إستهلال

قال تعالى:

بسم الله الرحمن الرحيم

{اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ * خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ * اقْرَأْ وَرَبُّكَ الْأَكْرَمُ * الَّذِي عَلَّمَ بِالْقَلَمِ * عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمْ }

صدق الله العظيم

سورة العلق الآية1-5

الإهـــداء

لروح أمي اهدي ثمرة مجهودي الذي لطالما نادت بتحقيقه وأسال الله لكي الرحمة وان يجعل هذا الجهد صدقة جارية لروحك وأسال الله منا القبول

مي التجاني محمد موسى

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Abstract

No wonder that grape fruit and orange are more popular fruits in Sudan. They are well known for their nutritional and medicinal properties, but for seeds oil of two plants fruit there is no enough studies reflecting the properties and benefits of them. This research aims to characterized and identify the properties and components of grape fruit and orange seeds oil. As the chemical analysis is important in formulating and developing new products and identifying the source of problems with unacceptable product in this work, phytochemical analysis, anti microbial activity, and physical properties were studied to identify the grape fruit and orange seeds oil. The Sudanese grape fruit (*citrus pardisi*) and orange (*citrus sinensis*) were collected from Algabraab field in November, 2014 the seeds were separated manually, dried at sunlight and grind. The oil was extracted by solvent extraction method using petroleum ether, a pale yellow colored oil (36.248)% for grape and(27.71)% for orange was obtained and characterized by physical properties and GC-Mass analysis . The crude extractions were obtained by solvent extraction method used methanol the yields percentage were (8.65) %for grape and (7.81)%for orange.

The phytochemical screening was carried out using the methods described by Martinez & Valencia, (1999), Sofowora, (1993), Harborne, (1984) and Wall et.al, (1952) with some modifications. The TLC analysis of crude extract proved the presence of many chemical compounds. Antimicrobial activity which determined by disc diffusion method carried for all extractions showed good results.

Fatty acids component like palmatic acid, linoleic and oleic and other compounds were found in the two oil samples. The physical properties results gave the grape fruit and orange seeds oil the vegetable oil behavior (can be used as edible oil), economic value health benefits and cosmetics manufacturing uses. The presences of saponin, alkaloids, tannins, favonoides, sterols, triterpens and cumarins in showed by phytochemical screening consider that the two planst seeds are active against bacteria and fungi.

ملخص

مما لا شك فيه أن البرتقال والقريب فروت من الفواكه الاكثر شهرة في السودان فهى معروفة لفائدتها الغذائية والطبية لكن لاتوجد دراسات كافية تعكس خصائص و اهمية زيوت الفاكهتين. وحيث أن الكيمياء التحليلية من أهم فروع الكيمياء المستخدمة لمعرفة مركبات وخصائص البذور لذلك فقد استخدمت في هذه الدراسة الكيمياء وخصوصا كيمياء النبات(Phytochemical). يهدف هذا البحث لتحديد و تحليل خصائص ومكونات زيت بذور القريب فروت والبرتقال. وكما هو معلوم اهمية التحليل الكيميائى فى تحديد الصيغ الكيميائية والمركبات الجديدة المؤثرة سالبا على بعض المركبات فقد تم اجراء تحليل فيتوكيميائى، الفعالية البيولوجية وبعض الخصائص الفيزيائية للزيت المستخلص.

جمعت عينات البرتقال والقريب فروت من حقل قرية الجابراب في ولاية نهر النيل في شهر نوفمبر من العام 2014. غسل الثمار وفصلت البذور يدويا جففت تحت اشعة الشممس ومن ثم طحنت تم استخلاص الزيت بواسطة جهاز السوكسلت واستخدام الايثر البترولي أولا وقد كانت نسب الزيت36.24% لبذور القريب و 27.71% لبذور البرتقال ومن ثم تم استخلاص الخام بواسطة الميثانول فكانت نسب الخام 8.65% لبذور القريب و7.81% لبذور البرتقال. حددت الخواص الفيزيوكيميائية للزيوت.ثم حددت مكونات الزيوت بواسطة جهاز الكتلة المطيافية ومن ثم تم التحليل الفيتوكيميائى لمستخلص الميثانول لبذور الفاكهتين للخام. تم فصل مكونات الخام باستخدام كروماتوغرافيا الطبقة الرقيقة كما تمت دراسة الفعالية البيولوجية بطريقة انتشار القرص لكل العينات المستخلصة. رصدت النتائج ودونت وخلص إلى أن بذور النباتين لهما فوائد صحية اقتصادية صناعية حيث أن زيوتهما تحتوى على الاحماض الدهنية مثل البالمتيك، اللينوليك والاوليك و الخصائص الفيزيائية لهذه الزيوت تشابه طبيعة الزيوت النباتية (زيوت الطهى) قيمة اقتصادية ويمكن ان تستخدم فى التصنيع الدوائي وتصنيع البوهيات و هناك فرصة لاستخدامها فى مستحضرات التجميل. وجود السابونين، الاكليدات، التانينات ،الفلافونات، الاستيرولات، ثلاثى الترابينات و الكومارينات فى التحليل الفيتوكيميائى لمستخلصات الخام تبرهن ان هذه البذور نشطة تجاه البكتريات و الفطريات.

List of content

Chapter Two
Material and Method

Chapter three

Result and discussion

List of tables

List of figure

Abbreviations

GC.Mas	Gas liquid chromatography –mass spectroscopy
TLC	Thin layer chromatography
RF	Retention value
Fig	Figure
Ph. Ph	phenolphthalein
I_A	Acid Value
S. G	specific gravity
I_P	peroxide value
BHT	butlyed hydroxy toluene
S_N	Saponifications Number
I_V	Iodine Value

Declaration

I hereby declare the work embodied in this thesis is my own. It has not been submitted for any other degree than the M.Sc. in chemistry of the Graduate College, Sudan University of Science and technology.

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Objectives

1. **Successive extraction of crude and oil from orange and grape fruits seeds.**
2. **Phytochemical analysis of crude extracts for seeds of two plants.**
3. **Assessment of anti microbial activity of all extracts of seeds of two plants.**
4. **Physicochemical analysis of grape and orange seeds oil.**
5. **Determination of chemical composition of the seeds oil of two plants.**
6. **Determination of chemical composition of the seeds crude extracts of two plants.**

1. Introduction

1.1.1 Back ground

The chemical analysis of foods is an important part of quality assurance program in food processing, from ingredients and raw materials, through processing, to the finished products (Suzanne, 2009). Chemical analysis is also important in formulating and developing new products, evaluating new processes for making food products, and identifying the source of problems with unacceptable product. For each type of product to be analyzed, it may be necessary to determine either just one or many components. The nature of the sample and the way in which the information obtained will be used may dictate the specific method of analysis.

In Greek phyto means plants phytochemicals are naturally occurring chemical compounds in plants.. They are responsible for plants organoleptic properties such as the smell of garlic or the deep purple of blue berries(Evans, 2008).Some phytochemicals considers as possible drugs for millennia, while other like carotenoids are significant as pro vitamins compounds. With the exception of dietary fiber, phytochemicals are not established as essential nutrients. There may be as many as 4,000 different compounds in plants that are regarded as phytochemicals with potential for biological activity. However, the term phytochemical is often used to describe a diverse range of biologically active compounds found in plants. Phytochemicals provide plants with color, flavor and natural protection against pests. Numerous epidemiological studies have indicated that a diet rich in fruit and vegetables offers considerable health benefits to humans. Among these benefits are:

1. Reduction of the risk of developing many forms of cancer (lung, prostate, pancreas, bladder and breast).
2. Reduction of the risk of cardiovascular diseases.

The majority of these beneficial effects are at least in part due to the presence of phytochemicals in vegetables and fruits. In this context phytochemicals may be defined as "non-nutrient" chemicals found in plants that have biological activity against chronic diseases.

Orange and grape fruit**:** These are two important and popular fruits in Sudan.

They are cultivated in all states in Sudan and known well as their nutritional and traditional medicinal properties (Ghazali, 1998), but rarely known in cosmetic manufacturing. They belong to the family Rutaceae and their Genus Citrus, they are known commercial as grape and orange and botanically as Citrus paradisi and Citrus sinensis .Availability of medicinal plants is not a problem, especially in developing countries. Orange and grape are tasty, juicy fruits. They are most important and widely grown fruit crops, with total global production reported to around 120 million tons per year. The trees are widely cultivated in tropical and subtropical climate, for its tasty juice and medicinal value. In worldwide trades citrus fruits generate about 105 billion dollars per year all over the world.

World Health Organization (WHO) estimates that about 80% of the population still depends upon herbal medicines for the treatment of various diseases due to easy availability economic reasons and less side effects.

1.1.2 Orange and grape seeds oil health benefits

As grape belongs to orange family so its composition is similar to orange and have same oil health behaviors. Also oil has important health benefits. Orange seeds oil contains palmatic acid, oliec acid and linoleic acid. Palmatic acid facilitates long term storage of energy in human cells. Oliec acid must be present in cells in adequate amount to produce other important fatty acids and fatty acids derivative which lower the risk of heart diseases. Linoleic acid a type of omega$_3$ fatty acid that is found in the plants is highly concentrated in the brain and may be important for cognitive and behavioral health along with normal growth and development Proanthocyanidins in human which, are very potent antioxidants help in diminishing the suns damaging effects and lessen free radical damage (Parle et.al, 2012).

