Sudan University of Science and Technology

College of Graduate Studies

Physiochemical characterization of mango seed oil

التوصيف الفيزيوكيميائى لزيت بذرة المانجو

A thesis submitted in partial fulfillment for requirement of the degree of master in chemistry

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November, 2016

الآية

قال تعالي:

أَوَلَمْ يَرَ الَّذِينَ كَفَرُوا أَنَّ السَّمَاوَاتِ وَالأَرْضَ كَانَتَا رَتْقًا فَفَتَقْنَاهُمَا وَجَعَلْنَا مِنَ الْمَاءِ كُلَّ شَيْءٍ حَيٍّ أَفَلاَ يُؤْمِنُونَ)

صدق الله العظيم

(سورة الأنبياء الآية (30)

DEDICATION

TO

My family and friends

ACKNOWLEDGEMENT

This research was prepared throughout the offers, advices and input of many people. I would like to convey my thanks to Dr. **Aziza Ali Karar** for the valuable assistance. Finally, I would like to thanks my family for moral and financial support.

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List of Appreviations

NCTC:	National collection of type culture, Colindale, England.
ATCC:	American type culture collection Rockville, Maryland, USA
Es:	Escherichiacol
Ps:	pseeudomonas aeruginosa
Sa:	staphylococcus aureus
Bs:	bacillus subtilis
Ca:	candida Alibican
GC-MS:	Gas chromatography mass spectroscopy
C.F.U:	Colony forming units.

Abstract

The purpose of the present study was to examine the physicochemical characteristics of mango seeds oil. Also using the extracted oil as antibiotic of some types of bacteria escherichiacoli , pseudomonas aeruginosa, staphylococcus aureus , bacillus subtilis and fungal , candida alibican.

Results of extraction showed that the percentage of extracted oil from mango seeds by using soxhlet and n-hexane as solvent was found to be 12.48%.

Analysis results of extracted oil by determined the physical and chemical properties showed that:

The density,(0.8958g/cm3) at ($25C^0$),refractive index ,(1.467) at (40 C^o), viscosity, (14.18mpa.s),PH , (6.2), acid value, (2.805mg/g), saponification value, (190mg/g), peroxide value, (5), iodine value, (49.173g/100g), ester value, (187.195).

Extracted oil was applied as antibiotic for some kinds of bacteria and fungi. The results obtained showed that the extracted oil did not give appositive results, where not lead to inhibition or killing of used bacteria.

The oil was found to contain high levels of stearic acid (38.62%) followed by oleic (30.72%), palmatic(6.68%) and lenoleic (2.85%).

Fourier transform infrared(FTIR) spectra showed the functional groups present in the oil.

Many parameters of mango seed oil from Sudan are comparable to those of typical mango seeds oil reported in the literature.

The physicochemical results and quality of mango seed oil from Sudan revealed that it could be employed for edible and commercial applications.

مستخلص

الغرض من الدراسة الحالية هو اختبار الخصائص الفيزيائية والكيميائية للزيت المستخلص من بذرة نبات المانجو.وجد ان المحتوى الزيتى لبذرة نبات المانجو والمستخلصة بمذيب الهكسان حوالى (12.48%).
نتائج الاختبارات الفيزيائية والكيميائية للزيت المستخلص كانت كالآتى:
الكثافة (0.8958جم/ سم) ,(عند درجة حرارة $25C^{o}$), معامل الانكسار1.467, (عند درجة حرارة $40C^{0}$) , اللزوجة ,(14.186ملىباسكال.ث), الرقم الهيدروجين (5.6) كمية الحمض الحر, 2.805 قيمة التصبن ، (190) رقم البيروكسيد ، (5) قيمة اليود (49.173) ، قيمة الاستر (187.195) .
تم استخدام الزيت المستخلص كمضادات حيوية لبعض انواع البكتريا والفطريات ، واظهرت النتائج ان الزيت المستخلص لا يعمل على تثبيط أو قتل البكتريا.
وجد ان الزيت يحتوى على نسبه عالية من حمض الاستياريك (38.62%) وحمض الاوليك(30.72 %) يليه بالمتيك(6.68 %) وحمض اللينوليك(2.85%) .طيف الامتصاص للاشعة تحت الحمراء اظهر بعض المجموعات الوظيفيه للزيت.العديد من نتائج الاختبارات على زيت بذرة المانجو السودانيه تم مقارنتها مع نتائج الاختبارات على زيت بذرة المانجو فى الدراسات السابقه وقد اظهرت تطابقا.اظهرت النتائج الفيزيائيه والكيميائيه جودة زيت بذرة المانجو السودانيه وبذلك يمكن استخدامه كزيت طعام بالاضافه للاستخدامات التجاريه الاخرى.

1. Introduction

1.1 Medicinal plant:

Medicinal plants are plants that have recognized medicinal uses. They range from plant which is used in the production of mainstream pharmaceutical product to plant used in herbal medicine preparation (***Lothtipour et al., 2008***)

Other medicinal plants may be cultivated, one of the advantages of cultivation is that it allows for greater control over growing conditions. Cultivation also allows for mass production, which makes production of the medicinal plant(s) commercially feasible as they can be processed in large numbers and priced low enough that people will be able to afford them (***WHO, 1991***).

According to the World Health Organization (WHO), 80% of the world population uses medicinal plants in the treatment of diseases and in African countries, this rate is much higher (***koyuncum, 1997***)..

Medicinal plants contain biologically active chemical substances such as coumarins, volatile oils, alkaloids, etc. In addition to these substances, plants contain other chemical compounds. These can act as agents to prevent undesirable side effects of the main active substances or to assist in the assimilation of the main substances. Opium juice, for example, contains other chemical compounds in addition to morphine, and reports show that it gives fewer side effects than morphine administered on its own. The saponins found in the left extracts of *Digitalis purpurea* also assist the cardio-active heteroclites to penetrate the blood more easily (***koyuncum, 1997***).

Folk medicines including herbal medicines and teas, in which the active compounds of the plants are directly used are the most commonly used of medicinal plants. The use of pure active compounds, obtained from medicinal plants, is also widely used. 400 compounds, derived from plants, are currently

used in the preparation of drugs such as vincristin and vinblastin. These compounds are used in the cure of cancer and can only be obtained from the plant *Catharanthus roseus* (Rose periwinkle). They cannot be produced synthetically, and their market price per year is 100 million dollars.

The indiscriminate collection, and lack of replacement of these plants, is cause for serious concern since many of them risk extinction. A plan to replace such species is urgently required, since these medicinal plants are essential for treatment of many important diseases where other cures have yet to be found (***koyuncum, 1997***).

1.2 Literature review

1.2.1 Previous study:

Vegetable fats and oils are widely used in the food, pharmaceutical, cosmetic and chemical industries and are normally obtained from oil seeds such as sesame seed, soy bean, cotton seed and oil ***(Gutcho, 1979; Birker and Padley, 1987; O'Brien, 1998).*** The physical, chemical and nutritional properties of fats and oils are limiting factors for their use in certain industrial sectors. Thus, obtaining nutritionally sound products for the food industry will greatly depend on the physical and/or chemical characteristics of the fat and oil formulations. Among these physical and chemical characteristics, thermal behaviour and phase changes are particularly important. In the food industry, for example, vegetable oil hydrogenation modifies its thermal behaviour and stability, making possible its use as a substitute for traditional saturated fats of animal origin as lard, butter, etc. ***(Bertoli et al., 1995; O'Brien, 1998).*** The antioxidant and tyrosinase inhibitory properties of extracts from mango seed kernel extracts contained phenolic components with a high antioxidant activity and also possess a tyrosinase inhibitory activity.They reported that the extracts had the most

effective antioxidants, which had the highest radical-scavenging, metal-chelatingand tyrosinase inhibitory activity. In conclusion, they recommended that mango seed kernel extracts are suitable for use in food, cosmetic, nutraceutical and pharmaceutical applications (***Maisuthisakul and Gordon, 2009***).

The lipid composition of mango seed kernels has attracted the attention of scientists in recent years because of their unique physical and chemical characteristics (***Hemavathy et al., 1988; Helmy, 1998***). Extracted and fractionated total lipids from Alphonso mango kernel into three lipid classes. Total lipid (11.6% of dry kernel) consisted of 96.1% neutral and 3.9% polar lipids, which comprised 2.9% glycolipids and 1.0% phospholipids (***Hemavath et al.,1987***). Also, extracted and fractionated total lipids from three different mango seed kernel varieties namely goleck, pairi and hindi (Rashwan, 1990). The neutral lipids varied from 95.2% to 96.2%, phospholipids from 2.7% to 3.3% and glycolipids from 1.1% to 1.4%. Triglycerides constituted the major fraction of the neutral lipids, for all varieties and accounted from 93.7% to 96.4%. The lipids of mango seed kernel consist of about 44–48% saturated fatty acids (majority stearic) and 52–56% unsaturated (majority oleic) (***Ali et al, 1985; Hemavathy et al., 1987; Mohamed and Girgis, 2005***).

1.3 Mango tree

Mango (Mangifera indica L.) fruit belongs to the family of Anacardiaceae in the order of Sapindales and is grown in many parts of the world, particularly in tropical countries. Over 1000 mango varieties are available worldwide. Of the available varieties, only a few are grown on commercial scales and traded ***(solis-Fuentes et al., 2011).***

When ripe, this delicious dessert fruit is particularly high in vitamin A. the fruit is also eaten green, processed into pickles, pulps, jams, and chutneys, and is frozen or dried.

Mango trees are usually between 3 to 10m tall but can reach up to 30m in some forest situations. The canopy is evergreen with generally spreading habit (***IanS.E.bally,2006***).

1.4 Taxonomical classification:

Kingdom : plantae

Class : mangoliopsida

Order : sapindales

Family : anacardiaceae

Genus : mangifera

Species : indica

Fig (1.1): Mango tree

1.5. Important uses of mango:

1.5.1 Food:

Mangos have long been recognized as more than just edible ripe fruit. The edible uses of the fruit include non-ripe fruit, seed, and processed products such as a chars, chutneys, preserves, etc. The fruit is eaten for its nutritional value, its medicinal value, and for pleasant flavour. Today mango and its flavour are added to many products, such as fruit juices, ice creams, wines, teas, breakfast cereals, mueslibars and biscuits (***IanS.E.bally,2006***).

1.5.2 Fuel:

Firewood and charcoal are the chief uses of the wood, because the trunk is usually too small to make it useful for timber (***FAO, 1986***).

1.5.3 Tannin or dyestuff:

The bark possesses 16% to 20% tannin and has been employed for tanning hides. It yields a yellow dye, or, with turmeric and lime, a bright rose-pink (***IanS.E.bally, 2006***).

1.5.4 Seed fat:

The presence of high amounts of the essential oleic suggests that the mango seed oil could be to be used in some as nutrient rich food (***Kittiphoom and Sutasinee, 2013***). The available stearic acid could be used in the production of detergents, soaps, and cosmetics such as shampoos and shaving cream products. Good levels of oleic were observed in the mango seed kernel oil (11.43-10.99% as in Abu-samaka and D. Night respectively). In exception of the oil extracted from Abu-samaka cultivar no linoleic acid was detected. Also, no margric acid was detected in D. Night. Oleic acid (omega-6) is a mono-unsaturated fatty acid found high concentrations in some vegetable oils such as olive oil (55-80%).

High concentrations of oleic acid help in lowering the levels of blood cholesterol and lower the risk of heart problems (***Tine, et al., 2005***).

