gaultheria oil) are obtained by enzymatic hydrolysis of the glycosides. In bitter almond seeds, amygdalin is acted on by emulsin resulting in a mixture of constituents from which the volatile oil may be distilled with steam. In black mustard seeds; the glycoside, sinigrin, is hydrolysed by myrosin with the production of volatile oil of mustard. In *Gaultheria procumbens* leaves, the glycoside gaultherin, is hydrolysed by gaulthrase with the production of winter green oil.





1.1.2.5-Supercritical method

Supercritical fluid extraction (SFE)

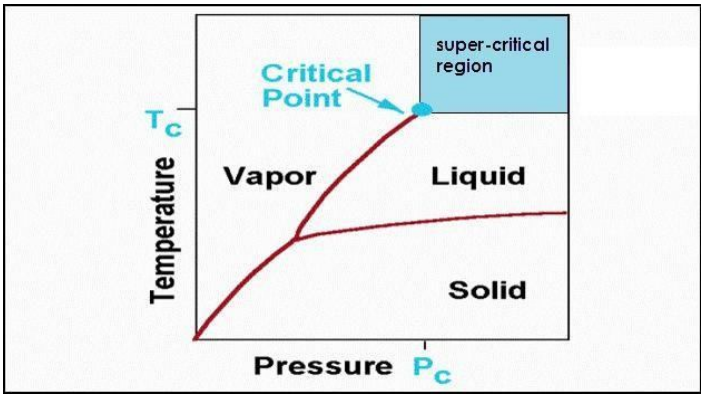
The drawbacks associated with the common methods used for isolation of the essential oils, have led to searching for new alternative extraction procedures from plants and/or to enhance the quality of the oil in the essential oils industry ,

The implementation of newer or better techniques is this mandatory to over come the common extractin problems. New alternatives , such as super critical CO2 (SC CO2) extraction, are introduced as effective methods for the isolation of high-quality

High pressure extraction is more effective and an efficient way to extract valuable constituents from botanicals.

The simplest way to explain this process is that you place the plant material in a pressure vessel and pump a particular liquified gas such as carbon dioxide or liquid solvent through it at a specific pressure and temperature simultaneously above its critical point.

Supercritical fluid extraction (SFE) is the process of separating any essential oil from the matrix using supercritcal fluid as extracting solvent.



Typical diagram of supercritical region, The lines that separate the liquid and vapor and solid phases are phase boundaries. Crossing a phase boundary causes a change in phase. They all cross at the Triple point. The liquid/vapor phase boundary comes to a stop at a particular temperature and pressure. This is the critical point. Beyond this is the critical region.

The pressure forces the solvent into the cell walls of the botanical and extract the desired constituents rapidly at supercritical region.

The process of separating the extract from the solvent. The extraction properties can be widely and precisely manipulated with subtle changes In pressure and temperature.

Commonly used supercritical solvents carbon dioxide, nitrous oxide, ethylene, propylene, Propane, n-pentane, ethanol and ammonia, of these, CO2 (crtitical point is

31.06ºC and 7.386 atm.p.) Is the solvent of choice for use in supercritical fluid extraction. because it is nonflammable, noncorrosive and inexpensive . In addition , CO2 has a low critical

temperature , which can help prevent thermal degradation

Physical methods of examination of volatile oils:

1. **Color:**
   Most oils are colorless when pure and fresh.

   Upon exposure to the air they acquire various colors:

Green……oil of wormwood, yellow…..oil of peppermint; red ….oil of origanum, brown……oil of cinnamon. Volatile oils can be made colourless by redistillation (rectification), The blue colour of oil of chamomile is an inherent property of the oil even when freshly distilled and said to be due to the highly unsaturated hydrocarbon chamazulene.

2. **Odor:**

   The odors of volatile oils are extremely variable. It is their most characteristic feature. The odor of an oil is sensibly modified by exposure to air.

3. **Taste:**

The tastes of volatile oils are almost as variable as their odors. Some are sweet, others have a mild, pungent, hot, acrid, caustic, or burning taste.

4. **Specific gravity:**

The specific gravity of a volatile oils vary approximately between 0.84 and 1.2. Those oils which are lighter than water, such as orange, caraway, coriander, lemon, turpentine and rosemary oils, are usually

rich in hydrocarbons, alcohols, esters and ketones.

Oils with specific gravities that approach or exceed1.0, e.g. anise, cinnamon, clove and sassafras oils, usually contain chiefly aldehydes, phenols, phenolic derivatives or certain esters. The specific gravity of any volatile oil is not absolutely constant, since it is influenced by such factors as the maturity of the plant from which the oil is obtained and the age of the oil, as well as by the methods of preparation and purification.