1.*2 Citrus paradisi* (grape fruit)

Grape fruit citrus paradisi is a subtropical citrus tree known for its sour to semi sweet fruit. Its hybrid originating in Barbados as an accidental cross between two introduced species , sweet orange (*C.sinensis*) and Pemelo or Shaddok *(C.maxima*). Both of which were introduced from Asia. In seventeenth century when found it was named to (Forbidden fruit) and it has also been misidentified with the Pemelo. It's known well in Nahr Elnil state with its nutrition and traditional medicinal properties. The plant cultivated in Algabrab village and along the River Nile from South Alhugna to East Alzydab on the two banks of great River Nile.(Ghazali, 1998)

Table 2.1 Chemical analysis of *citrus paradisi* (grape fruit)

Nutrients		**Vitamins**		**Minerals**	
Component	**Values(g)**	**Vitamins**	**Values (%)**	**Minerals**	**Values(%)**
Energy	1.381kg (33k cal)	Vitamin C	33.3mg (40%)	Potassium	148mg(3%)
Carbohydrate	8.41g	Folate(B_9)	10mg(13%)	Calcium	12mg(1%0
Sugar	7.31g	Choline	7.7mg(12%)	Iron	6mg(1%)
Dietary fiber	1.1g	Panothenic acid	0.283mg(6%)	Magnesium	9mg(1%)
Protein	0.69g	Vitamin B_6	0.043mg(3%)	Manganese	0.101mg(1%)
Fat	0.10g	Thiamine(B_1	0.037mg(3%)	Phosphorous	8mg(1%)
		Niacin(B_3)	0.269mg(2%)	Zinc	0.07mg(1%)
		Riboflvin(B_2)	0.020mg(2%)		
		Vitamin E	0.13mg(1%)		

1.2.1. Classification

The plant authenticated and identify by taxonomist Yahia Suliman Mohammed at Herbarium of Medicinal and Aromatic plant and Tradition Medicine institute (Sudanese National center for research)

Binomail name: *Citrus paradisi*
Kingdom: Angiosperms
Sub division: Edicots
Class: Rosids
Family: Rutaceae
Genus: *Citrus*
Species: *C. ×paradisi*

1.2.2 Description

The ever green grape fruit trees usually grow to around 5-6 meters although they can reach 13-15 meters. The leaves are dark green up to 15cm in long and thin. It produces 5cm white four – petaled flowers. The fruits is yellow orange skinned and generally an oblate spheroid in shape it ranges in diameters from 10-15cm. The flesh is segmental and acidic varying in color depending on cultivars which include white pink and` red pulps of varying sweetens (generally the redder varieties are sweeter).

1.2.3 History

One ancestor of the grape fruit was Jamaican sweet orange (citrus sinensis) itself an ancient hybrid of Asian origin. The other was the Indonesian Pemelo (C.maxima). One story of fruits origins is that a certain captain shaddock brought Pemelo seeds to Jamaica and bred the first fruit, however it probably originated as a naturally occurring hybrid between the two plants. Sometime after they had been introduced the hybrid fruits, then called the forbidden fruit was first documented in 1750 by Welshman Rev Griffih Hughes who described specimens from Barbodos in the natural history of Barbodos , currently the Grapefruit is said to be one of the seven wonders of Babodo

1.2.4 Drug interaction

Grape fruit juice have been found to inter act with numerous drugs in many cases resulting in adverse effect. This happens in two ways, one is that grape fruit can block an enzyme which metabolizes medication if the drug in blood can become too high leading to an adverse effect. The other effect is that grape fruit can block the absorbed then not enough of it is in the blood to have atherapeatic effect.

2.1.5 Seeds

Seeds are greenish to pale whitish. flattened and angular. The seed is generally poly embryonic. The embryos are either “zygotic”, or “nuclear. The zygotic embryos are derived from pollination of the ovary “i.e” sexual reproduction and therefore are not always similar in horticultural qualities to the parent tree. The nuclear embryos are derived wholly from the mother plant and show very similar characteristics to the parent plant.

2.1.6 Seeds extract

Grape fruit seeds extract has been shown to have strong antimicrobial properties against fungi. It is also believed to have antimicrobial properties for bacteria, however there are no known studies that demonstrate its efficiency .Additionally, although it is promoted as a highly effective plant based presentive by some natural personal care manufacturers studies have shown that the apparent anti microbial activity associated with seeds extracted preparation is merely dye to contamination with synthetic preservatives .There is a popular myth that grape fruits contain high amount of sperimidine a simple poly amine that may be related to aging. The myth probably relies on the confusion between spermidine and putrescine while citrus fruits show high amounts of putrescine they contain very little spermidine. Investigation in cancer medicine (pharmcodynamics its inhibiting effect on the metabolism of some drugs may allow smaller doses to be used which can help to reduce costs. (Megan, 2015)

1.2.7 Oil extraction

Grape seeds are usually discarded as a part of wine making process. The extraction and sale of grape seeds oil can be a profitable sideline. In the twentieth century grape seeds oil began to be processed and sold in much higher volume, primarily in the United States and Europe. Many stores sell pure grape seeds oil for various applications because each seeds yields small amount of oil is usually extracted chemically. The chemical extraction doses have an impact on the flavor of oil but it makes seeds oil affordable for people who want to produce it. The oil is known for being light in color and flavor with a thin of nuttiness. It is unsaturated oil and contains beneficial compounds such as linoleic acid

1.2.8 Uses of seeds oil

There are two primary uses for grape seeds oil cosmetics and cooking applications. When purchasing grape seeds oil for using in food, make sure that is clearly marked as food grade. The oil should be stored in cool dark place until it is used, unless it has been heavily stabilized. In cosmetics grape seeds oil is widely used. It is often combined with other oils to make massage oil, since it glides well on the skin and it also has moisturizing properties and appears to assist with skin repair as it has mildly astringent and antiseptic qualities.

1. 2.9 Seedsoil benefits

The oil has important health with good source of essential fatty acids and vitamin E. The poly phenols and flavnoids found in this oil contain strong antioxidant compounds (flvanoid, oligomerric, procyanidin) credibly strong antioxidant about 50 times stronger than antioxidant like vitamin C and E, so this compound provide protection against cellular and tissue damage caused by free radicals. Oil can help strength and repair damaged or broken capillaries and blood vessels this in turn can help to improve circulation and alleviate conditions like varicose vein spider veins and hemorrhoids. Seeds oil can boost the health of heart and cardiovascular system by lowering the level of bad LDL cholesterol and increase the level of good HDL cholesterol which can reduce the risk of coronary disease in addition two studies showed that taking Grape seeds oil and chromium helped in lowering the bad LDL cholesterol (parle etal,2012).

The oil contains lioleinc acid which is un saturated fatty acid that can prove beneficial for people having diabetes. Oil has anti flammatory properties for which can provide relief against pain and swelling caused by arthritis and even in conditions like rheumatoid arthritis. This oil can provide great relief. Many studies showed that seeds oil help in preventing colon, prostate, stomach, breast and lung cancer cells. The antioxidant found in grape seeds oil help in preventing cancer. Omega $_3$ omega$_6$ and omega $_9$ are fatty acids found in grape seeds oil. These fatty acids benefit us by forestalling any heart disease and influence the biological properties in our body (Okiekh et al, 2013) (Parle et al, 2012) (Nwobi et al, 2006). Oil contains Proanthocyanidins which are very potent antioxidants that help in diminishing the suns damaging effects and lessen free radical damage (Harvard, 2014)

1.3 *Citrus sinensis* (orange)

Sweet orange (*Citrus sinensis*) is the world's most commonly cultivated fruit tree. It belongs to the Rutaceae family which comprises mandarins, limes, lemons, grapefruits, sour and sweet oranges. Citrus fruits are of immense economic value; occupying the top position in fruit production. Orange trees are widely cultivated in tropical and subtropical climates for the sweet fruit, which is peeled or cut (to avoid the bitter rind) and eaten whole, or processed to extract orange juice. Orange is well known in Sudan, the fruits are cultivated in Nahr Elnil State. It is known for its nutritional and medicinal proprieties throughout the state. From times immemorial whole orange plants including ripe and unripe fruits, juice, their peels, leaves and flower were used in traditional medicine and beauty

Table 2.2 deferent varieties of citrus sinensis (orange)

No	Spice	Description
1	Mosambi	Fruits are light yellowish orange in color, rough surface with prominent streaks on the rind, oblate to spherical, apex broad, rind thick, well defined segments numbering 9-12, peeling difficult, pulp light yellow, juice sweet.
2	Malta (common)	Fruits are orange yellow, smooth surface, shape spherical, medium to large in size, thickness of the rind medium, segments 10. well defined, pulp orange abundant juice, good flavor.
3	Malta (blood red)	Fruit skin is yellow with scarlet blush rind, is relatively thin, light and glossy pulp corn colored and red streaked, early ripening, pulp sweet abundant juice red colored, pleasant flavor .
4	Sathgudi	Fruit is smooth and have attractive orange color, spherical shape, size variable, rind medium thick, segments 10 to12, pulp orange colored, abundant juice, good flavor.

1.3.1 Classification

The plant authenticated and identify by taxonomist Yahia Suliman Mohammed at Herbarium of Medicinal and Aromatic plant and Tradition Medicine institute (Sudanese National center for research)

Binomail name: *Citrus sinensis*
Kingdom: Angiosperms
Sub division: Edicots
Class: Rosids
Family: Rutaceae
Genus: *Citrus*
Species: *C. ×sinensis*

1.3.2. Description

Classic orange (*citrus sinensis*), mandarin orange (*citrus reticulate*) and tangelos (*citrus* , share similar characteristics. These evergreen trees bring dense shade to your yard year -round. They produce tasty fruit, grow at a steady place of two feet per year and live 50 to150 years. The height and growth habit of orange trees is similar. Orange and tangelo trees grow to25feet tall, while mandarin orange trees grow to 20 feet tall. Leaves are 4-10cm long arranged, alternately are ovate in shape with crenulated margins .Trees have thin smooth and gray –brown to greenish bark. Most species are single- trunked with very hard wood, canopy widths range from slender to broad depending on species; leaves are smooth, oval 5-15cmx 2-8cm dark green, and glossy possessing a distinctive smell often similar to the fruit. Petioles are generally winged, leaves are uni foliate, ovate ovular elliptical with acute to obtuse tips, and contain some oil glands which are released when crushed. Young twigs are green and angled in cross- section, and branches are spineless and circular in cross section. The orange fruit is hesperidia. It is a type of berry that ranges widely in size, shape, and color juice quality. Fruits are globosely in shape. Wild orange fruit has smooth skin and the petiole wings are entire. The petioles of sour orange leaves are much larger than that of sweet orange. Orange is round *citrus* fruits with finely textured skins that are of course green yellowish in color.