1.6 Phytochemical study of *Mango (mangifera indica):*

Chemical constituents of mangifera indica are always an interest. The different chemical constituents of the plant, especially the polyphenolics, flavonoids, triterpenoids. Mangiferin a xanthone glycoside major bio-active constituent, isomangiferin, tannins & gallic acid derivatives.The flower yielded alkyl gallates such as gallic acid, ethyl gallate, methyl gallate, n-propyl gallate, n-pentyl gallate, n-octyl gallate, 4-phenyl gallate, 6-phenyl-n-hexyl gallate and dihydrogallic acid. Roots of mango contain the chromones, 3-hydroxy-2-(4'-methylbenzoyl)-chromone and 3-methoxy-2-(4'-methybenzoyl)-chromone. The leaves and flowers yield an essential oil containing humulene, elemene, ocimene, linalool, nerol and many others. The fruit pulp contains vitamins A and C, β-carotene and xanthophylls. An unusual fatty acid, cis-9, cis-15-octadecadienoic acid was isolated from the pulp lipids of mango. Phenolic Antioxidants, free sugars and polyols isolated and analyzed from Mango Stem Bark (***Shaha et al., 2010)***.

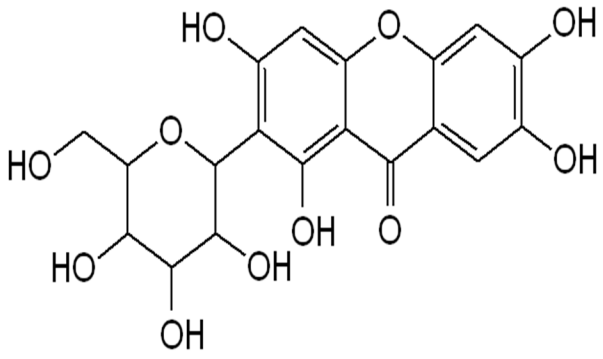

Fig (1.2) : Structure of Mangiferin

1.7. Toxicity:

The sap which exudes from the stalk close to the base of the fruit is somewhat milky at first, also yellowish-resinous. It becomes pale-yellow and translucent when dried. It contains mangiferen, resinous acid, mangiferic acid, and the resinol, mangiferol. It, like the sap of the trunk and branches and the skin of the unripe fruit, is a potent skin irritant, and capable of blistering the skin of the normal individual. As with poison ivy, there is typically a delayed reaction. Hypersensitive persons may react with considerable swelling of the eyelids, the face, and other parts of the body. They may not be able to handle, peel, or eat mangos or any food containing mango juice. A good precaution is to use one knife to peel the mango, and a clean knife to slice the flesh to avoid contaminating the flesh with any of the resin in the peel ***(Morton, 1987)***.

The leaves contain the glucoside, mangiferine. In India, cows were formerly fed mango leaves to obtain from their urine euxanthic acid which is rich yellow and has been used as a dye. Since continuous intake of the leaves may be fatal, the practice has been outlawed. When mango trees are in bloom, it is not uncommon for people to suffer itching around the eyes, facial swelling and respiratory difficulty, even though there is no airborne pollen. The few pollen grains are large and they tend to adhere to each other even in dry weather. The stigma is small and not designed to catch windborne pollen. The irritant is probably the vaporized essential oil of the flowers which contains the sesquiterpene alcohol, mangiferol, and the ketone, mangiferone. Mango wood should never be used in fireplaces or for cooking fuel, as its smoke is highly irritant ***(Morton, 1987)***.

1.8. Mango oil:

Mango kernel oil has been used in the cosmetics industry as an ingredient in soaps, shampoos and lotions because it is a good source of phenolic compounds (***Soong and Barlow, 2004***) including microelements like selenium, copper and zinc (***Schiber et al., 2003***). In addition, the extract of mango seed kernel exhibited the highest degree of free-radical scavenging and tyrosinase-inhibition activities compared with methyl gallate and phenolic compounds from the mango seed kernel and methyl gallate in emulsion affected the stability of the cosmetic emulsion systems (***Zhao et al., 2007***).

On a dry basis, the kernel contains (7.1–15)% crude fats . This fat has attracted considerable interest from scientists due to its unique physical and chemical characteristics, which are similar to those of cocoa butter, illipe, shea, kokum and sal butter . It is a promising, safe and natural source of edible fats, a sit does not contain any trans fatty acids .

On the nutritional and toxicological studies of the mango seed kernel, indicated that mango seed kernel fat is promising and a safe source of edible oil and was found to be nutritious and non-toxic so that it could be substituted for any solid fat without adverse effects (***AhmedE.M.Abdalla,2006***).

Table (1.1) : Fatty acid composition(w/w%) of extracted oil with hexane solvent(*Kittiphoom, 2013*):

Fatty acid	Hexane extract
Palmitic acid(16:0)	8.97
Stearic acid(18:0)	37.37
Oleic acid(18:1)	43.77
Linoleic acid(18:2)	6.76

1.9. Oil properties:

1.9.1 Physical properties:

1.9.1.1 Pour point:

The temperature bellow which an oil will not flow. If the ambient temperature is bellow the pour point. The oil will essentially behave as a solid (***Allan and Joullie, 1960***).

1.9.1.2 Distillation characteristics:

As the temperature of the oil is raised, different compounds reach their boiling points inturn and are distilled off. This determines the volatility of oil and controls the rate and extent of evaporation (***Neseyanive, 1981***).

1.9.1.3 Density:

A part of determination whether or not an oil will float, its density can often give a general indication of other properties of the oil. Oils with low densities tend to have low viscosities and cotain a high proportion of volatile components (***Catherine, 1934***). the density of crude oils is often expressed as **API** gravity where :

API = (141.5/s.g**)** – 131.5

Where s.g =specific gravity, density relative to pure water.

API = American petroleum institute

API values range from bellow 10 to over 40 but it should be appreciated that high API values relate to low density oils and vice versa.

1.9.1.4 Viscosity:

The viscosity of oil is its resistance to flow through a capillary tube. since viscosity involves the size and the shape of the macro molecular, it was considered as the one of the most important analytical and commercial parameter. The viscosity of a solution may have a complicated variation with

composition. Due to possibility of hydrogen bonding among the solute and solvent molecules.

More hydroxyle groups make high viscosities, because a network of hydrogen bonds is formed between the molecules. High viscosity oils flow with difficulty while oils with low viscosity are highly mobile and spread quickly. Viscosity decreases as temperature increase ***(Bancraff, 1932***).

1.9.1.5 Wax content:

The behavior of oil may be influenced by wax content. Oils with wax contents greater than about 10% tend to have high pour points and if the ambient temperature is below this, the oil will be either a solid or a highly viscosity liquid. High wax contents can also help to stabilize water-in-oil emultions.

1.9.1.6 Refractive index:

The refractive index (n) of a substance with reference to air is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the substance. It varies with the wavelength of the light used in its measurement. Refractive indexes(n) , are stated in terms of the wavelength of the sodium D-line (589.3nm) at a temperature of 19.5°C unless otherwise specified.

The index of refraction is related to the unsaturation of fatty acid being altered besides, by the presence of abnormal or unusual acids, in which the presence of the hydroxyl group raises the refractive index.

1.9.1.7 Infrared spectroscopy:

Infrared spectroscopy has been applied to solid lipids to provide information about polymorphism, crystal structure, conformation and chain-length, but the commonest use of traditional IR spectroscopy has been the recognition and quantitation of trans unsaturation in acids and esters where unsaturation is

predominantly cis, using neat liquids or solutions. One trans double bond absorbs at 968 cm^{-1}. Additional trans centres increase the intensity but do not change the frequency unless they are conjugated when small changes are reported.

There is no similar diagnostic absorption for a cis olefin but Raman spectra show strong absorption bands at 1665±1 cm^{-1} (cis olefin), 1670 ±1 cm^{-1}(trans olefin), and 2230 ± 1 cm^{-1} and 2291 ± 2 cm^{-1} (acetylenes) for the type of unsaturation indicated.

Carbonyl compounds have a strong absorption band in the region 1650-1750 cm^{-1}. The wavelength varies slightly with the nature of the carbonyl compound as in the following saturated and unsaturated compounds: aldehydes (1740-1720 and 1705-1680 cm^{-1}), ketones (1725-1705 and 1685-1665 cm^{-1}), acids (1725-1700 and 1715-1690 cm^{-1}) and esters (1750-1730and 1730-1715 cm^{-1}).

Most oils with the usual mixture of saturated and unsaturated acids have similar infrared spectra. Additional bands associated with less common functional groups include hydroxyl (3448cm^{-1}), keto (1724 cm^{-1}), cyclopropene (1852 and1010 cm^{-1}). Epoxide (848 and 826 cm^{-1}), allene (2222 and 1961 cm^{-1}), vinyl(990 and 909 cm^{-1}) and conjugated enyne systems (952 cm^{-1}) (***FrankD.,2000***).

1.9.1.8 Gas chromatography-mass spectrometry (GC-MS):

Chromatography detection systems usually have automatic readout of the peak area, as well as retention time. With complex mixtures, it is not a simple task to identify the many peaks. The appearance of chromatographic peak at a particular retention time suggests but not guaranteed the presence of a particular compound. Instruments are commercially available in which the gas effluent is automatically fed into a mass spectrometer where they are positively identified

according to the mass (formula weight and fragmentation pattern).this analytical technique is called gas chromatography-mass spectroscopy(GC-MS).

The MS is a sensitive and elective detector and when a capillary GC column is used this technique is capable of identifying and quantifying unbelievably complex mixture of trace substances. MS produces, separates, and detects ions in gas phase. A sample with moderately high vapour pressure is introduced in an inlet system, operated under vacuum and at high temperature. It vaporizes and carried to ionization source. Analyte molecules are typically neutral and must be ionized. This is a ccomplished by various means but typically neutral and must be ionized. This is accomplished by various means but typically is done by bombarding the sample with high . energy electrons in an electron-impact source. The ion are separated in the spectrometer by being accelerated through a mass separator. separation are actually accomplished based on the mass-to-charge (m/e)ratios of the ions. One rule for identifying the molecular ion is the nitrogen rule. In applying this M^+ is taken as highest mass, ignoring isotope contributions. such a molecular mass will be an even number if it contains an even number(0,2,4,…) of nitrogen atoms. So otherwise be an odd number when it contains an odd number of nitrogen atoms, and fragmentation. For example organic molecules seldom lose more than four H atoms, to give M-4 fragments. other losses we would expect to see are methyl groups(M-15),NH_2 or O (M-16),OH or NH_3 (M-17),and C_2H_2 (M-26). So there should be no losses of 4 to 14 or 21 to 25 mass units. The most common used ionization source is the electron-impact source, where the gaseous molecules are bombarded by a high-energy beam of electrons. Usually 70 eV, generated from a tungsten filament. An electron that collides with a neutral molecule may impact sufficient energy to remove an electron, resulting in a single charge ion:

$M + e^- \longrightarrow M^+ + 2\,e^-$

Where M is the analyte molecule and M^+ is the molecular ion or parent ion. The M^+ ions are produced in different energy states and the internal energy (rotational, vibrational , and electronic) is dissipated by fragmentation reactions, producing fragments of lower masses, which are themselves ionized or converted to ions by further electron bombardment(***Christian, 2004***).the mass spectrum is a plot of ion abundance versus m/e ratio. The most abundant ion gives rise to the tallest peak in the mass spectrum, called the base peak. The simple removal of an electron from molecule yield an ion whose weight is the actual molecular weight of the original molecule. The molecular ion is a radical – cation since it contains an odd electron as well as a positive charge. For determination of molecular weight of the original correspond to the higest mass in spectrum, the ion must have an odd number of electrons and the ion must be capable of forming the important fragment ions in the spectrum. The molecular ion break a part into fragments and the peaks corresponding to the mass –to-charge for these fragments appear in the mass spectrum. The fragment (ions) formed are cations. Often fragmentation involves the loss of a neutral fragment this fragment does not appear in the mass spectrum. The most common mode of fragmentation involves the cleavage of one bond. In this process the odd-electron molecular ion yields an odd- electron neutral fragment and an even-electron fragment ion. The neutral fragment that is lost is radical and the ionic fragment is carbocation. Ease of fragmentation to form ions increase in the order:

$CH_3^+ < RCH_2^+ < R_2C^+ < R_3C^+ < H_2C{=}CH\text{-}CH_2^+ < C_8H_5\text{-}CH_2^+$

The cleavage of two bonds yields radical-cation and a small molecule. Fragmentation processes may involve rearrangements (migrations of groups) and secondary fragmentation of fragments are also possible (***pavia, 1996***).