5. Optical activity:

The rotatory power of some of the volatile oils varies within relatively wide limits. This determination should never be omitted in their examination, however, since it frequently serves as a valuable means of detecting adulteration with inactive. substances, such as alcohol, or with substances of different rotatory power from that of the oil being examined, e.g. lemon oil (+ 57º to + 65.6º) adulterated with turpentine oil (about + *25*° to - 40°).

6. Refractive Index:

The measurement of the refractive indices of volatile oils is required to performed at 20ºC, except in the case of rose oil, which is measured at30ºC. The index of refraction does not vary greatly with different official volatile oils, the values being between about 1.46 and 1.61 at 20°C. In some cases, however, this determination may serve for the detection of extraneous matter.

7. Congealing temperature or point:

Most essential oils solidify only at low temperatures, Consequently, in practice, this determination is carried out with only a few oils, such as anise oil, eucalyptus oil and fennel oil, which contain large amounts of the readily crystallizable constituents anethole and eucalyptol. The higher the congealing temperature of these oils, the more they are valued. An abnormally low congealing temperature of an volatile oil indicates the partial removal of the characteristic constituent for which the oil is valued or the addition of extraneous matter, such as alcohol.

8. Distilling range or limits:

Volatile oils that are composed of mixture of hydrocarbons, alcohols, esters, etc., boil between certain limits of temperature, frequently separated widely. Consequently, the official standards usually designate the temperature or range of temperature at which a definite percentage of the oil distills; e.g. not less than 90% of turpentine oil should distill between 154°C and 170°C; not less than 95% of pine oil should distill between 200ºC and 225°c, and less than 10% of dwarf pine needle oil should distill below 165°C.

9. **Solubility :**

The volatile oils are generally soluble in organic solvents, such as absolute alcohol, ether,. chloroform, benzene carbon disulfide, etc. They dissolve more or less readily in dilute alcohol according to the nature of their components. Oils containing a large percentage of

oxygenated substances often produce turbid solutions with light petroleum or carbon disulfide because of the separation of water, small quantities of which are dissolved in such oil. The test of the solubility of a volatile oil in dilute alcohol gives valuable data relative to its purity.

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Chapter Two

Materials and Methods

2-Materials and Methods

2.1-Materials

2.1.1-Plant material

The fruits of black cardamom were purchased from the local market – Omdurman, Sudan.The plant was kindly authenticated by institute of Aromatic and Medicinal Plants – Khartoum, Sudan.

2.1.2-Instruments

A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 μm, thickness) was used GC-MS analysis.

2.1.3-Test organisms

Black cardamom oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in Table (1).

Table 1 : Test organisms

Ser.No	Microorganism	Type
1	Bacillus subtilis	G+ve
2	Staphylococcus aureus	G+ve
3	Pseudomonas aeroginos	G+ve
4	Escherichia coli	G-ve

5	Aspergillus niger	Fungi
6	Candida albicans	Fungi

2.2 – Methods

2.2.1- Extraction of oil

Dry-powdered tubers of Amomum Sublitum (200g) were macerated with n-hexane at room temperature for 48h. The solvent was removed under reduced pressure and the oil was kept in the fridge at 40°C for further manipulation.

2.2.2- Esterification of oil

A Methanolic solution of sodium hydroxide was prepared by dissolving (2g) of sodium hydroxide in 100ml methanol. A stock solution of methanolic acid was prepared by mixing (1ml) of concentrated sulphuric acid with (99ml) methanol. The oil (2ml) was placed in a test tube and (7ml) of alcoholic sodium hydroxide were added followed by (7ml) of alcoholic sulphuric acid. The tube was stopped and shaked vigorously for five minutes and then left overnight.(2ml) of supersaturated sodium chloride were added, then (2ml) of n- hexane were added and the tube was vigorously shaked for five minutes.The hexane layer was then separated.(5µl) of the hexane extract were mixed with 5ml diethyl ether . the solution was filtered and the filtrate(1µl) was injected in the GC-MS vial.

2.2.3-GC-MS analysis

Black cardmoum fixed oil was analyzed by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm, diameter ; 0.25 μm, thickness) was used. Helium (purity; 99.99%) was used as carrier gas. Oven temperature program is given in Table 2, while other chromatographic condition are depicted in Table 3.