1.3 .3 History

Orange tree is most cultivated and rarely found in the forests. It was first cultivated in south china and northern India**.** The Persian orange grow widely in southern Europe after it is introduced to Italy in the 11th century. It was primary for medicinal purposes. Italian traders might have introduced it to the Mediterranean area after 1450 AD Christopher Columbus took seeds of orange , lemons and citrons to Haiti and the Caribbean on his second journey of sea in 1493 AD. Portuguese Navigators have also been credited with bringing orange trees to the Mediterranean region around 1500 AD. After identification of the orange fruit it was so highly regarded that wealthy person grew orange in private conservatories called orangeries. Spaniards introduced the sweet orange into South America and Mexico in the 1500s.In 1646 orange was well known in Europe. They were introduced in Florida by Spanish explorer Juan Poncedeleon in1753 and were introduced to Hawaii . The first record of citrus as a medical was done by Theophrastus in 350 BC and, was introduced as fruit by Alexander. In early European history, writers wrote about Persian citrus, that it had wonderful fragrance and was thought to remedy for poisoning. sweetener, and repellant to mothers. Alexander used orange fruit as a perfume first and then as food. Orange trees have existed on American soil years before the detection of independence and commercial cultivation in Florida in (1870s) and California in (1870s). Orange consumption in the US before 1880s however remained in sufficient by way of transforming. They include Orange in their normal diet until around 1880s when refrigeration and mass transport system via rail/road was developed after which there was nation – wide recognition. (Parle et al, 2012)

1.3.4 Orange benefits

a. Botanical properties

Botanical properties found in orange and other natural foods are essential for product formulators to create natural and safe products. Herbs roots, flowers, fruits and seeds of plant have been used in the manufacturing of medicinal and cosmetic products for hundred years.

b. Medicinal properties

All parts of orange including seeds and leaves are rich in vitamin C which is a strong antioxidant. Compounds found in orange peels lower cholesterol. Natural fiber in orange helps prevent atherosclerosis and protect against Rheumatoid Arthritis. Eating orange may prevent ulcers and reduce the risk of stomach and colon cancers (Oikeh et al, 2013)

1.3.5 Seeds

Seeds are greenish to pale whitish, flattened and angular. The seed is generally poly embryonic, The embryos are either “zygotic”, or “nuclear. The zygotic embryos are derived from pollination of the ovary “i.e” sexual reproduction and therefore are not always similar in horticultural qualities to the parent tree. The nuclear embryos are derived wholly from the mother plant and show very similar characteristics to the parent plant.

a. Properties of seeds oil

The extracted oil is used in food flavorings, health supplemental and beauty products. Alternative medicine practitioners often use plant extracts in their treatment diseases such as arthritis cancer and heart disease through many generation. Natural products manufacturers have gained extensive knowledge on the capabilities safety on plant extract including orange seeds extract uses. Essential oils extracted from orange seeds provide flavorings for water, cake frosting other food items when added to bath and body products. Orange seeds oil provides a pleasing citrus aroma and when added to cleaning product it provides a fresh scent as well as cleaning and detergent abilities. It is also great condition agent when used in hair care product and with dried pulp can be ground into a powder for use in cosmetics and skin conditioning product. Since orange seeds provide a high protein residue suitable for human and animal consumption, they are often used in cattle feed and fertilizer mixture.(Harvard, 2014)

b. Seeds oil health benefits

Orange seeds oil contains palmatic acid oliec acid and linoleic acids. Palmatic acid facilities long term storage of energy in human cells. Oliec acid must be present in cells in adequate amount to produce other important fatty acids and fatty acids derivative which lower the risk of heart disease. Lionleic acid; a type of omega3 fatty acid that is found in the plants is highly concentrated in the brain and may be important for cognitive and behavioral health along with normal growth and development in human.(Nwobi et al, 2006)

1.4 Extraction

Extraction is one of human kind's oldest chemical operations. The preparation of a cup of coffee or tea is a type of oldest extraction methods of flavored and odor components from dried vegetable matter with hot water. Aqueous extracts of bay leaves, stick cinmon, peppercorns, and cloves are used as food flavorings along with alcoholic extracts of vanilla and almond. For the last century and half , organic chemist have been extracting , isolating , purifying and characterizing the myriad compounds produced by plants that have been used for centuries as drugs and perfumes substances such as Quinine from Cinchona bark, Morphine from the Opium Poppy , Cocaine from Coca leaves and Menthol from Peppermint oil.(fieser and Williamson, 1964)

1.4.1 Identification

Extraction defined by explaining the isolation methods of compounds from its sources or compounds from materials and this comes almost by suitable solvent which is used. . The separation of active compounds (secondary metabolites compounds) from and carbohydrates (primary metabolites compounds) is called extraction. Chemist are using many types of extraction in this section, the isolation of oils from its source is another part of extraction according to the species of solvent. So we can say extraction is the word circulation the words isolation, separation, purification, distillation, crystallization, and fractionation compounds from others.

Extraction is subdivided in three types. The organic chemist commonly employs, in addition to solid/ liquid extraction, two other types of extraction liquid/liquid extraction and acid / base extraction.

Liquid/liquid extraction and acid/base extraction are employed in the majority of organic reaction because it is usual to have the product crystallized from reaction mixture or to be able to distill the reaction product directly from the reaction mixture.

1.4.2 Solid / liquid Extraction

In this part of extraction always used a solid of material extract by a suitable solvent to yield oils, crud and ash. The extract always contain secondary metabolizes compounds of plant. Soxhlet instrument be a greet apparatus to use in solid/liquid extraction process, for exhaustive extraction of solid mixtures and even of dried leaves or seeds. The solid is packed into filter paper (thimble) solvent vapor rises in the large diameter tube on the right, and condensed solvent drops onto the solid contained in the filter paper to leaches out soluble material and after initialing and automatic siphon, carries it to the boiling flask where non volatile extracted materials accumulate .

The same solvent cycle is repeated by vaporized. Substance of very slight solubility can be extracted by bro longed operation.

Soxhlet extraction occasionally a crude product (often of natural occurrence) is obtained from which particular component may be isolated by repeated extraction with a hot solvent. For this purpose soxhlet extraction apparatus usually employed.

1.4.3 Liquid /liquid Extraction

After chemical reaction has been carried out the organic product is often separated from inorganic substances by liquid/liquid extraction for example

$2CH_3\,CH_2CH_2\,CH_2OH + 2NaBr + H_2SO_4 =$

$2CH_3\,CH_2CH_2\,CH_2Br + 2H_2O + Na_2SO_4$

1-butanol is heated with an aqueous solution of sodium sulfate. The 1-bromo butane is isolated from the reaction mixture by extraction with ether a solvent in which 1-bromo butane is soluble and in which water and sodium sulfate is aqueous mixture. The ether is less dense than water and floats on top. It can be removed and evaporated to leave the bromo compound free of inorganic substance.

1.4.4 Acid / Base Extraction

The third type of extraction, acid/base extraction involves carrying out simple acid/base reactions in order to separate strong acids, weak organic acids, natural organic compounds and basic organic substances. If we use benzoic acid, phenol, naphthalene and aniline as example of four types of compounds we can notice that the four organic compounds are dissolved in ether. The ether solution is shaken with a saturated aqueous solution of sodium bicarbonate, a weak base will react only with the strong acid (benzoic acid) to form the ionic salt, sodium benzoate which dissolves in the aqueous layer and is removed. The ether solution now contains just phenol, naphthalene and aniline. A 10% aqueous solution of sodium hydroxide is added and mixture shaken. The hydroxide a strong base will react only with the phenol a weak acid to form sodium peroxide an ionic compounds that dissolves in the aqueous layer is removed. The ether now contains only naphthalene and aniline, shaking it with dilute hydrochloric acid remove aniline a base as ionic anilinium chloride. The aqueous layer is removed. Evaporation of the ether now leaves naphthalene, the neutral compound .The other three compounds are recovered by adding acid to sodium benzoate and sodium phenolate and base to the anilinium chloride to regenerate the covalent compounds benzoic acid, phenol, and aniline .

1.4.5 Properties of solvent

The solvent used for extraction have many properties: Recrystallization solvent should readily dissolve the substance to be extracted, it should have low boiling point so that it can readily be removed, it should not react with the solute or other solvent, it must not be flammable or toxic, it should be relatively inexpensive and it should not be miscible with water. Diethyl ether usually referred to simply as ether is probably the most common so it is used for extraction, but it is extremely flammable.

Ether has high solvent power for hydrocarbons and so it is highly volatile that it is easily removed from the extract at room temperature.

Ether is useful for isolation of natural products that occur in animal and plant tissues having high water content. Although often preferred in research work because of these properties. Ether avoided in industrial processes because of the fire hazard, high solubility in water, loss of solvent recovery, incident to volatility, and the oxidation on long exposure to air or a peroxide and in a dry state it may explode. The alternative immiscible solvents sometimes preferred, even though they do not have all favorable properties of ether are petroleum ether, ligroin, benzene, carbon tetrachloride, chloroform, dichloromethane, 1,2-dichloroet and 1-butanol. The chlorinated hydrocarbon solvents are heavier to dissolve in water hence, after equilibration of aqueous and non aqueous phases, the heavier lower layer is drawn off and the upper layer extracted further and discarded. Chlorinated hydrocarbon solvents advantages are free from fire hazard, but their higher cost impedes against their use.

1.5 Oils and fats

1.5 .1 Identification

Fats and oils are water insoluble substance of plant or animal origin which consist predominantly of glycerol as of fatty acid or triglycerides (Daniel,1979). The word of fat is ordinary used to refer to triglycerides (that are solid or more correctly, Semi solid at ordinary temperatures. Whereas the word (oils) used for triglycerides that are liquid under the same conditions .No clear distinction can be made between the two words, they are used inter changeably unless the distinction between solid and liquid substance is important.

1.5 .2 Composition

The oils and fats are mixtures of lipids. They are mainly Triacylglycerols (generally >95%) accompanied by diacylglycerols, mono-acylglycerols and free fatty acids, but they may also contain phospholipids, free sterols and sterol esters, tocols (tocopherols and tocotrienols), triterpene alcohols, hydrocarbons and fat-soluble vitamins. This refers to as crude oils when it is first extracted. During refine some of the minor components are removed, wholly or in part, useful materials may be recovered. For example, phospholipids are separated during degumming and deodorizer distillates contain fatty acids along with valuable sterols, sterol esters, tocols, etc.(Fran, 2004)(Daniel, 1979).

2.4.2.1 Triglycerides

Structurally triglycerides are the condensation product of one molecule of glycerol with three molecules of fatty acids to yield three molecules of water and one molecule of a triglyceride. When the three fatty acids are the same a simple triglyceride be produced. When they are different a mixed triglyceride is produced. Simple triglycerides are easily prepared from glycerol and fatty acids but mixed triglycerides of known structure are obtained by specialized methods only.