1.9.2. Chemical proparties :

1.9.2.1. Acid value:

Acid value is the number of mgs of potassium hydroxide required to neutralize the free acid in one gram of substance. The amount of free acid present in most of the fixed oils is generally low, and the acid value is used to eliminate low grade and rancid oils which tend to have higher acid value (***Backett and Stenlke, 2002***).

1.9.2.2 Saponification value:

The average length of the chain of the fatty acids incorporated into a fat or an oil is expressed by the saponification number, which is defined as the number of mg of potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of one gram of substance. The saponification value is a measure of both free and combined acids. All edible oils have saponification value lying between (188-196). The saponification value is a measure of the equivalent weight of the acids presents, and is therefore useful as an indication of purity. Adulteration with mineral oils would be shown by low saponification values where as rancidity which leads to the formation of low molecular weight acids would be indicated by an abnormally high saponification values. The acid value of most edible oil is small relative to the saponification value (***Backett and Stenlke, 2002***).

1.9.2.3 Ester value:

Ester value is defined as the number of mg of potassium hydroxide required to neutralize the acids resulting from the complete hydrolysis of one gram of materials. Ester value is a measure of the combined acids present in the substance ***(Backett and Stenlke, 2002***).

1.9.2.4 Iodine value:

The degree of unsaturation of a fat or an oil is described by the iodine number , which is defined as the weight of iodine absorbed by 100 parts by weight of the substance . it is a measure of the unsaturated compounds present in +the substance, and is based upon the addition of halogen cross a carbon –carbon double bond. Most edible oils have iodine values in the range 87-62.

A quantitative measurement of the unsaturation present is obtained, provided that, certain experimental conditions are complied with, since there is a tendency with both reagents for substitution reaction as well as addition to take place(***Backett and Stenlke, 2002***).

1.9.2.5 Aldehydes and ketones in oils:

The determination of aldehydes depends upon the reaction with hydroxylamine hydrochloride, which is expressed by the following equation

$$R\text{-}CHO + NH_2OH.HCl \longrightarrow R\text{-}CH\text{-}N\text{-}OH + H_2O + HCL$$

The liberated hydrochloric acid can be titrated with standard alkali (***Backett and Stenlke, 2002).***

1.9.2.6 Acetyl values in oils:

Acetyl value is defined as the number of mg of potassium hydroxide required to neutralize acetic acid liberated by hydrolysis of one gram of the acetylated substance. Acetyl value is a measure of the free alcohols present in the substance and is calculated from the difference between the saponification value of the acetylated and un acetylated substances. Acetyl value is used in the control of cholesterol waxes (***Backett and Stenlke, 2002***).

1.10. Extraction of fixed oil:

Over the centuries, four basic methods of extracting vegetable oil from the various seeds were developed. Those four methods are: the first was boiling in

water leading to a partial separation of oil, which was skimmed off the top of the vessel. The second was the cage-type press in which pressure was put on stationary mass by levers, screw jacks or hydrolic cylinders and the vegetable oil flowed from the compressed mass to collecting rings bellow. Both these methods are more or less obsolete, the third method is the mechanical screw press, and fourth is solvent extraction (***Bredeson, 1978***).

1.11. Solvent extraction:

Solvent extraction has been defined as a process for transporting materials from one phase to another, for the purpose of separating one or more compounds from mixtures. Various solvents have been used commercially and other have been proposed to be used encouraging laboratory result (***Jonhnson and lusas, 1983).***

1.12. Soxhlet extractor:

a soxhlet extractor is a piece of laboratory apparatus invented in 1879 by von soxhlet. It was originally designed for the extraction of a lipid from a solid material. Typically, a 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 (***Jensen and William, 2007***).

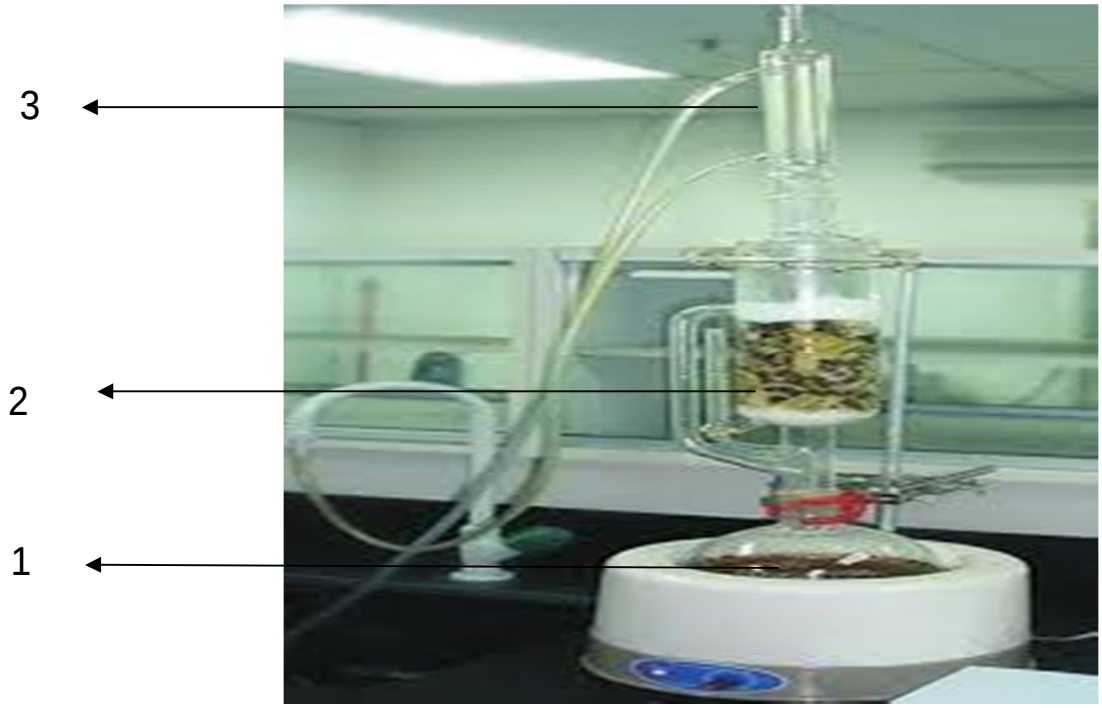

Fig (1.3): Soxhlet extractor

1.12.1. Description:

A soxhlet extractor has three main section: a percolator (boiler and reflux) which circulates the solvent, a thimble (usually made of thick filter paper) which retains the solid to be laved, and a siphon mechanism, which periodically empties the thimble (***Soxhlet and Franzvon,1879***).

In this method the sample is dried, ground into small particles and placed in a porous cellulose thimble. The thimble is placed in an extraction chamber (2), which is suspended above a flask containing the solvent (1) and above a condenser (3). The flask is heated and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask. At the end of the extraction process, which lasts a few hours, the flask containing the solvent and lipid is removed.

In some device a funnel allows to recover the solvent at the end of the extraction after closing a stopcock between the funnel and the extraction chamber. The solvent in the flask (1) is then evaporated and the mass of the remaining lipid is measured. The percentage of lipid in the initial sample can then be calculated (***Soxhlet and Franzvon,1879***).

1.12.2. Operation:

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 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 trans port any solid material to the still pot . this cycle may be allowed to repeat many times ,over hours or days.

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 ***(Soxhlet and Franzvon.,1879).***

1.13. Aims of this work:

The aims of this work are to extract and analyze the fixed oil of mango seed kernel.

In this study physical and chemical proparties of mango seed oil were investigated, and so the anti-bacteria and antifungal activities of the mango seed oil.

CHAPTER TWO

2. MATERIALS & METHODS

2.1. Materials:-

2.1.1Collection of sample:

Mango fruits are purchased from Khartoum north local market. The seeds were washed and air dried and the kernels were removed manually from the seeds.

2.1.2 Equipments:

- Sensitive balance (AND, Japan).
- Soxhlet extractor (duran ceuland).
- Rotator evaporation (BUCHI 461Water bath made in switzerland).
- Refractometer (DR-A1-PLUS, England).
- Pycnometer (Gay-lussac, Germany).
- Viscometer (Ostwald, Japan).
- pH meter ELLCDL1120 pH meter.
- Infrared spectrometer (FT.IR 8400S, Japan).
-GC-MS (QP2010 Ultra, shimadzu, japan).
- Water bath (Kottermann, Germany).
- Oven (FN. 400, NO. 03-162,Turkey)
- Thermometer (Hallenkamp, England).

2.1.3. Chemicals:

- N-hexane.
- Ethanol .
-potassium hydroxide.
- Sodium hydroxide .

- Sulfuric acid .
- Hydrochloric acid .
- Distilled water.
- Chloroform.
- Glacial acetic acid .
- Potassium Iodide.
- Starch.
- Phenolphthalein Indicator.
- Sodium thiosulphate.
- Di chloromethane.
- Iodine mono chloride .
- Super saturated sodium chloride solution.
- Buffer solution (pH= 7).

2.2. Methods:-

2.2.1 Preparation of the sample:

The seeds were washed and air dried and the kernels were removed manually from seeds. The kernels were chopped and dried. The dried material was ground in a hammer mill into a powdery form and kept in a closed dark glass bottle and stored at 4° C until utilization.

2.2.2 Extraction of oil:

292.0g of mango seeds kernel were extracted with n-hexane using soxhlet extractor apparatus for about 8 hours. The Extract was then filtered through filter paper and the solvent was evaporated under reduced pressure using rotary evaporator apparatus. The obtained oil was subjected to different physical, chemical and antimicrobial studies.

2.3. Physical analysis of mango oil:

2.3.1 Determination of density:

The pycnometer was cleaned with ethanol and dried in an oven. The dry pycnometer was filled with water to proper point on pycnometer; the pycnometer was wiped dry with towel and weighed. After that the pycnometer was emptied and dried in an oven, and then the dry pycnometer was filled with the oil sample in such a manner to prevent entrapment of air bubbles. Carefully any oil that has come out of the capillary was wiped off, and weighed. Both weights were carryout in the same temperature ***(A.O.A.C., 2000)***.

2.3.2 Determination of refractive index:

The refractive index of oil sample was determined using refractometer.