Table:2 Oven temperature program

Rate	Temperature(C)	Hold time (min^{-1})
_	60.0	0.00
10.00	300.0	0.00

Table 3: chromatographic conditions

Column oven temperature	1300.0 °C
Injection temperature	280.0 °C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	93.1KPa
Total flow	50.0ml/min
Column flow	1.50ml/sec

Linear velocity	44.7cm/sec
Purge flow	3.0ml/min
Spilt ratio	-1.0

2.2.4-Antimicrobial assay

2.2.4.1-Preparation of bacterial suspensions:-

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in (100ml) of normal saline to produce a suspension containing about 108-109 colony forming units per ml. The suspension was stored in the refrigerator at 4°C unit used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilution of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

2.2.4.2-Preparation of fungal suspensions

Fungal cultures were maintained on dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with

sterile normal saline, and the suspension was stored in the refrigerator until used.

2.2.4.3-Testing for antibacterial activity

The cup-plate agar diffusion method was adopted , with some minor modification, to assess the antibacterial activity. (2ml) of the standardized bacterial stock suspension were mixed with (200ml) of sterile molten nutrient agar which was maintained at 45°C in the water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle and in each of these plates which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for one of the test solution. Separate Petri dishes were designed for standard antibacterial chemotherapeutics (ampicillin and gentamycin).

The agar discs were removed, alternate cups were filled with (0.1 ml) samples of each test solution using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours.

The above procedure was repeated for different concentrations of the test solutions and the standard chemotherapeutics. After incubation, the diameters of the resultant growth inhibition zones were measured in triplicates and averaged.

Chapter Three

Results and Discussions

3-Results and Discussion

3.1-The GC-MS analysis of *Ammomum sublatum* essential oil

GC-MS analysis of ***Ammomum sublatum*** oil was conducted and the identification of the constituents was initially accomplished by comparison with the MS library (NIST) and further confirmed by interpreting the observed fragmentation pattern.Comparison of the mass spectra with the database on MS library revealed about 90-95% match.

3.2.1- Constituents of oil

The GC-MS spectrum of the studied oil revealed the presence of 74 components. The following components were detected as major components in the chromatogram:

-9-Octadecenoic acid methyl ester(19.32%)

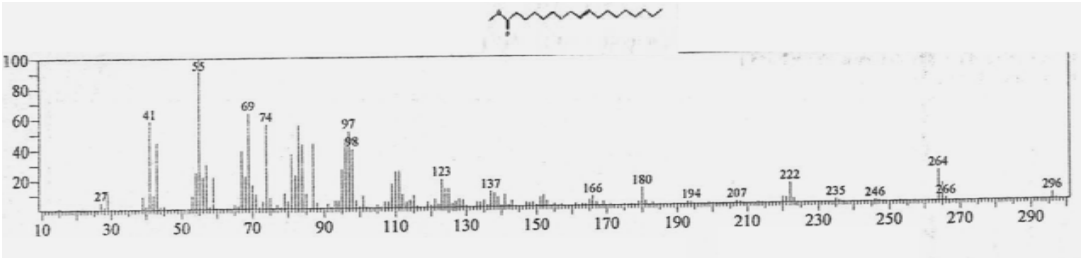

Fig. 1: Mass spectrum of 9-octadecanoic acid methyl ester

The EI mass spectrum of 9-octadecanoic acid methyl ester is shown in Fig.1.The peak at m/z 296, which appeared at R.T. 15.496 in total ion

chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^+$ .The peak at m/z265 corresponds to loss of a methoxyl function.

-Hexadecanoic acid methyl ester(10.09%)

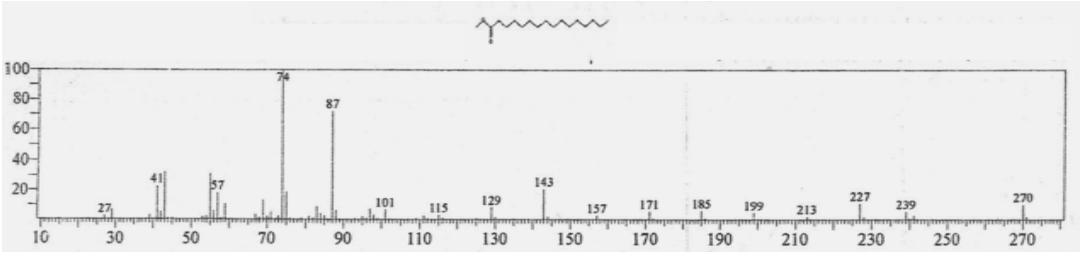

Fig. 2: Mass spectrum of hexadecanoic methyl ester

The EI mass spectrum of hexadecanoic acid methyl ester is shown in Fig. 2.The peak at m/z 270, which appeared at R.T. 13.308 in total ion chromatogram, corresponds to $M^+[C_{17}H_{34}O_2]^+$ .The peak at m/z239 corresponds to loss of a methoxyl function.