H
I
H - C - Fatty acid
I
H - C - Fatty acid
I
H - C - Fatty acid
I
H

Fig 4.1

1.5.2.2 Unsaturated fatty acids

A large number of unsaturated fatty acids occur naturally and are more difficult to isolate, purify and characterize as the saturated acids. Their study further complicated by fact that they are less stable and readily converted to position and geometric isomers. The naturally occurring acids usually contain an even number of carbon atoms in most cases the double bonds have the cis configuration and preferred position for a double bonds between the ninth and tenth carbon atoms in the fatty acid chain. Butter fat is possible the best example of common fat which is known to contains unsaturated fatty acids with number of carbon atoms (18,19) . Fatty acids with as many seven double bonds have been reported as component of fats. Those containing one, two and three double bonds and 18carbon atoms are the most important unsaturated fatty acid of vegetable and animal fats. Those with four or more double bonds and 20-24carbon atoms are found principally marine oils.

1.5.2.3 Nonglyceride components

Nonglyceride component of fats and oils found in crude fats and oils are less in refined oils. In the most, some of these are completely substantially removed, from the crude fats during refining. The components of are:

a. phosphatides

Phosphatides which are associated with fats and oils in many plant and animal tissues consist of a polyhydric alcohol (usually, but not always glycerol) which is esterified with fatty acids and also with phosphoric acid. The phosphoric acid is in turn combined with a basic nitrogen- containing compound such as Choline, Betaine or ethanol amine. Two common phosphatides are Lecithin and Cephalin. Sphingomyelins is another common group of phosphatides. Lipositols or Inositol phosphatides are another common type of vegetable oil phosphatides.

b. Raffinose and Pentosans

Raffinose and Pentosan are also non glyceride compounds they have been found in considerable quantity in sell lings from crude cotton seed oil

c. Sterols

The collective name sterols have been assigned to crystalline neutral, unsaponifiable alcohol of high melting points. The sterols are characteristic minor components of all natural oils and fats.

d. Component of nutritional significance

Fats and oils are important sources of the fat soluble vitamins A, D and E

e. Mineral content

The analysis of fats and oils to their content of heavy metals is difficult, and relatively little data are yet available various crude vegetable oils may contain much as 0.1-0.3ppm of copper, 0.1-0.7ppm of manganese and 1.0-5.0ppm iron. Fats often contain appreciable amount of nickel after hydrogenation for example 0.1-0.2ppm.

1.5.3 Specifications

Major oils and fats are usually named by their biological source (such as soybean oil or butter fat), but each oil/fat has a range of physical, chemical and compositional parameters by which it can be recognized. Traditional physical properties include density, measures of melting behavior (if solid), refractive index and viscosity. To these must now be added several chromatographic and spectroscopic properties. Chemical properties include:

1- Iodine value (a measure of average unsaturation),

2- Saponification value or saponification equivalent (a measure of average
acacyl chain length).

3- Acetyl value (a measure of free hydroxyl groups).

4- Acid value (an indicator of quality, measuring free or unesterified acids).

5- Peroxide value (indicators of quality measuring oxidative deterioration· component acids.

6- Component triacylglycerols.

7- Minor components as groups or as individual compounds. .(Frank, 2004)

1.5.4 Vegetable oil.

Vegetable oils are those oils which extracted from plant crops containing sufficient part of plant fruit, seeds, leaves..etc (Liezel, 2011). “Vegetable oil” is the term given to any oil from a plant source and so this will include oils such as olive oil, sunflower oil, and many others.

1.5.4.1 Benefits of oil

Vegetable oils find wide use in pharmaceutical, industrial, nutritional and cosmetic products. These include products such as cooking oils, margarine, salad dressings, food coatings, paints, plasticizers, lubricants, hydraulic fluids, glycerol, synthetic fibers, lecithin, printing inks, medicines, face masks, hand creams, shower gels, soaps, detergents and many more too numerous to mention here. To appreciate the role of commodity oils and fats in daily life, it is useful to have a basic knowledge of their major use and it is sufficient to note here that about 80 percent of world production is used as human food, a further 6 per cent in animal feed (providing further food for humans), and the remaining 14 percent is the basis of the oleochemical industry (Gunstone& Hamilton, 2001) in contrast to the much larger petrochemical industry.

1.5.4.2Classification of vegetable oil

The vegetable oils maybe further subdivided into three categories:

1. By-products, where the crop is grown for another purpose other than seed oil: cotton (fabric), corn (grain), soybean (protein-rich meal).
2. Tree crops, which are generally slow to mature but then produce crops. Regularly for many years: palm, palmkernel, coconut and olive.
3. Crops, which have to be replanted each year to produce an annual harvest and where decisions about cultivation are made each sowing season by a large number of individual farmers: rape/canola, sunflower and groundnut.

1.5.4.3 Vegetable oils component

Vegetable oil component consist of tricylglcerols component which modify the properties of oil, they are classified as follows:

a. Major component of vegetable oil

The major components present in vegetable oils are triacylglycerols consisting of fatty acids bound to a glycerol backbone. The major oil like castor oil , cocoa butter oil ,coca nut oil , corn oil ,cotton seed oil, ground nut oil ,linseed oil olive oil ,palm oil, palmkernel oil, rapeseed oil, rice bran oil, safflower oils, sesame oil ,soybean oil , sunflower oil and tall oil.

b. Minor components of vegetable oils

Several of the minor components of vegetable oils such as 1,2diacylglycerols and 1,3 diacylglycerols, as well as free fatty acids are also found in oils. Several minor components are present in vegetable oils of which many of these components are associated with the color and distinctive taste of different types of oils as well as their anti-oxidant properties.2,1 monoacylglycerols and diacylglycerols are such components but if found in significant quantities in a vegetable oil, these often give an indication of adulteration or aging of the oil. This is due to the fact that upon hydrolysis of triglycerides, the mono- and diacylglycerides form. This hydrolysis occurs naturally in two ways, either enzymatically in the vegetable or fruit, or during storage due to the presence of water and long term exposure to oxygen in air.

Minor components of vegetable oils include:

- Pigments, including chlorophyll and caretonoids
- Alcohols
- Sterols, including free sterols and sterol esters
- Tocols, including tocopherols, tocosterols and tocotrienols
- Phospholipids
- Squalene
- Hydrocarbons, including alkanes, alkenes, polycyclic aromatic hydrocarbons and carotenes

1.5.5 Physical properties of vegetable oil

The physical characteristics of vegetable oil are dependent on such factors as seed or plant source, degree of unsaturation, length of carbon chains, isomeric forms of the fatty acids, molecular structure of the glycerides, and processing. Physical properties of vegetable oils, that are of most practical importance. Included flavor, melting points, plasticity, fluidity, color, viscosity, emulsification, specific gravity, solubility, refractive index, and polymorphism. In addition to their being important in the commercial manufacture of food products, many physical methods of testing and analysis are more accurate and less time-consuming as compared with chemical analysis.(Lawson, 1995)

1.5.5.1 Flavor

All oils consumed in the United States are preferred to be either as bland in flavor as possible or to have a "butter like" flavor. This preference depends on the intended use of the flavor of lard was at one time considered to be desirable in the United States, but today blandness or absence of flavor in shortenings is much preferred. The normal flavor of lard is still considered desirable in some parts of Europe. Unrefined (virgin) olive oil also has a distinct flavor that is prized quite highly by people living in areas surrounding the Mediterranean Sea and by people in the United States whose families were originally from countries in the Mediterranean area, including Spain and Italy. In addition, the sales of olive oil in the United States have doubled over the past 5 years because of its perceived nutritive value. Crude vegetable oils such as cottonseed, soybean, and palm oils have distinctive undesirable flavors, but these oils are processed to attain the desired bland, neutral flavor.

1.5.5.2Color

Whiteness of color is generally preferred except with butter, margarine, and some liquid and plastic shortening that are intentionally given an added yellow color, usually obtained through the use of minute quantities of beta-carotene. In addition, these yellow coloring materials must be added near the end of processing in order to minimize their loss.

1.5.5.3 Viscosity

Viscosity is a measure of internal friction between molecules. In general, the viscosity of oils decreases slightly with an increase in unsaturation; therefore, viscosity is increased slightly by hydrogenation. Oils or fats containing a greater proportion of fatty acids of relatively low molecular weight are slightly less viscous than ones of an equivalent degree of unsaturation but containing a higher proportion of high-molecular-weight acids. The viscosity of highly polymerized oils is much greater than that of normal oils. Viscosity is occasionally referred to in determining the condition of fats used in deep frying. During the use of oil in the frying kettle, the viscosity of a frying fat or oil will tend to increase as oxidation and polymerization increase. This can be related to polymer development and tendency toward foaming.

1.5.5.4 Iodine value

The Iodine value of the oil can be expressed as the degree of unsaturation and it is defined as the number of grams of iodine that will react with the double bonds in 100 g of oil. The higher the iodine value, the more the unsaturation of a specific oil or fat. The typical iodine value of crude soybean oil is in the 125-135 range. A processed salad oil will have an iodine value of about 110-115. A typical semisolid shortening made from partially hydrogenated vegetable oils will have an iodine value of 85-95.

1.5.5.5 Melting point

The melting point is the temperature at which a solid fat becomes a liquid oil. Each individual pure fatty acid has a specific melting point. As oils and fats are essentially mixtures of various fatty acids as triglycerides (e.g., stearic, oleic, linoleic, etc.) Oil and fat do not have sharp melting points. For example, as the temperature of a shortening rises, some fatty glycerides melt, and as the temperature drops, some portions of this product resolidify. The amount of the fat that is solid at a given temperature can be determined analytically. The melting point of a specific oil or fat is the temperature at which that particular product is completely melted. For vegetable shortenings, the complete melting point is about 120°F (49°C). Fluid shortenings also have a complete melting point of about 120°F (49°C). The complete melting point for a specific product may be quite misleading in a study of its physical properties. More pertinent is knowledge of the ratio of solids to liquids at room temperatures.

1.5.5.6 Polymorphism

Polymorphism is the ability of fat and oil crystals to exist in more than one crystal form or modification. These crystal forms are alpha, beta-prime, and beta, in order of increasing stability. The change is said to be monotropic, that is, always proceeding in the solid phase from lower to higher stability. The forms differ in crystalline structure and in melting points. Both are of appreciable importance in fat products that have solid contents at use temperatures such as shortenings (plastic) and fluid shortenings, which are pourable and pump-able. Some investigators believe there are more than three polymorphic forms for glycerides. An example is an intermediate polymorph between beta-prime and beta, said to have large crystals.