The sample chamber containing the lens was opened and cleaned with acetone then plugged to source of light. The equipment was calibrate with a drop of water, after which a drop of the oil sample was added into the sample chamber and closed. The adjustment knob was turned crossed the cross bar then the reading were taken at 40°C (***A.O.A.C., 2000***).

2.3.3 Determination of viscosity:

The method of determining viscosity (kinematic viscosity) utilized the capillary tube viscometer.

The oil sample was placed into a glass capillary U-tube and the sample was drawn through the tube using suction until it reached the start position indicated on the tube under gravity.The narrow capillary section of the tube controls the oils flow rate. Once the oil meniscus touched the lower line (***A.O.A.C., 2000***).

2.4. Chemical study of mango oil:

2.4.1 Determination of acid value(A_v) :

1.0g of oil sample was Dissolved in 5ml of a mixture of equal volumes of ethanol (96%) and light petroleum ether previously neutralized with (0.1M) potassium hydroxide, using 0.1ml of phenolphthalein indicator. The solution was titrated with 0.1M potassium hydroxide until the pink colour persists for at least 15s .

The acid value was calculated as followed:

Acid value = 5.61*N/M

Where:

N: volume of alkaline

M: weight of sample

2.4.2 Determination of saponification value(S_v):

4.0g of potassium hydroxide were dissolved in 20 ml of water and sufficient ethanol (96%) was added to produce 1000ml.The mixture was allowed to stand over night and the clear liquid was poured off. 1.0g of the substance was weighted into a 200-ml flask, and 25 ml of the ethanolic solution of potassium hydroxide was added, the solution was boiled under a reflux condenser for 1 hour, rotating the content frequently. While the solution is still hot, the solution was titrated with 0.5M hydrochloric acid using 1ml of phenolphthalein indicator. The operation was repeated without the substance being examined (blank).

The saponification value was calculated from the expression:

saponification value = 28.05v/w

Where :

V: the difference between the two titrations and w is the weight, in g, of substance taken.

2.4.3 Determination of peroxide value (P_v):

5.0g of the sample was placed in a 250ml conical flask fitted with aground-glass stopper. 30ml of a mixture of 2 amounts of chloroform and 3 amounts of glacial acetic acid were added, then Shaked to dissolve the substance and 0.5ml of saturated potassium iodide solution was added, then Shaked for exactly 1min.then 30ml of water was added and the mixture was titrated with 0.01M sodium thiosulphate. The titrant was added slowly with continuous vigorous shaking, until the yellow colour is almost disappeared, then 5ml of starch solution was added and the titration was continued ,with vigorously shaking, until the colour is disappeared (A1ml of 0.01M sodium thiosulphate). A blank test was carried out under the same conditions(A2ml of 0.01M sodium thiosulphate). The volume of 0.01M sodium thiosulphate used in the blank titration must not exceed 0.1ml.The peroxide value was calculated by using the formula:

Peroxide value =10(A1-A2)/M

2.4.4 Determination of Iodine value:

0.25g of the studied sample was dissolved in 10ml of dichloromethane in a dry iodine flask, 20ml of iodine mono chloride solution was added, The stopper was inserted, and allowed to stand in the dark at (15 -25)C° for 30 minutes. 15 ml of dilute potassium iodide solution was placed. The stopper was carefully removed, the stopper and the sides of the flask were rinsed with 100ml water, shaked and titrated with 0.1M sodium thiosulphate, using starch as indicator. At the same time the operation was carried out in exactly the same manner, but

without the substance being examined. The iodine value was calculated from the expression:

Iodine value=1.269v/w

Where:

V: The difference, in ml, between the titrations and w is the weight, in g ,of the substance taken.

2.4.5 Determination of ester value (E_v):

Ester value was calculated from the saponification value (S_v) and the acid value(A_v).

2.5. Biological activity:

2.5.1. Preparation of the studied organisms:

2.5.1.1. Preparation of bacterial suspensions:

1.0 ml aliquots of a 24 hours broth culture of the studied bacteria was aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours. The bacteria growth was harvested and washed off with 100ml sterile normal saline, to produce a suspension containing about 10^8-10^9 C.F.U/ml. The suspension was stored in the refrigerator at 4°C till used.

The average number of viable bacteria per ml of the stock suspension was determined by means of the surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for 2 hours at room temperature for the drops to dry and then incubated at 37°C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02ml) was multiplied by 50 and by the

dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.

2.5.1.2. Preparation of fungal suspension:

The fungal cultures were maintained on sabouraud dextrose agar, incubated at 25° C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

2.5.2. In vitro testing of extracts for antimicrobial activity:-

2.5.2.1. Testing for antibacterial activity:

The cup-plate agar diffusion method was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts.

1.0 ml of the standardized bacterial stock suspension 10^8-10^9 C.F.U/ml were thoroughly mixed with 100ml of molten sterile nutrient agar which was maintained at 45°C. 20ml aliquots of the inoculated nutrient agar were distributed into sterile petri-dishes. The agars were left to set and in each of these plates 4 cups(10mm in diameter)were cut using a sterile cork borer(no.4)and agar discs were removed. Alternate cups were filled with 0.1ml sample of each of the oils dilutions in methanol using automatic microliter pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 18 hours. Two replicates were carried out for each extract against each of the test bacteria. After incubation the diameter of the resultant growth inhibition zones were measured (Table2.1).

Table (2.1): Type of bacterial and proparties:

Microorganisms	Amino acid and gram stain
Bacillus subtilis	NCTC 8236 (Gram + ve bacteria)
Staphylococcus aureus	ATCC 25923 (Gram+ve bacteria)
Klebsiella pneumonia	ATCC 53657(Gram –ve bacteria)
Pseudomonas aeruginosa	ATCC 27853 (Gram –ve bacteria)
Escherichia coli	ATCC 25922(Gram –ve bacteria)

2.5.2.2 Testing for antifungal activity:

The same method as for bacteria was adopted. Instead of nutrient agar , sabouraud dextrose agar was used. The inoculated medium was incubated at 25°C for two days for the candid albicans(Table 2.2).

Table (2.2): Type of fungal and proparties:

microorganism	Amino acid
Candida albicans	ATCC7596

2.6. Spectroscopic analysis of the extracted oil:

2.6.1 FTIR analysis:

1.0 drop of extracted oil was placed between two plates of solid sodium chloride .The plates were transparent to the infrared light and did not introduce any lines onto the spectra. The drop formed a thin film between the plates, and then the plates were taken to FTIR instrument.

2.6.2. Determination of fatty acid composition by GC-MS:

2.6.2.1Preparation of methyl esters of the fatty acid:

2ml of the mango seeds oil was taken and transfer to glass test tube, then 7ml of methanolic sodium hydroxide and 7ml of methanolic sulfuric acid was added, after that gentle shaking was made for three minutes then the test tube was placed in test tube rack for overnight, after that a suitable volume from super saturated sodium chloride was added and a suitable volume from normal hexane was added then the mixture was well shaken. After three minutes two layers separated, the upper layer is the organic layer which contains the fatty acid and the lower layer is the aqueous layer. From the upper organic layer 5µL was taken and diluted with 5ml diethyl ether then 1.0g sodium sulphate as drying agent was added. After that the sample was taken to GC-MS. Methanolic sodium hydroxides were prepared by dissolving 2.0g of sodium hydroxide in 100 ml methanol. Methanolic sulfuric acid was prepared by taken 1ml from concentrated sulfuric acid in 99 ml methanol.

2.6.2.2 GC-MS analysis:

GC-MS was carried out on sample of oil used GC-MS (QP 2010Ultra) instrument.

CHAPTER THREE

3. RESULTS& DISCUSSION

3.1Results:

3.1.1. Oil content:

Table (3.1) : Percentage yield of Extracted Oil

Weight of sample(g)	Weight of oil(g)	Yield%
292	23.393	12.48

3.1.2. Physical properaties of extracted oil:

Table (3.2): Physical Properties of Extracted Oil:

Test	Value
Density	0.8958 g/ml
Viscosity	14.186 mpa.s
Refractive index	1.467
Melting point	35 $^{\circ}$C
pH	5.6

3.1.3. Chemical proparaties of extracted oil:

Table (3.3) :Chemical proparties of extracted oil:

Test	Value
Acid value	2.805
Saponification value	190
Peroxide value	5.0
Iodine value	49.173
Ester value	187.195

3.1.4. FTIR analysis of extracted oil:

Table (3.4) :Characteristic absorption peaks of extracted oil

Functional group	Characteristic absorption (cm^{-1})
Alkyl (C-H) stretch	3005
Hydroxyl (O-H)	2855-2924
Carbonyl (C=O)	1747
Alkenyl (C=C)	1647
C-O	1163-1238
Alkyl(C-C)	871
CH_2 bending	1465

3.1.5. GC-MS analysis of extracted oil:

Table (3.5): Retention Time (R_t) and (area%)of the Compounds Identified in the Extracted Oil:

Peak	R.T	Area	Area%	compound
1	3.123	195128	1.21	Oxime
2	4.029	1274380	7.92	Glycerin
3	4.766	45599	0.28	2-ethyl-hexanol
4	11.372	464091	2.88	Butylated hydroxyl ltoluene
5	15.828	1074898	6.68	Hexadecanoic acid
6	17.483	459054	2.85	9,12octadecandienoicacid(z,z)
7	17.528	4944959	30.72	9,octadecanoic acid(z)
8	17.743	6216627	38.62	Methyl stearate
9	19.502	337107	2.09	Methyl18-methylnonadecanoate
10	20.413	142832	0.89	Phenol,2,2-methylenebis[6-(1,1-dimethylethyl)-4-methyl]
11	22.545	944277	5.87	9,19-cyclolanost-24-en-3-ol
		16098952	100.00	

3.1.6. Biological activity of extracted oil:

Table (3.6): biological activity of extracted oil

Biological Activity	Antibacterial activity				Antifungal activity
microorganism	Ec	Ps	Sa	BS	Ca
Inhipition zone	0	0	0	0	0

3.2. Discussion

3.2.1. Percentage yield of oil:

The percentage yield of extracted oil from the mango kernel seeds was found to be 12.482 %.The oil yield levels reported in this work for mango seed are useful in food industries. The comparison of the yield percentage of the extracted oil from mango seed in sudan with variety from Congo results obtained showed that the mango seeds contained 13% crude oil (***Dhingras and Kapoor, 1985***). Variation in oil yield may be due to the differences in variety of plant, cultivation climate, ripening stage, the harvesting time of the seeds kernels and the extraction method used.

3.2.2. physical Proparties:

3.2.2.1 Density:

The density of extracted oil shown in table (3.2) equal to 0.8958g/ml is small value when compared to the density of water.

3.2.2.2 Refractive index:

The refractive index of extracted oil shown in table (3.2) equal to 1.467 mpa. They were in close agreement with values reported for conventional oil as for soybean (1.466-1.470) and palm kernel (1.449-1.451) (***De Bussy, 1975***). The high refractive index of mango kernel seems to confirm the high number of carbon atoms in their fatty acids. Refractive index increase also as the double bond increases.

3.2.2.4 FTIR result:

The results of Fourier transform infrared (FTIR) spectra analysis of mango seed oil were presented in table (3.4) indicates that:

The absorption observed at 3005$cm^{-1}$is due to the carbon –hydrogen single bond (C-H) stretching vibration.

The absorption range of 2852-2924cm^{-1} recorded is characteristic of oxygen hydrogen single bond (O-H) stretching vibration.

The absorption observed at 1747cm^{-1} is due to the carbon –oxygen double bond (C=O) stretching vibration.