-9,12-Octadecadienoic acid methyl ester(8.15%)

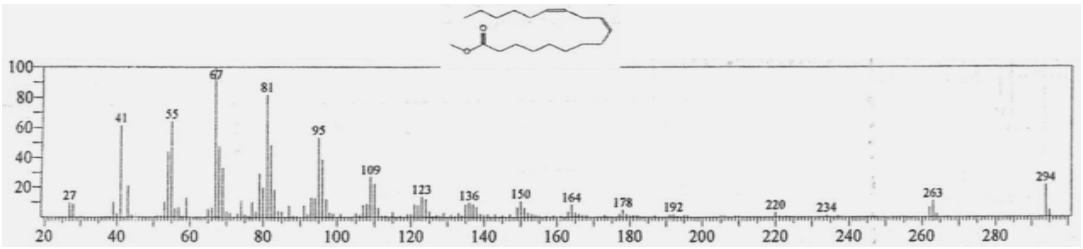

Fig. 3: Mass spectrum of 9,12-octadecanoic acid methyl ester

The EI mass spectrum of 9,12-octadecanoic acid methyl ester

is shown in Fig. 3.The peak at m/z 294, which appeared at R.T. 15.432 in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^+$ .The peak at m/z263 corresponds to loss of a methoxyl function.

Methyl stearate(3.97%)

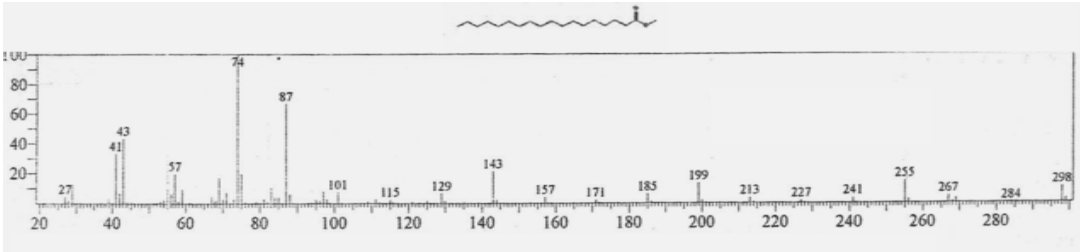

Fig. 4: Mass spectrum of methyl stearate

The EI mass spectrum of methyl stearate is shown in Fig. 4.The peak at m/z 298, which appeared at R.T. 15.783 in total ion chromatogram, corresponds to $M^+[C_{19}H_{38}O_2]^+$ .The peak at m/z267 corresponds to loss of a methoxyl function.

3.2-Antimicrobial activity

The oil was screened for antimicrobial activity against standard organisms. The average of the diameters of the growth inhibition zones are shown in Table (1) .The results were interpreted in terms of the commonly used terms (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) .Tables (2) and (3) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table (1) : Antibacterial activity of ***Ammomum sublatum*** oil :M.D.I.Z (mm)

Drug	Conc.(mg/ml)	Ec	Ps	Sa	Bs	Ca
Ammomum sublatum oil	100	16	16	11	29	22

Table (2) : Antibacterial activity of standard chemotherapeutic agents :M.D.I.Z (mm)

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	40 20 10	15 14 11	30 25 15	- - -	- - -
Gentamycin	40 20 10	25 22 17	19 18 14	22 18 15	21 15 12

Table (3) : Antifungal activity of standard chemotherapeutic agents against standard fungi

Drug	Conc. mg/ml	An.	Ca.
Clotrimazole	30 15 7.5	22 17 16	38 31 29

- Sa.*: Staphylococcus aureus*
- Ec.*: Escherichia coli*
- Pa.: *Pseudomonas aeruginosa*
- An.: *Aspergillus niger*
- Ca.: *Candida albicans*
- *Bs.: Bacillus subtilis*
- M.D.I.Z: Mean diameter or growth inhibition zone (mm). Average or two replicates, inhibition zone >=15: sensitive, <15: resistant.

The oil showed activity against all test organisms,but it was partially active against *Staphylococcus aureus* . It showed significant activity against *Bacillus subtilis* and the fungus *Candida albicans*..

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