1.5.5.7 Emulsification

Triglycerides with three fatty acids attached to a glycerol molecule have minimal emulsification properties. However, monoglycerides with only one fatty acid attached to a glycerol molecule with two free hydroxyl groups (OH) on the glycerol molecule take on some of the properties of both fats and water. The fatty acid portion of the molecule acts like any other fat and readily mixes with these fatty materials, whereas the two OH groups mix or dissolve in water; thus monoglycerides tend to hold fats and water together

1.5.5.8 The refractive index

The refractive index of vegetable oils and its fatty acids is a very important characteristic because of its utility in analytical procedures. It is based on the ratio of the speed of a light wave in air as compared to its speed in the fat substance. The procedure is easy, speedy, and requires a very small sample. It is very useful for identification purposes, establishing purity, and observing the progress of reactions such as catalytic hydrogenation.

1.5.5.9 Specific gravity

Specific gravity is a comparison of the weight of an oil with that of the same volume of water. The specific gravity of vegetable oils is usually about 0.910-0.920 g/m1 at 25°C (77°F). As the temperature increases, the specific gravity of the oil or fat decreases. As oils/fats

1.5.5.10 Acidity and saponification value

The level of free acid is an item in most specifications for crude and refined oils. It is measured by titration with standard sodium hydroxide solution. The amount of alkali required to hydrolyse (saponify) a fat is a measure of the average chain length of the acyl chains. Though this value is affected by unsaponifiable material it is also present in the oil. This parameter may be reported as `saponification value' (SV) or `saponification equivalent' (SE). These are inversely related by the expression SV = 56100/SE. With increasing chain- length, saponification equivalent rises, but saponification value falls. Typical saponification values for some common oils include coconut 248±265, palmkernel oil 230±254, cocoa butter 192±200, palm oil 190±209, cottonseed 189±198, soybean 189±195, sunflower 188±194, corn 187±195, groundnut 187±196, olive 184±196 and rape 182±193. When a natural fat or oil is hydrolysed, it gives fatty acids (soluble in aqueous alkali), glycerol (soluble in water) and other material (insoluble in aqueous alkali). The latter can be extracted with an appropriate organic solvent and is described as unsaponifiable or non-saponifiable material. It includes sterols, tocopherols, hydrocarbons, long-chain alcohols, etc. There is a growing interest in these compounds and methods of analyzing this fraction.

1.5.6 Extraction of vegetable oil

The separation of oils from vegetable materials is constituted and specialized branch of oil technology. The widely differing characteristics of oils materials from diverse source have given rise to extraction processes such as rendering pressing and solvent extraction. All extraction processes however have certain in objects common. These are first to obtain the oil source uninjured and as free as possible from undesirable impurities. Second is to obtain the oil in as it is consistent with the economy of the process and third to produce an oil cake or residue of greets possible value.

The extraction of vegetable oil is more difficult matter vegetable materials, and in particular some of the oil seeds contain a large proportion of solid material associated with the oil. Different ways of quantitatively extracting lipid from a sample are available and depend on the nature of the matrix in which the lipid exists. For seeds oil the oil is generally extracted from crushed seed by the soxhlet procedure using hexane or a suitable hydrocarbon fraction such as that boiling between 40 and 60C. This method provides a sample of oil which can also be used for further tests.

1.5.7 Refining of vegetable oil

Refining test is run to determine the expected level of free fatty acids color, flavor, and other important characteristics in the oils' final forms. These tests determine their ultimate quality as finished brands. In addition, tests can determine for which type of finished oil or fat products they are suitable. The first step in the plant refining procedure is to react the crude oil with an alkaline material to remove the free fatty acid An excessive quantity can contribute to an unsatisfactory flavor in a fat and detract from a fat's frying life. When the very weak alkali of an exact measured strength reacts with the free fatty acids, the product is soap.

$RCOOH + \quad NaOH = \quad RCOONa \ + \ H_20$

Free fatty acid   alkali   soap   water

This soap must be removed, and this is done by passing the mixture of fat and soap through a continuous centrifuge machine that separates the fat or oil from this soap which is sometimes referred to as "foots." The refined fat or oil is water washed to remove the final traces of soap and is centrifuged again. Finally, the refined material is dried to remove the residual water. Other very minor materials, such as phosphatides, proteins, carbohydrates, waxes, and gums that have not been previously removed by the degumming process are also separated out during refining. Oils that are low in phosphatide content, such as palm or coconut, may be physically refined The latest continuous refining techniques have greatly contributed to the efficiency of refining, the speed of refining, and the improved quality of the refined oil. The term of other very minor materials, such as phosphatides, proteins, carbohydrates, waxes, and gums, that have not been previously removed by the degumming process are also separated out during refining. Oils that are low in phosphatide content, such as palm or coconut, may be physically refined. The latest continuous refining techniques have greatly contributed to the efficiency of refining, the speed of refining, and the improved quality of the refined oil.

1.5.8 Bleaching of vegetable oil

The usual method of bleaching is by adsorption of the color pigments on an adsorbent material. (Fuller's earth) The colors are adsorbed onto the earth or clay, and then the earth or clay is filtered from the oil. Continuous methods of bleaching are generally more efficient than the older batch-bleaching methods. The usual method of bleaching is by adsorption of the color pigments on an adsorbent material. Fuller's earth, for example, is a natural bleaching earth consisting basically of hydrated aluminum silicate. Within recent years, natural earths or clays have been replaced to a considerable extent by acid-activated clays or earths that have considerably more bleaching power. Activated carbon is also used as a bleaching adsorbent to a limited extent.

1.5.9 Hydrogenation of vegetable oil

This is the manufacturing process that permits the production of both plastic solid shortenings and fluid shortenings from liquid oils

Hydrogen adds directly to the points of unsaturation in the fatty acids. The hydrogenation process was developed as a result of the need to increase the oxidative stability of the oil or fat and convert liquid oils to fluid shortening and plastic shortening by increasing solids or greater utility in certain food uses. Hydrogenation is extremely important in using soybean oil, our most important source oil. Soybean oil now provides 60-65% of the total visible fat in the U.S. diet. Crude soybean oil contains about 8% linolenic acid, which is very reactive with oxygen. The linolenic acid content must be reduced to around 2% or less to have acceptable oxidative/flavor stability properties in most food operations, and hydrogenation is presently the only practical way to achieve this. Fatty acids with two double bonds, such as linoleic, can be hydrogenated to form fatty acids with one double bond, such as oleic, or with no double bonds, such as stearic. Likewise, fatty acids with three double bonds (such as linolenic) can be hydrogenated to form fatty acids with two double bonds, one double bond, or no remaining double bonds. As the degree of hydrogenation increases, the saturation of the fat and its firmness at room temperature also increase. Obviously, its melting point increases too, as does its degree of oxidative stability under storage conditions. Hydrogenation is accomplished in a reactor where hydrogen gas is bubbled through the oil at a suitable temperature and pressure in the presence of a catalyst. The catalyst accelerates the reaction but is not consumed in the reaction. It may enter into temporary combination with the reactants, but such combinations are unstable and are invariably broken down at the completion of the reaction to again yield the catalyst in a relatively unchanged form.

1.5.10 Fractionation of vegetable oil

Fractionation is the removal of solids at selected temperatures. The most widely practiced form of fractionation is that of crystallization wherein a mixture of triglycerides is separated into two or more different melting fractions based on solubility at a given temperature. The term "dry fractionation" frequently is used to describe fractionation processes such as winterization or pressing. Winterization is a process whereby a small quantity of material is crystallized and removed from edible oils by filtration to avoid clouding of the liquid fractions at refrigeration temperatures. Originally, this processing was applied to cottonseed oil by subjecting the oil to ambient winter temperatures, hence the term "winterization." Today many oils, including cottonseed and partially hydrogenated soybean oils, are winterized. A similar process called "dewaxing" can be utilized to clarify oils containing trace amounts of clouding constituents Pressing is also a fractionation process sometimes used to separate liquid oil from solid fat. The process squeezes or "presses" the liquid oil from the solid fat by means of hydraulic pressure. This process is used commercially to produce hard butters and specialty fats from such oils as palm kernel and coconut. Solvent fractionation is the term used to describe a process for the crystallization of a desired fraction from a mixture of triglycerides dissolved in a suitable solvent. Fractions may be selectively crystallized at different temperatures after which the fractions are separated and the solvent removed. Solvent oils, and some salad oils from a wide array of edible oils.

1.5.11 Deodorization of vegetable oil

Deodorizing is the process that makes possible the production of a bland, neutral flavor so that the finished shortening or other fat food product will not impart any flavor of its own to the final fried, baked, or other food product. Each product to be deodorized has been formulated from non hydrogenated oils and/or hydrogenated stocks that have received their proper degree of hydrogenation. Deodorizing is accomplished in a "still" or steam distillation flavors present in the refined, bleached, and hydrogenated oil. This is feasible because of the differences in volatility between the triglycerides and the substances that give flavors and odors to fats.

1.6 Secondary metabolites compound

Secondary metabolites compound is a type of natural product. Natural products are organic compounds that are formed by living systems. The elucidation of their structures and their chemistry, synthesis and biosynthesis are major areas of organic chemistry. Naturally occurring compounds may be divided into three broad categories. Firstly, those compounds which occur in all cells and play a central role in the metabolism and reproduction of those cells. These compounds include the nucleic acids and the common amino acids and sugars. They are known as primary metabolites**,** the high molecular weight polymeric materials such as cellulose, lignins and proteins which are form the cellular structures. Finally, there are those compounds that are characteristic of a limited range of species. These are the secondary metabolites**. (**Hanson, 2003).

1.6.1 Biological activity

Secondary metabolites on the other hand have often attracted interest because of their biological effect on other organisms. The biologically active constituents of medicinal, commercial and poisonous plants have been studied throughout the development of organic chemistry. Many of these compounds are secondary metabolites. It has been estimated that over 40% of medicines have their origins in these natural products. A number of screening programmes for bioactive exist and have led to new drugs, for example; taxol which is the same species used for the treatment of various cancers. Natural products often have an ecological role in regulating the interactions between plants, micro organisms, insects and animals. They can be defensive substances, anti feedants, attractants and pheromones. Chemotaxonomy provides another reason for examining the constituents of plants. Phytochemical surveys can reveal natural products that are "markers" for botanical and evolutionary relationships.

1.6.2 Classes of secondary metabolites

At first sight, the structures of secondary metabolites may seem to be bewilderingly diverse. However, the majority of these compounds belong to one of a number of families, each of which have particular structural characteristics arising from the way in which they are built up in nature, **i.e.** from their biosynthesis. The classes of secondary metabolites are:

*Polyketides and fatty acids.

*Terpenoids and steroids.

*Phenylpropanoids.

*Alkaloids.

*Specialized amino acids and peptides.

*Specialized carbohydrates.