The absorption observed at 1647cm-1 is due to the carbon –carbon double bond (C=C) stretching vibration.

A range of 1163-1238cm-1absorption recorded is characteristic of carbon-oxygen single bond (C-O) stretching vibration.

The absorption observed at 871cm^{-1} is due to the carbon-carbon single bond (C-C) stretching vibration .

The 1465 cm^{-1} absorption is likely to be for CH_2 bending (scissoring). And at 721cm^{-1} for CH_2 bending (rocking).

The 1417cm^{-1} absorption is likely to be for CH_3 out of plane bending, and 1377cm^{-1} for CH_3 in plane bending.

3.2.2.5 Gas chromatography mass spectroscopy (GC-MS):

Gas chromatography mass spectroscopy analysis reveals that the main fatty acid compositions in mango seeds oil, which are characterized in table (3.5).

Mango seed oil is characterized by a high content of stearic acid and oleic acid as a principal fatty acid, there are different amount of minor fatty acid, palmitic, lenoleic acid. The minor component of anti oxidant (butylated hydroxyl toluene), oxime, methyl 18-methylnonadecanoate, triterpenoid(9,19-cyclolanost-24-en-3-ol).

3.2.3. Chemical Proparties:

3.2.3.1 Acid value:

The acid value of extracted oil was found to be about 2.805. Since the acid value is used to determine the ability of any oil for resisting the rancidity or not. The low acidity of extracted oil indicates that; the mango seed was almost free from hydrolytic rancidity brought almost by lipases and enables the direct use of such an oil in industries without further neutralization.

3.2.3.2 Saponification value :

The Saponification value is use to measure both free and combined acid as an indication of purity, and the low saponification value shows that; the oils are adulterated with mineral oils equal to 190. This indicates that the oil contains a high amount of triglycerides which effects its ability to form soap.

3.2.3.3 Iodine value :

The Iodine value is use to study the saturation and un saturation of compound, which are present in the substance. The iodine value of extracted oil was determined and its value is 49.173.

3.2.3.4 Peroxide value :

The Peroxide value is use to determine the rancidity of the oils. All edible oils have peroxide values less than 10. The peroxide value of extracted oil is equal to 0.960 this indicates that; mango kernel oil had a high quality due to the low level of peroxide value.

3.2.4. Biological Activity:

One experiment was conducted to test the effectiveness of mango oil as an antimicrobial for certain types of bacteria escherichiacoli, pseudomonas aeruginosa, staphylococcus aureus , bacillus subtilis and fungul , candida alibican. All the extracted mango seed oil doesn’t have an activity against the tested microorganisms.

3.3. Recommendation:

The study recommended the usage of mango seed kernel for oil production as a substitute for vegetable oil. Further, it has the potential to be utilized for extraction of ingredients that could be used for the development of some industries rather than to be discarded as a waste.

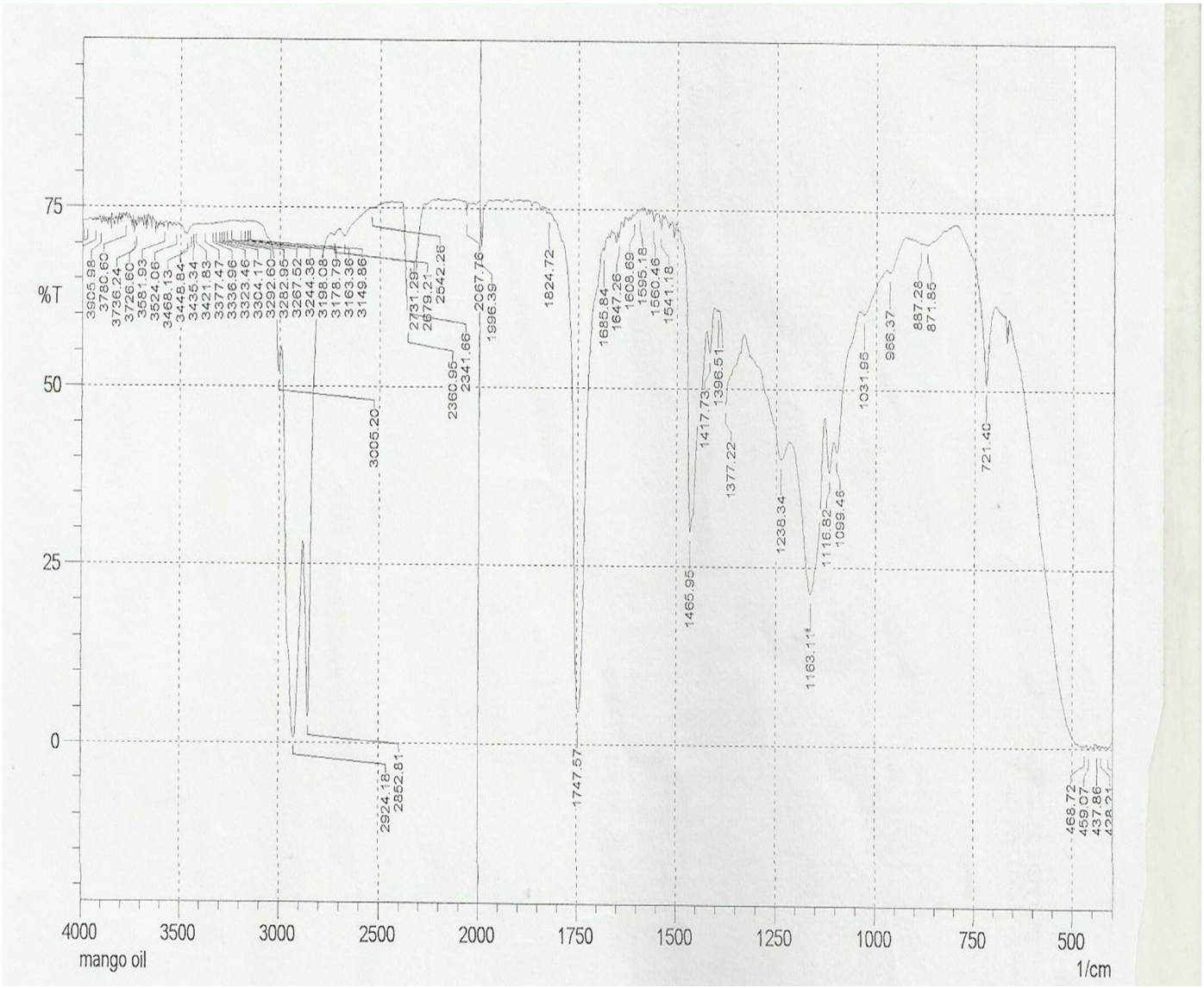

Fig (3.1): FTIR spectra of extracted oil.

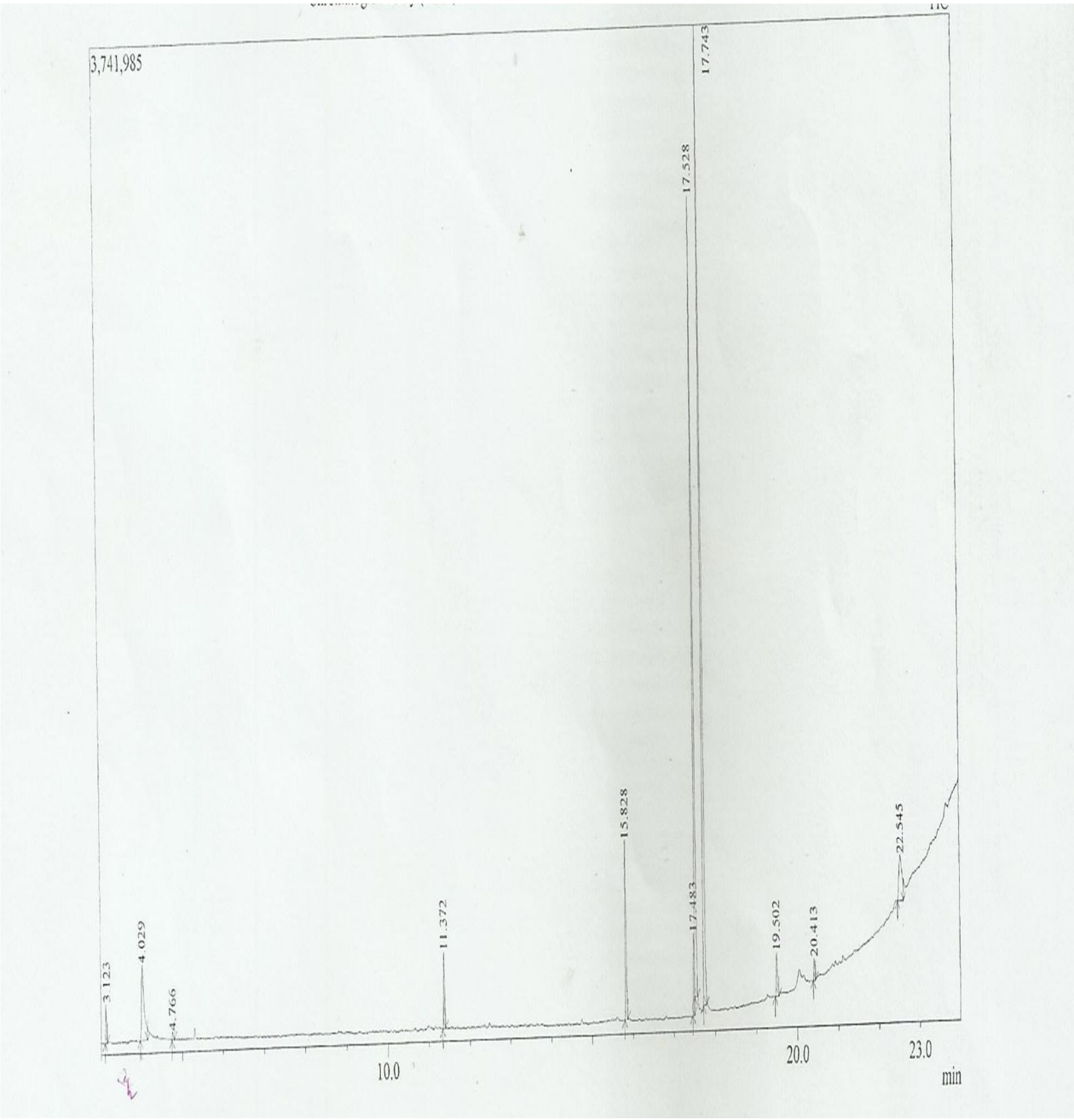

Fig (3.2): GC-MS spectra of the mango seed oil.