1.6.2.1 Polyketides and fatty acids

Polyketides are formed by the linear combination of acetate (ethanoate) units derived from the "building block" acetyl co-enzyme A. Polyketides are natural products that are formed by the stepwise condensation of acetate (ethanoate) units. In the result carbon chain alternate carbon atoms come from the methyl and carboxyl groups of the acetate building block. Some highly unsaturated polyacetylenes derived from polyketides.

The idol condensation between carbonyl activated carbon and another carbonyl group gives β-hydroxy ketone. The condensation followed by enolization of poly carbonyl compound creates phenol (fig 5.1).

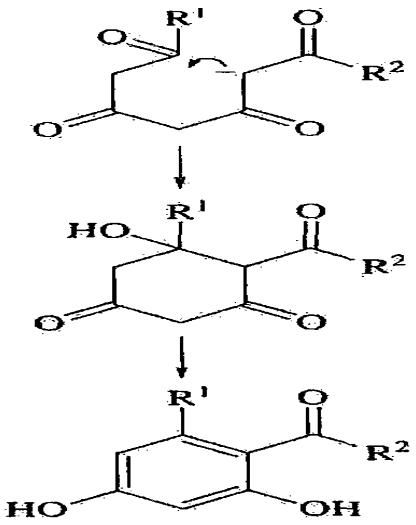

Fig 5.1

1.6.2.2 Terpenoids and steroids

Terpenoids and steroids are assembled in nature from isoprenoidC_5units derived from isopentenyl (3-methylbut-3-en-1 -yl) pyrophosphate. These C_5units are linked together in a head-to-tail manner. They have a characteristic branched chain structure the terpenes are compounds that are built up from isoprene units. Their structures are divisible into the C_5 isoprene linked C-C-C-C in a head-to-tail manner. This isoprene rule, developed by Ruzicka in 1921, provides a useful guide in structure determination. The origin of the C_5 unit (isopentenyl pyrophosphate(fig 5.2).

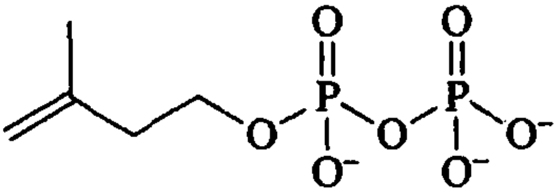

Fig 5.2

The terpenes are classified by the number of these C5isoprene units that they contain. The classes are:

Monoterpenoids, C 10.
Sesquiterpenoids, C15.
Diterpenoids, C20.
Sesterterpenoids, C25.
Triterpenoids, C30.
Carotenoids, C40.

Natural rubber is a polyisoprenoid substance. The steroids are derived from the tetracyclic triterpenoids. Isoprene units are sometimes found as components of other natural products. Most of the terpenes have cyclic structures. The majority of the terpenoid cyclizations which take place in living systems are of an acid catalysed type. The branched chain nature of the isoprenoid backbone coupled with readily protonated functional groups (e.g. alkenes) lend themselves to acid-catalysed rearrangements during the course of these biosynthetic reactions. Consequently, there are some terpenoid skeletons that do not appear, on first inspection, to be derived from the regular combination of isoprenoid units.

a. Monoterpenoids

Monoterpenoids are major components of the aromas of plants. These volatile natural products, known as essential oils, form the basis of the perfumery and flavoring industries. Distillation of these oils meant that it was possible to obtain quantities of these terpenes for structural studies. The structures of many of the simple monoterpenes were established between 1890 and 1920 through the work of Wallach, Wagner, Tiemann, Semmler and Perkin. Although modern gas chromatographic analyses of these oils often shows them to be highly complex mixtures of terpenoid and non-terpenoid natural products, one or two monoterpenes usually predominate.

b. Sesquiterpenes

Some sesquiterpenesare found in the higher boiling portions of essential oil. These include caryophyllen from oil of cloves, humulene from oil of hops, cedrene from cedar wood oil and longifolene from Indian turpentine oil (Pinus ponderosa). These structures illustrate the diversity of the carbon skeletons found among the sesquiterpenes. Sesquiterpenoid lactones are common biologically active constituents of plants of the Compositae family.

c. Diterpenoids

Many of the diterpenoids are wood resin products. Abietic acid is the major component of colophony, the wood rosin obtained from Pinus and Ahies species. It is used in varnishes and resin soaps. Although crude samples of the resin acids were isolated in the 19th century, there was considerable confusion concerning their identity. The carbon skeleton of abietic acid was not fully established until the work of Ruzicka in the 1930s, and the stereochemistry was not clarified until 1948 by Barton.

d. Triterpene

The simplest triterpene, squalene was first isolated from fish liver oils. Subsequently, it has been found in plant oils and mammalian fats. The common tetracyclic triterpenelanosterol is a major constituent of wool fat and its esters are found in lanolin cream. Other triterpenes, such as the a- and β-amyrins are found in wood resin and the bark of many trees. More highly oxidized and degraded triterpenes are exemplified by limonin which is a bitter principle obtained from lemon and orange seeds. Although limonin was first isolated by Bernay in 1841, its structure was not finally established until 1960 asthe result of a collaborative effort involving work by Barton in the UK, Jeger and Arigoni in Switzerland and Corey in the USA. Other members of this group have been obtained from the heartwood of trees in the Meliaceae and Rutaceaefamilies.

e. Steroid

The steroids are derived from tetracyclic triterpenes and possess acyclo penta penhydrophenanthrene backbone. The availability; crystalline and well-defined conformation of the steroids have meant that they have become suitable substrates with which to investigate the influence of steric factors on reaction rates and mechanisms. Cholesterol forms an important constituent of lipid membranes, and the more highly degraded steroids include the steroid hormones. Many sterols occur as glycosides typified by the steroidal saponins (fig 5.3).These are responsible for the foaming produced by many plants.

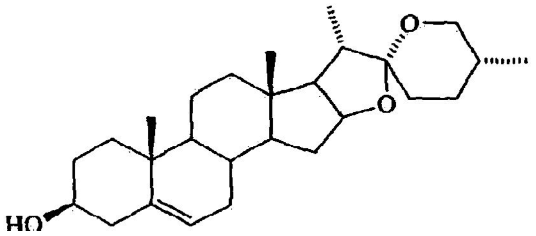

Fig 5.3

f. Carotenoids

The carotenoids are red or yellow pigments that are found in many plants. Thus β – caroten (fig 5.4) provides the red colouring matter of carrots and lycopene is the deep-red pigment of tomatoes. The carotenoids are important as precursors of vitamin A, which plays acentral role in vision. The carotenoids are good anti-oxidants and contribute beneficial effects to many foods.

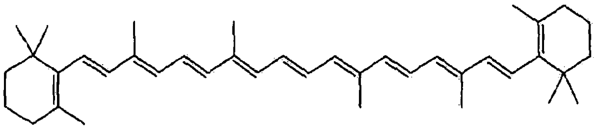

Fig 5.4

1.6.2.3 Phenylpropanoids

The recognition that many naturally occurring aromatic compounds possessed a three-carbon chain attached to the ring (fig 5.5) led to them being grouped together as the phenylpropanoids. Aromatic compounds that are biosynthesized by this route can be distinguished by their oxygenation pattern from those that are of polyketide origin. Hydroxylation occurs at the position para to the C_3chain and then in the met position, rather than on alternate carbon atoms .The structures of a number of simple phenylpropanoids which are widespread plant products were established in the late 19th century. Cinnamic acid (1.56, R = H), Chydroxycinnamic acid (coumaric acid, 1.56, R = OH), 3,4-dihydroxycinnamic acid (caffeic acid) and their methyl ethers **(e.g.** ferulic acid) are found in the free state and as their esters. Neutral compounds such as eugenol (an aromatic constituent of clove oil) are components of essential oils. The important aromatic amino acids phenylalanine, tyrosine and tryptophan are formed by this route. The cyclization of the 2-isomers of the alkenes leads to coumarinssuch as umbelliferone. Some of these compounds are associated with the photosensitizing properties of plants of the Umbelliferae**.** Oxidative coupling of two phenylpropanoid units, such as coniferyl alcohol. leads to the lignancarbon skeleton, exemplified by pinoresinol Podophyllotoxin from the Hindu drug Podophyliumemodi, is an interesting cytotoxic agent.

Fig 5.5

1.6.2.4 Alkloids

Some of the first natural products to be isolated from medicinal plants were alkaloids. When they were first obtained from plant material in the early years of the 19th century it was found that they were nitrogen containing bases which formed salts with acids. Hence they were known as the vegetable alkalis or alkaloids. This ability to form salts and to complex with metal ions helped their separation and detection in the era before chromatography. Many alkaloids have neuro active properties and interact with the receptors at nerve endings. This is not surprising, since many alkaloids have fragments buried within their overall structure which resemble the natural substances (the neurotransmitters) that bind to these receptors.

Alkaloids may be grouped according to their plant sources, e.g. Aconitum, Amaryl lidaceae, Cinchona, Curare, Ergot, Opium, Senecio and Vinca. Another classification is based on the structure of the ring system containing the nitrogen atom (e.g**.** piperidine, isoquinoline, indole). This can reflect their biosynthetic origin from amino acids such as ornithine, lysine, phenyl alanine, tyrosine and tryptophan A group of alkaloids possess piperidine or pyrrolidinerings. In 1820, two French chemists, Caventou and Pelletier, were able to isolate the active principle quinine from Cinchona ledgerianu bark. It contains the quinoline ring(fig 5.6) system and its correct structure was established by Rabe in 1907. It is used as an ant malarial drug and as a bitter substance in tonic waters. Another big family of alkaloids is the indole alkaloids. They are derived from the amino acid tryptophan and they occur in tropical plants from the Apocyanaceae (Asyidosperma, Rautolfiaand Viptca) and Loganiaceae (Stryclnos) families. These alkaloids include poisons such as strychnine Although most of the structure of which was established by Robinson in 1946. Other compounds in this series have useful medicinal properties. Reserpine from Rauwlfia bark is used in the treatment of mental disease. The dimeric Vinca alkaloids are used in the treatment of leukemia and other cancers. The ergot alkaloids, from the ergot fungus Claviceps purpurea are also indole alkaloids and are amides of lysergic acid**.**

The purine carbon skeleton is found in the alkaloids caffeine and theophylline, which are stimulants that occur in coffee and tea. Kinetin is a cytokinin plant growth substance that stimulates cell division in plants. In some alkaloids, such as the steroidal alkaloids and the toxic diterpenoid alkaloids from Aconitum species, the nitrogen atom is

inserted after the carbon skeleton has been formed. In a biosynthetic these might be considered as terpenoids.