Peak at R.time: 3.123

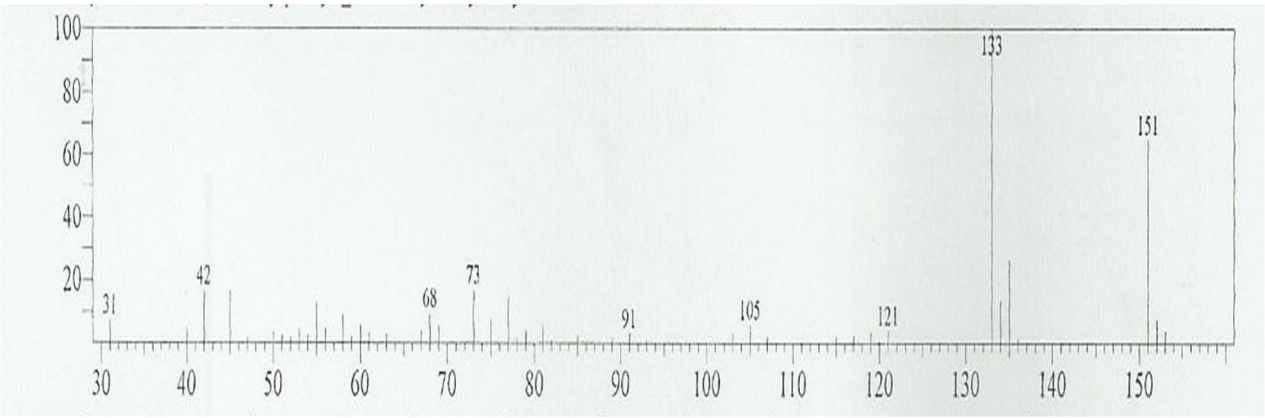

Figure (3.3) : GC.MS spectrum of oxime

Peak at R. Time: 4.29

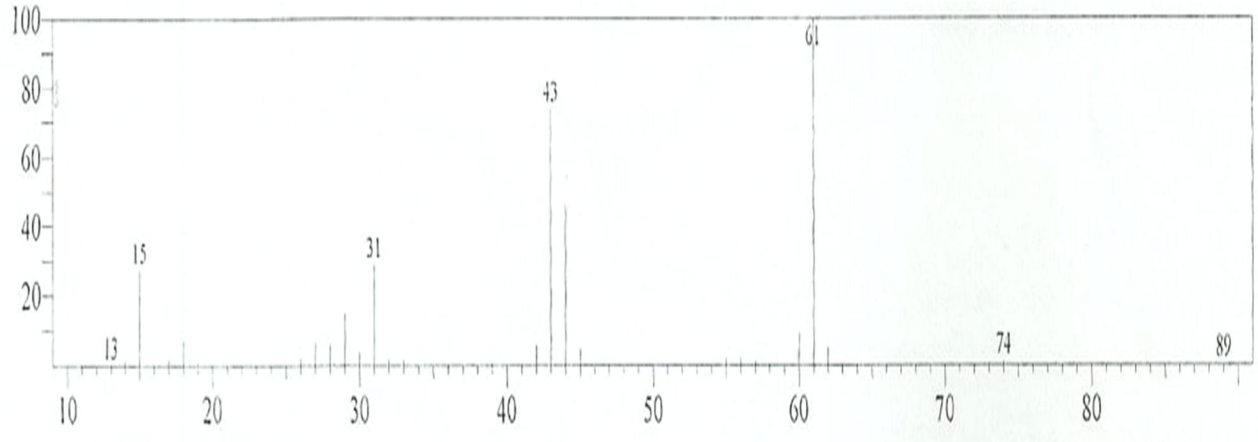

Figure (3.4): GC-MS spectrum of glycerin.

Peak at R.Time:4.766

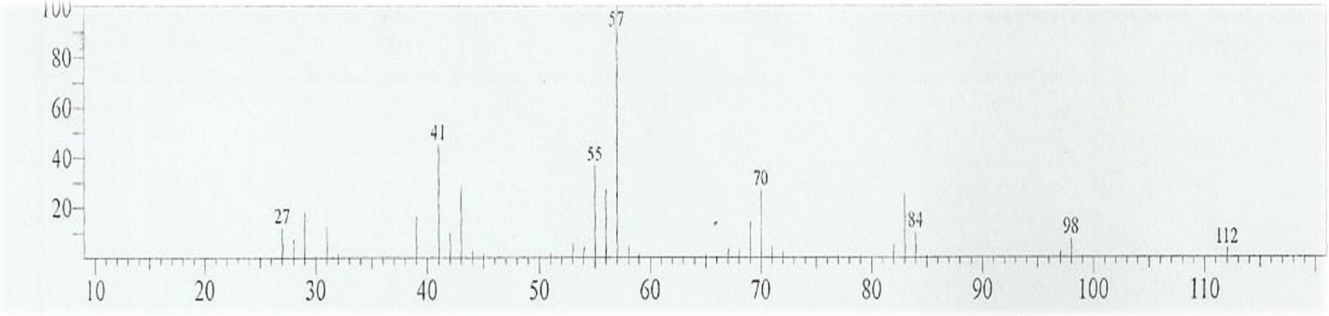

Figure (3-5): GC-MS spectrum of 2-ethyl- hexanol

Peak at R.Time: 11.372

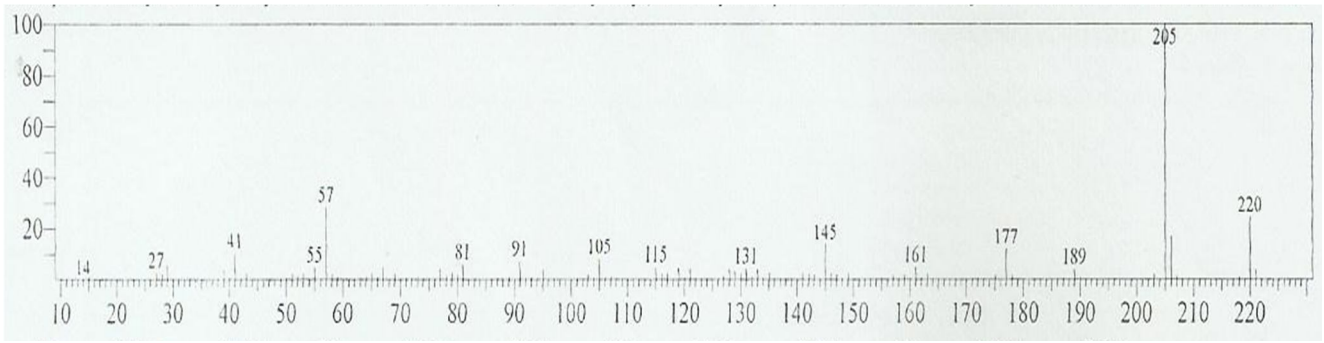

Figure (3.6) :GC-MS spectrum of butylated hydroxytoluene.

Peak at R.Time: 17.483

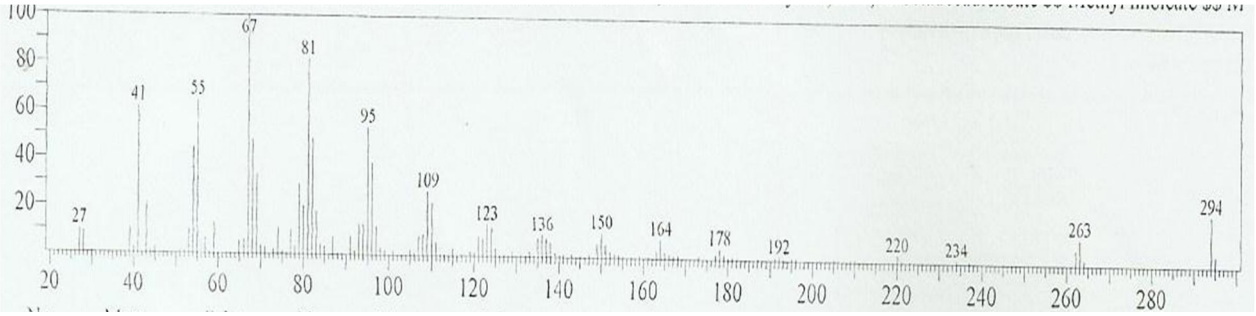

Figure (3.7):GC-MS spectrum of 9,12-octadecadienoic acid(z,z), lenoleic acid.

Peak at R.Time: 17.528

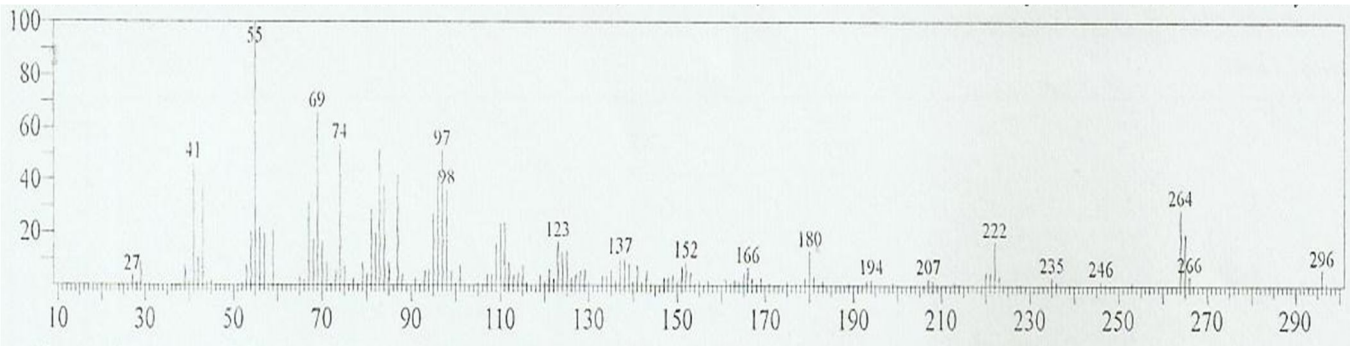

Figure (3.8): GC-MS spectrum of 9-octadecenoic acid(z),oleic acid.

Peak at R.Time:17.743

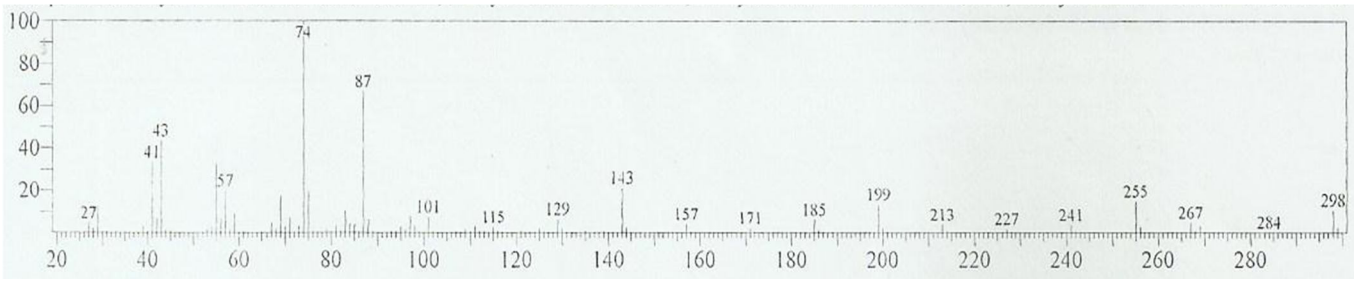

Figure (3.9): GC-MS spectrum of methyl stearate, stearic acid.

Peak at R.Time: 19.502

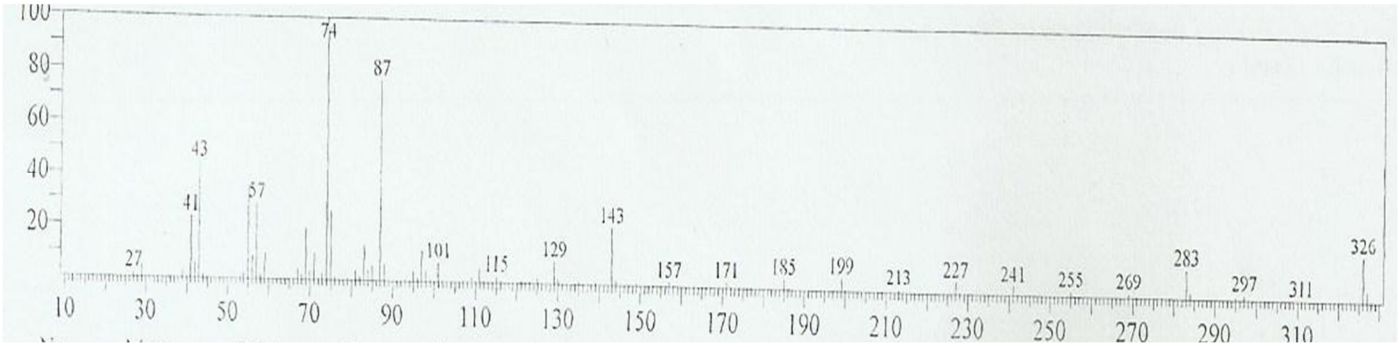

Figure (3.10): GC-MS spectrum of methyl 18-methylnonadecanoate.

Peak at R.Time: 20.413

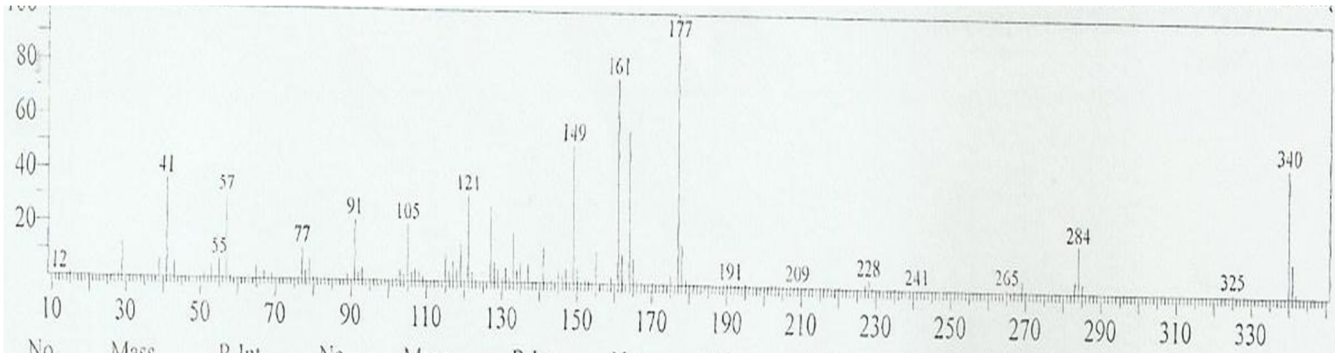

Figure (3.11): GC-MS spectrum of phenol,2,2`-methylenebis[6-(1,1-dimethylethyl)-4-methyl-]

Peak at R.Time: 22.545

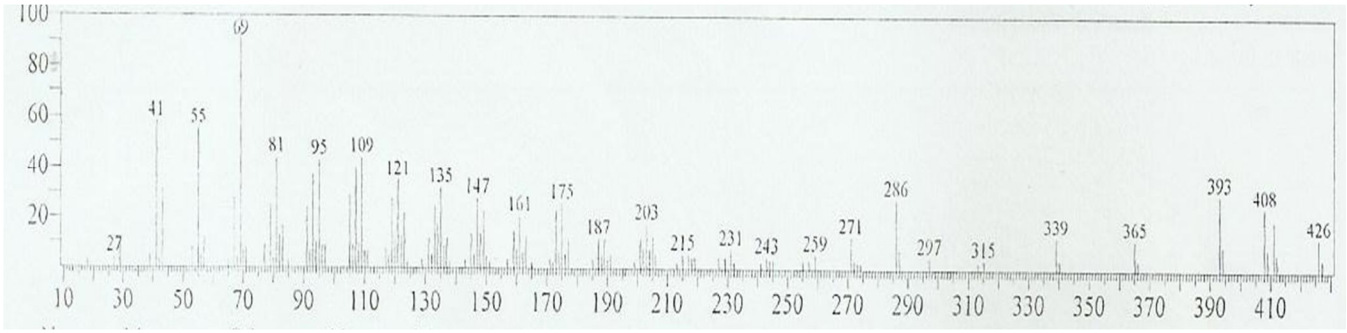

Figure (3.12): GC-MS spectrum of 9, 19-cyclolanost-24-en-3-ol.

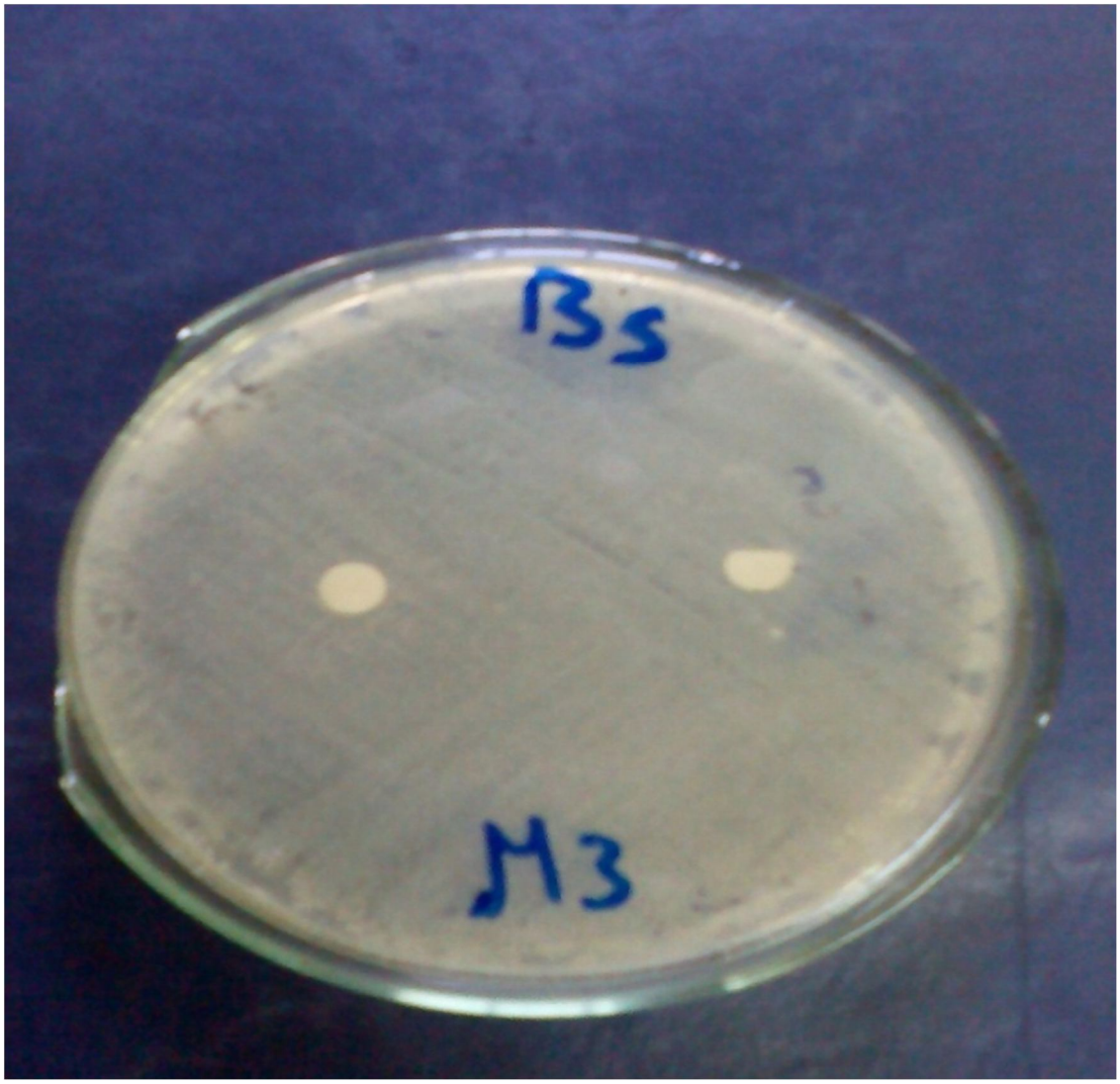

Fig (3.13): Antibacterial activity of mango oil (bacillus subtilis)

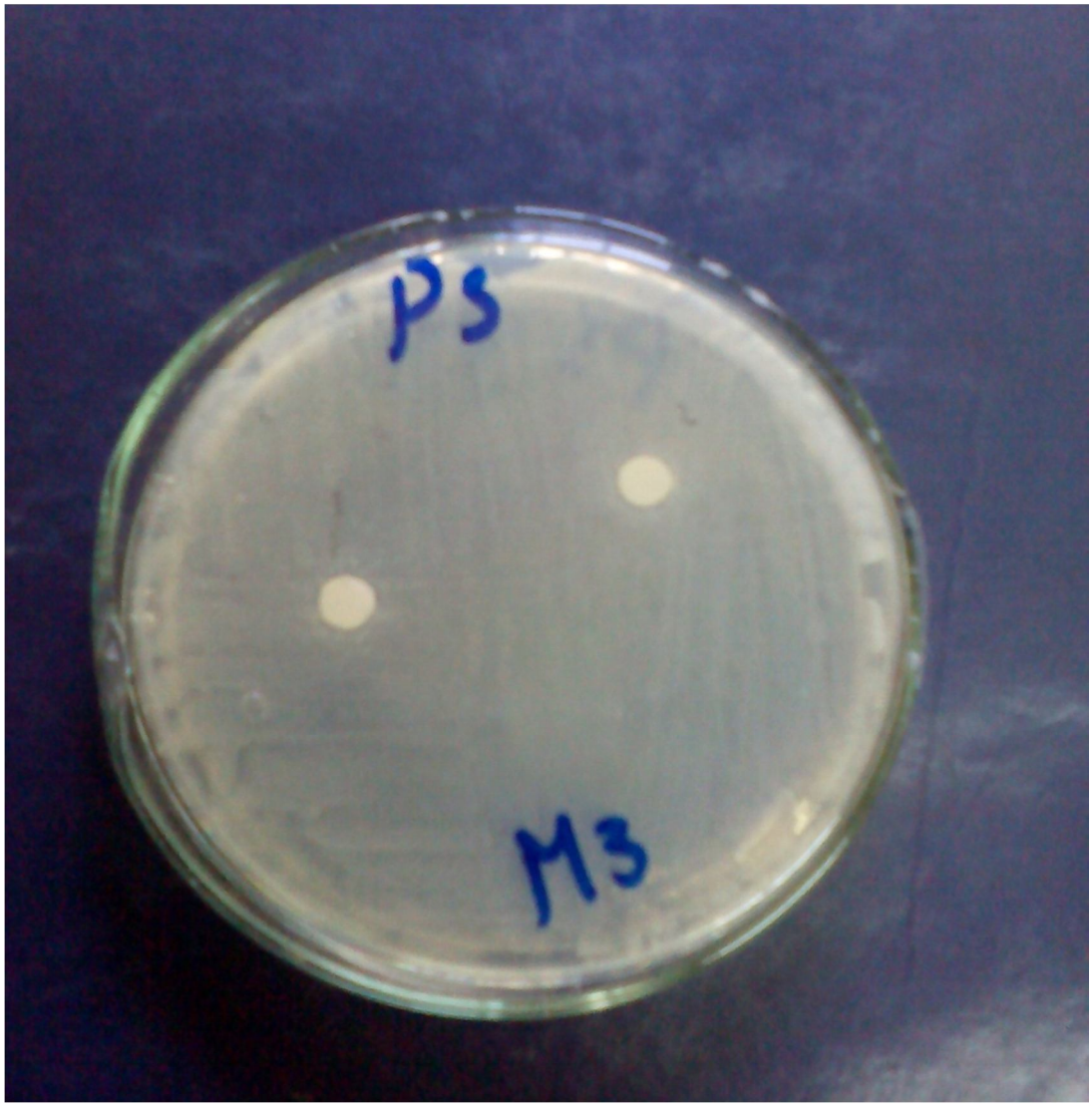

Fig (3.14): Antibacterial activity of mango oil (Pseudomonas aeruginosa)