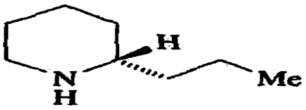

Fig 5.6

1.6.2.5 Antibiotic derived from Amino Acid

The alkaloids are not the only group of secondary metabolites that are derivedfrom amino acids. Amino acids not only form the building blocks for the large peptides and proteins but also for smaller peptides that are converted into the β-lactum antibiotics such as the penicillins(fig 5.7) and cephalosporin(fig 5.8). The diketopiperazine antifungal agents produced by Tricoderma and Gliocladium species, such as gliotoxin(fig 5.9)are also derived from amino acids.

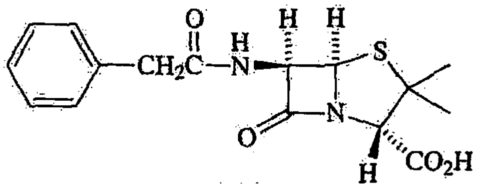

Fig 5.7

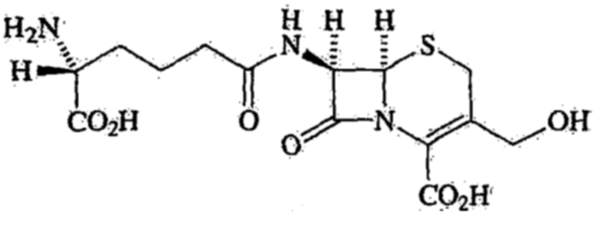

Fig 5.8

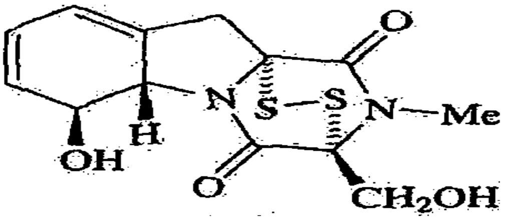

Fig 5.9

1.6.2.5 Vitamins

There are a number of natural products that are essential for life but which cannot be produced by the body. The recognition by Hopbins, Grijns and others at the start of the 20th century that diseases such as scurvy, rickets and beri-beri, which arose from deficiencies in the diet, might be associated with a requirement for particular compounds present in foods led to the search for these essential factors. These natural products became known as vitamins. As each vitamin was isolated, it was identified by a letter of alphabet. As their structures became known, these compounds acquired a trivial name.

A number of vitamins play a role as 'co-enzymes in the function of the particular enzymes such as those involved in biological oxidation, enzyme. This biological function of the vitamins places them in the class of primary metabolites. However, the elucidation of their structures and aspects of their chemistry and biosynthesis link them with secondary metabolites. In the period following 1920, bioassay-guided fractionation using test animals led to the isolation of various vitamins. They are present in their natural sources in low concentrations, and consequently very small amounts of material were available for structural studies. Nevertheless, the structures of many of the vitamins were established in the 1930s and confirmed by synthesis. Material then became available for biological studies and diaetary supplements. The presence of the fat-soluble vitamin A in egg and in fish oils was established in 1913-191 5, and its structure was elucidated by Karrer in 1931. The recognition that the disease beri-beri, which was prevalent in South East Asia, was a. deficiency disease and that it could be cured by an extract obtained from rice polishing, led Funk in 191 1 to coin the name vitamin for these essential dietary factors. Vitamin B $_1$(thiamine) was isolated in 1926 and in quantity by Williams in 1934. Its structure(fig 5.7) was established in 1936 and confirmed by synthesis. Other members of this group of vitamins include riboflavin (vitamin B_2) pyridaxamine (vitamin B_6) biotin. folic acid and vitamin B12 . Vitamin B_{12} was isolated in 1948 and its structure established by a combination of chemical work (Todd) and X-ray crystallography (Hodgkin) in 1954. It is one of the most potent of the vitamins, being active in the treatment of pernicious anemia at the level of micrograms. Vitamin C (ascorbic acid,) is important in the prevention of scurvy. Its structure was established in 1932. Vitaxriin D, a steroid, Vitamin E, tocopherol is an antioxidant and vitamin K(, a quinone (1961, is an antihaemorrhagic factor.

1.7 Isolation and analysis of natural product

Secondary metabolites, with some exceptions, occur in amounts that are less than 0.01% of the dry weight of the plant. Extraction of I kg of dry plant material is likely to yield 'less than 100 mg of a natural product. These compounds may be unstable and present as part of a complex mixture. The isolation, separation and purification of these natural products require considerable skill. The extracted must contain compound like phenols, lignins, cumarins, stilbenses, quinines, tannins, flavonoids, anthocyans, glycosides, terpense, saponins, essential oil, fatty acids, alkaloides, fixed oils, alkamides and poly sacharides constituents.

1.7.1 Extraction of natural product

Natural product extraction is to isolate an unknown compound responsible for a particular biological activity (Rirchard J.P Cannel, 1998). or to isolate a certain compound known to be produced by a particular organism or to extract a group of compounds within an organism that are all related in some way, such as by a common structural feature. Another type of extract is to isolate all of the metabolites produced by one natural product source that are not produced by a different “control” source, e.g., two species of the same genus, or the same organism grown under different conditions.

Extraction might be to purify sufficient amount of a compound to characterize it partially or fully. More specifically to provide sufficient material allow for confirmation or denial of a proposed structure.

Natural products may be obtained from the crushed biological material by extraction with a solvent such as petroleum ether, chloroform. ethyl acetate or methanol. Several solvents of increasing polarity may be used. Thus lipid material (waxes, fatty acids, sterols, carotenoids and simple terpenoids) can be extracted with non-polar solvents such as petroleum ether, but more polar substances such as the alkaloids and glycosides are extracted with methanol, aqueous methanol or even hot water. Many alkaloids are present as their salts with naturally occurring acids such as tartaric acid.

Commercial extractions may use tone quantities of plant material, and a range of different extraction procedures including steam distillation have been used. Recently, commercial procedures have been developed using super-critical carbon dioxide as a mild solvent, but because of the pressures involved this requires quite complicated apparatus. The initial extraction is followed by a separation into acidic, basic and neutral fractions.

Asolution of the extract in an organic solvent (such as ethyl acetate) is shaken with an inorganic base (such as aqueous sodium hydrogen carbonate) to remove the carboxylic acids as their water-soluble sodium salts. The more weakly acidic phenols may only be extracted with a sodium hydroxide solution. Extraction of the original solution with an acid such as dilute hydrochloric acid will remove the bases such as the alkaloids as their salts. The neutral compounds remain behind in the organic phase. The acids and the phenols may be recovered from the aqueous solution of their sodium salts by treatment with dilute hydrochloric acid and re-extraction with an organic solvent, and the bases may be recovered by treatment of their salts with ammonia and re-extraction. As the instrumentation for the structure elucidation of organic compounds becomes ever more effective, and allows the use of increasingly small amounts of material, it most difficult operation in phytochemical research becomes to isolation and purification plant constituents. Although the chemical properties of functional groups and moieties contained in compounds such as aldehyde, phenols and alkaloids can be exploited for their separation from other materials. Such methods might not fractionate components of the same class's in this matter area that new techniques are constantly being developed (Saunders, 2008).

1.7.2 Sublimation

Sublimation may sometimes be possible on the whole drug, as in the isolation of caffeine from tea or for the purification of material present in a crude extract. Modern equipment employs low pressures with a strict control of temperature.

1.7.3 Distillation

Fractional distillation has been traditionally used for the separation o f the components of volatile mixtures. In phytochemistry it has been widely used for the isolation of the components of volatile oils. On a laboratory scale it is not easy by this method to separate minor components o f a mixture in a pure state and gas chromatography is now routinely used (quantitative value)

Steam distillation is much used to isolate volatile oils and hydrocyanic acid from plant material.

1.7.4 Fractional liberation

Some groups of compounds lend themselves to fractional liberation from a mixture. As an example, a mixture of alkaloid salts in aqueous solution when treated with aliquots of alkali will give first the weakest base in the Free State followed by base liberation in ascending order of basicity. If the mixture is shaken with an organic solvent after each addition then a fractionated series o f bases will be obtained. A similar scheme can be used for organic acids soluble in water-immiscible solvents; in these cases, starting with a mixture of the acid salts, it is possible to fractionally liberate the acids b y addition of mineral acids.

1.7.5 Fractional crystallization

A method much used in traditional isolations and still valuable for the resolution of often otherwise intractable mixtures the method exploits the differences in solubility of the components of a mixture in a particular solvent. Frequently derivatives of the particular components are employed (picrates of alkaloids, osazoneso f sugars).

1.7.6 Adsorption chromatography

Of the various methods of separating and isolating plant constituents the chromatographic procedure' originated by Tswett is one of the most useful techniques of general application. The use of charcoal for the decolorization and clarification of solutions is well known: colored impurities are adsorbed by the charcoal and colorless solution results on filtration. All finely divided solids have the power to adsorb other substance on their surfaces to a greater or lesser extent: similarly, all substances are capable of being adsorbed, some much more readily than others. This phenomenon of selective adsorption is the fundamental principle of adsorption chromatography the general process of which may be described with reference to one of Tswett's original experiments. Adsorption chromatography has proved particularly valuable in the isolation and purification of vitamins, hormones, many alkaloids, cardiac, glycosides, Anthraquinones…etc. It is commonly employed as a clean-up 'technique for the removal of unwanted materials from plant extracts prior to assay. Thin-layer chromatography with adsorbents such as alumina is an Adaptation of the method.

1.7 .7 Partition chromatography

Partition chromatography was introduced by Martin and Synge in l94l for the separation of acetylated amino acid and was first applied to the separation of alkaloids by Evans and Partridge in 1948.The method Has now been largely superseded by the more sophisticated HPLC but it retains the advantage of being inexpensive to set up and operate the separation of the components of a mixture is, as in counter-current extraction, dependent on differences in the partition coefficients f the components between an aqueous and an immiscible organic liquid.

The aqueous phase is usually the stationary phase and is intimately mixed with a suitable 'carrier' such as silica gel, purified kieselguhr or powdered glass packed in a column as in adsorption chromatography.

The mixture to be fractionated is introduced on the column, in a small, volume of organic solvent and the chromatogram is developed with more solvent or successively with different solvents of increasing eluting power. When the water is the stationary phase the solute undergoing separation travel down the column at different speeds depending on their partition coefficient between the two liquid phases; the use of a buffer solution as aqueous phase widens the scope of the technique as ionization constants and partition coefficients are exploited in effecting separation.