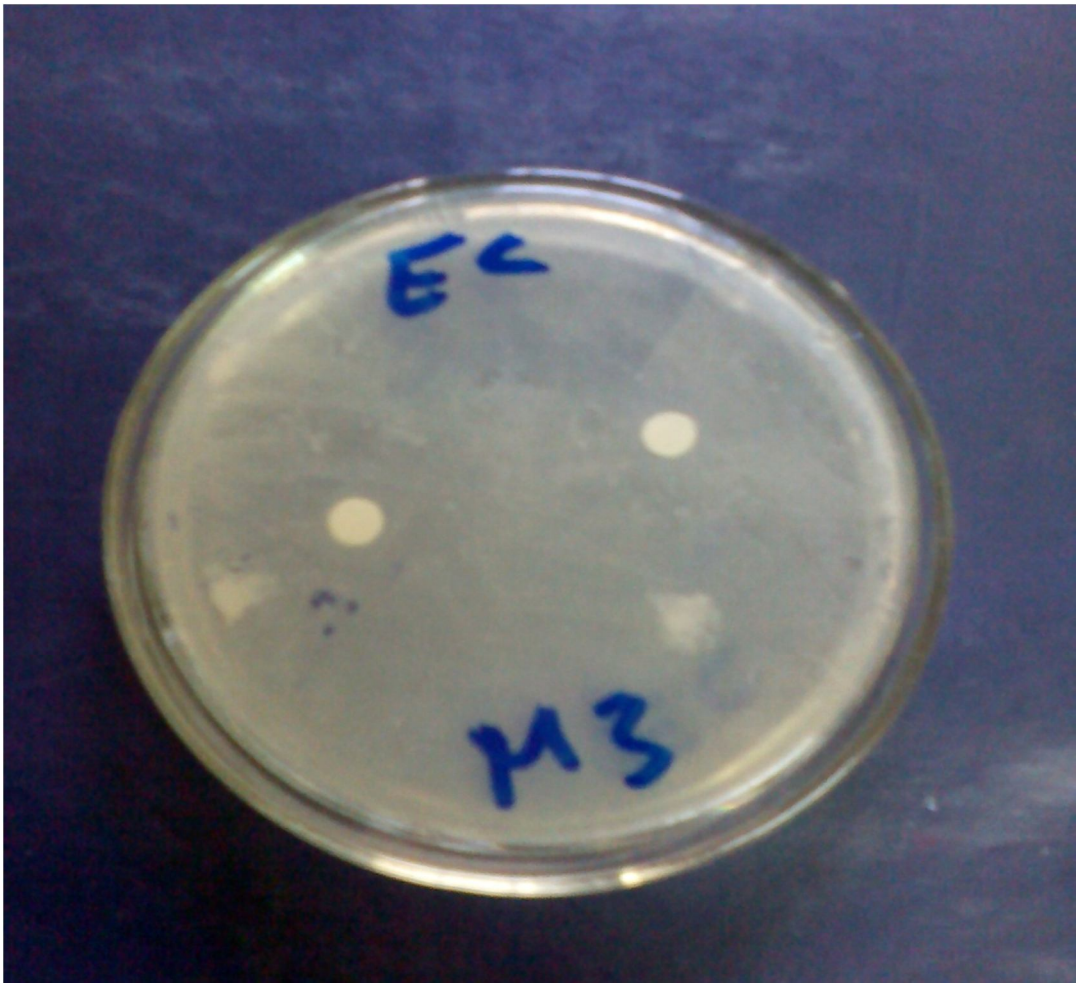

Fig (3.15): Antibacterial activity of mango oil (escherichiacoli)

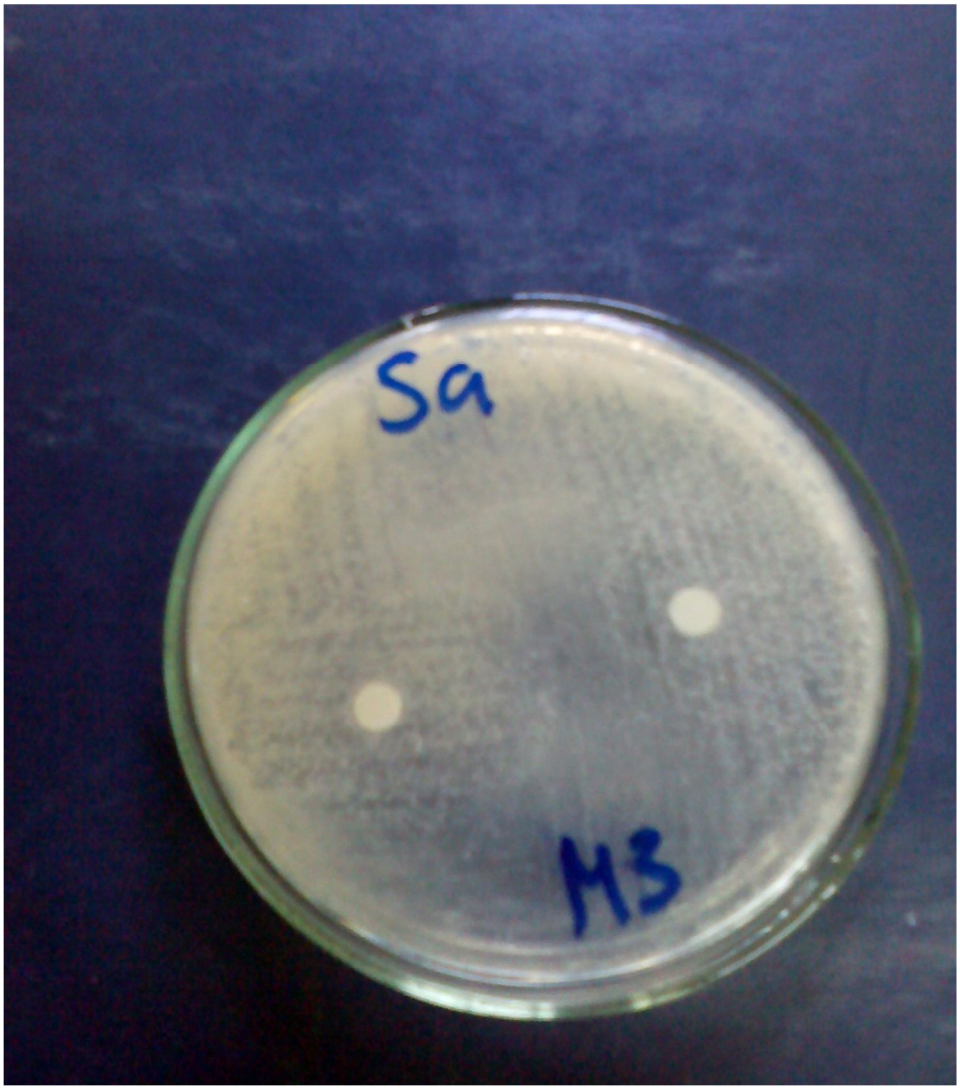

Fig (3.16): Antibacterial activity of mango oil (Staphylococcus aureus)

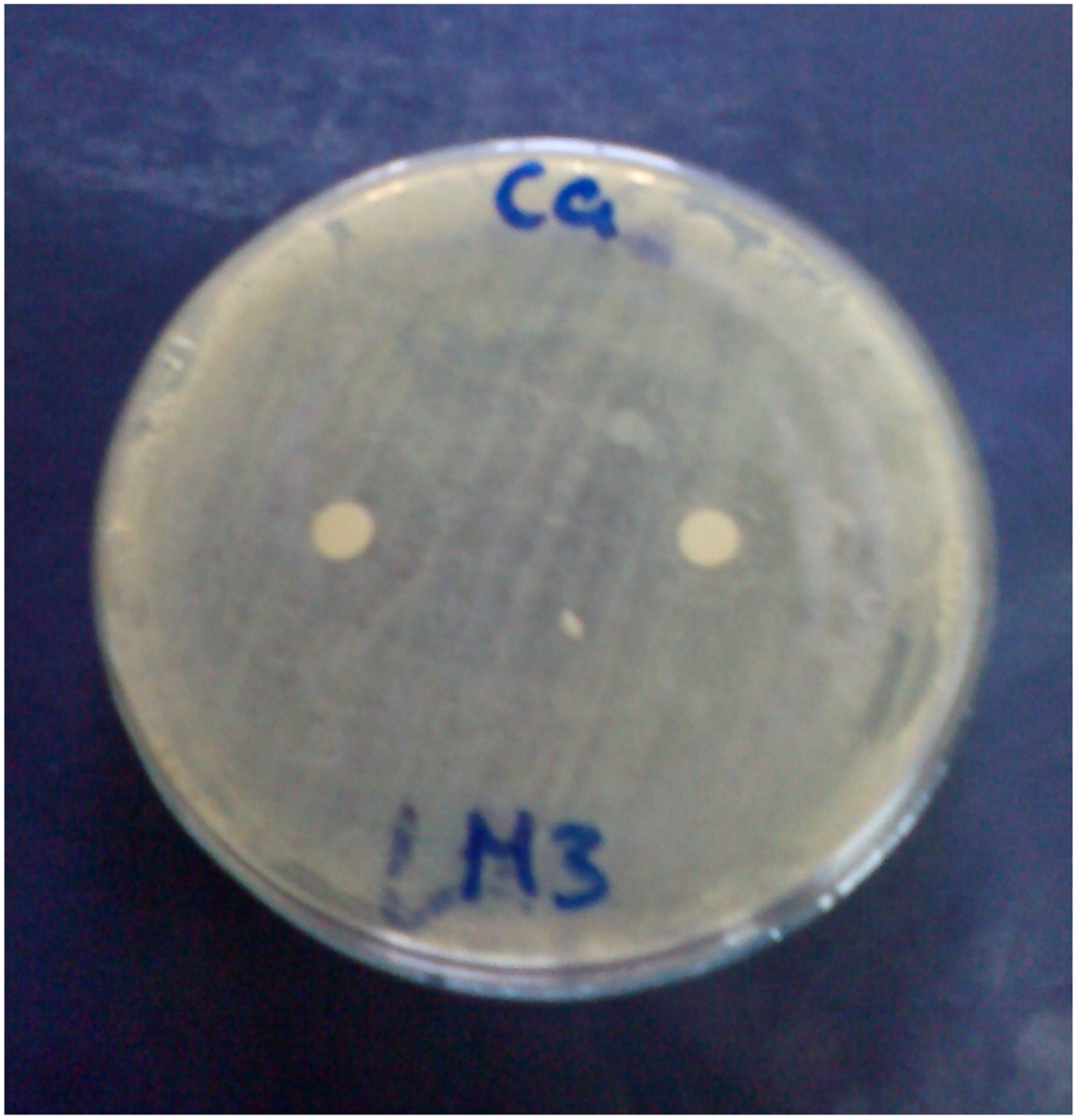

Fig (3.17): Antifungal activity of mango oil (candida)

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