The separated zones may be located by methods similar to those employed in adsorption chromatography with water as the aqueous phase, the positions of separated zones of acids or alkalis may be shown by employing a suitable indicator dissolved in the water. This method is clearly not applicable to buffer acid- or alkali-loaded columns. In these cases complete elution of the separated zone is often necessary. The elaute is collected in aliquot portions and estimated chemically or physically for dissolved solute. A graph of the analytical figure (titration, optical rotation. optical density, refractive index, etc.) for each fraction of elaute may then be plotted to show the degree of separation of the solutes. The fractionations obtained in partition chromatography are influenced to a considerable degree by the displacement effect of one solute on another and advantage is taken of this in displacement development, in which the chromatogram is developed with a solution of an acid or a base that is stronger than any in the mixture to be separated. The effect is for the stronger acids or bases displace the weaker ones, resulting in a rapid clear-cut separation of the constituents. For the elution development of these separated zones it is essential that there is no distortion of the zones. Since the front of one band follows immediately on the tail of the preceding less acidic or less basic component.

2. Materials and methods

2.1 Material collection

Citrus Senensis (Orange) and Citrus paradisei (Grape fruits) were collected from Algabraab Fields (River Nile State of Sudan) at November -2015. The two types of plants were cleaned carefully. The seeds were separated manually and washed using tap water and twice with distilled water. Then sun dried for three days with about twelve hours per day to reduce the moister content.

2.2 Chemicals

Ethyl acetate ($CH_3COOC_2H_5$), (ALFA CHEMICA), India.

Benzene (C_6H_6), (SDFCL), India.

Di Chloro Methane (CH_2Cl_2), (LOBA CHEMIE), India.

Silica gel GF 254 for TLC, (SDFCL), India

Methanol (CH_3OH), (ALPHA CHEMICA), India.

Ethanol 96% (CH_3CH_2OH), (ALPH CHEMICA), India.

Ethanol 80% (CH_3CH_2OH), (ALPHA CHEMICA), India.

Chloroform (CH_3Cl_3), (SDFCL), India.

Toluene (C_7H_8), (SDFCL), India.

Hexane fraction from petroleum (SDFCL) India.

Acetone (C_3H_6O), (SDFCL), India.

Petroleum ether (SDFCL) India.

Potassium Iodide (KI), (BDH), Chemical England.

Potassium Hydroxide (KOH), (SDFCL), India.

Ferric Chloride Anhydrous ($FeCl_3$), (CDH), India.

Aluminum Chloride ($AlCl_3$), (BDH), England.

Sodium Thiosulfate (NaS_2O_3), (CDH), India.

Glacial Acetic Acid (CH_3CH_2COOH), (DUKSAN), Korea.

Formic Acid (CH_3COOH), (DUKASN), Korea.

Hydro chloric acid (HCl). (LOBA). India.

Sulfuric Acid Conc (H_2SO_4), (BDH). England.

Ammonium Hydroxide ($NHCl_l$), (BDH), England.

Mercuric Cloride ($HgCl_2$), (BDH), Chemical England.

Hydrogen Peroxide3% (H_2O_2), (Wafrafarma), Sudan.

Acetic Anhydride ($(CH_3.CO)O$), (BDH), England.

Saline Solution (NaCl), (WafraFarma, Sudan.

Vanillin ($CH_3O.C_6H_3(OH).CHO)$), (BDH), Chemical England.

Starch ($C_6H_{12}O_6$ $(C_6H_{10}O_5)_n$), (CHADWEIL HEATH), England.

PenolPhthalien ($C_{20}H_{14}O_4$), (LOBA CHEMIE).

Gelatin Salt (NH_2-R-COOH), (CHADWEIL HEATH), England.

2.3 Microbiological media

Nutrient Agar (Himedial lab PVT .Ltd) India

Sabourand Dextrose (Himedial lab PVT .Ltd) India

Bacterial microorganisms:

Bacillus subtilis	NCTC 8236 (Gram + ve bacteria)
Staphylococcus aureus	ATCC 25923(Gram +ve Bacteria)
Escherichia coli	ATCC 25922(Gram -ve bacteria)
Pseudomonas aeruginosa	ATCC 27853 (Gram -ve bacteria)

Fungul microorganism

Candida albicans ATCC7596

National Collection of Type Culture (NCTC), Colindale, England.

American Type Culture Collection (ATCC) Rockville, Maryland, USA.

2.4 Glassware

Conical flask 100ml, volumetric flask 100&200ml, Erlenmeyer flask 100ml

Washing bottle, brusher, starrier, glass slide, burette 50ml, pipette 25ml,

Density bottle 25ml, glass stopper, dropper, test tubes cylinder, capillary tubes

Beakers 50,100&400ml, separating funnel, funnel, Petri dishes, vials, stand

Glass plate for TLC, TLC Rack, TLC plate Ruler, TLC Ruler, TLC Tank, Cotton wool

Filter papers, square Ruler, spatula, fume cupboard and indicators Pump.

2.5 Instruments

Lab Balance IND, A and B company, India.

Soxhlet, ACHTUNG, Duran Company, England.

Rotavapour, RE111, Buchi, Swizerland.

Water bath, ACHTUNG, Duran Company, England

Incubator, Heraeus, schultheiss, Germany.

Autoclave, HGT, England.

Spryder, DESAGA, HEIDELBERG, Germany.

Oven, B and T, Asearle company, England.

UV Instrument, DESAGA, SARSTEDT GRUPPE, Germany.

GCMS-QP2010plus, SHIMADZU company, Japan.

2.6 Preparation of solutions

2.6.1 HCl (2M)

6.0 ml of concentrated HCl were added to distilled water in 500 ml beaker. The solution was transferred quantitatively to 1000 ml volumetric flask and the volume was completed to the mark. The solution was shaken and kept for further use**.**

2.6.2 HCl (0.5M)

Was prepared by diluting25 ml of HCl (2N) to 100 ml in a volumetric.

2.6.3 NH4OH (10%)

10.00g of NH4OH were dissolved in distilled water in 50 ml beaker. The solution transferred quantitatively to a volumetric flask 100ml and the volume was completed to the mark. The solution was shaken and kept for further use

2.6.4 $AlCl_3$(1%)

1.0 g of Alcl3 was dissolved in distilled water in 50 ml beaker. The solution was transferred quantitatively to a volumetric flask 100ml and the volume was completed to the mark. The solution was shaken and kept for further use

2.6.5 KOH (0.5 M)

9.0g of KOH were dissolved in distilled water in 500 ml beaker. The solution was transferred quantitatively to a volumetric flask 1000ml and the volume was completed to the mark. The solution was shaken and kept further for use.

2.6.6 KOH(0.1M)

Was prepared by diluting20 ml of KOH(0.5M) to100 ml in a volumetric.

2.6.7 `Mayer,s reagent

1.36g of mercuric chloride and (5.0)g of potassium iodide were dissolved in distilled water in 100ml volumetric, the solution was completed to the mark, shaken and kept for further use.

2.6.8 Gelatin

10g of gelatin powder was dissolved in sterile water in 500ml beaker by gently swirling mixture for 15 min in $60C^0$ in water bath. The solution was cooled and kept for further use.

2.6.9 Thio sulfate (0.1 M)

15.8g of $Na_2S_2O_3$ were dissolved in distilled water in 500 ml beaker. The solution was transferred guantitatively to volumetric flask 1000ml, the volume was completed to the mark. The solution was shaken and kept for further use.

2.6.10 Thiosulfate (0.01M)

Was prepared by diluting 10ml $Na_2S_2O_3$ (0.1M) to100ml in a volumetric (100)ml.

2.6.11 Phenolphethalein

1.00g of phenolphethalein (Ph.Ph) powder were dissolved in 60ml ethanol in 100 ml volumetric flask. The solution was completed to the mark by distilled water; the solution was shaken and kept for further use

2.6.12 Starch

1.0g of starch was prepared as a paste and added to a boiling water ins100ml beaker. The solution was cooled to room temperature and transferred to 200 ml volumetric flask. The volume was completed to the mark with distilled water. the solution was shaken and kept for further use

2.6.13 Potassium hydroxide 3%

3.0 g potassium hydroxide were dissolved in 100 ml methanol.

2.6.14 Ferric chloride 5%

5.0 g ferric chlorid were dissolved in 100 ml methanol and few drops of hydrochloric acid.

2.6.15 Iodine vapors

5 g of iodine crystal were taken in tank and the tank heated gently in water bath for 10 minutes to induce iodine vapors.

2.6.16 Dragendoroff' reagent:

a/ 0.85 g bismuth sub nitrate were dissolved in 10 ml glacial acetic acid and 40 ml distilled water.

b/ 20 g potassium iodide were dissolved in 50 ml distilled water.

Solutions (A and b) were left over night, equal volumes of solution a and b were mixed and 20 ml of glacial acetic acid added then completed to 10 ml with distilled water.

2.6.17 Vanillin-sulphuric acid 2%

2.0 g of vanillin was dissolved in 100 ml con sulphuric acid and kept in dark bottle.

2.7 Methods

2.7.1 Extraction of crude and oil from grape fruit and orange seeds:

Extraction was carried out according to method described by (Sukhdev et al, 2008)

152.6 g of Citrus pardisi and 147.9 g of Citrus senensis were coarsely powdered separately using mortar and pestle and successively extracted with petroleum ether and 80 % methanol using soxhelt extractor apparatus. Extraction carried out for about six hours for petroleum ether and eight hours for methanol till the color of solvents at the last siphoning time returned colorless. Solvents were evaporated under reduced pressure using rotary evaporator apparatus. Finally extracts allowed to air in petri dishes till complete dryness and the yield percentages were calculated as followed:

Weight of extract obtained / weight of plant sample X100

2.7.2 Phytochemical screening

Phytochemical screening for the active constituents was carried out according to the method described by (Martinez & Valencia (1999), Sofowora (1993), Harborne (1984) and Wall et al (1952)) with many few modifications.

2.7.2.1 Identification of tannins:

0.5 g of each extract was washed three times with petroleum ether, dissolved in 10 ml hot saline solution and divided in two tests tubes. To one tube 2-3 drops of ferric chloride added and to the other one 2 – 3 drops of gelatin salts reagent added. The occurrence of a blackish blue color in the first test tube and turbidity in the second one denotes the presence of tannins.

2.7.2.2 Test of sterols and triterpenes:

5.00 g each the extract was washed three times with petroleum ether and dissolved in 10 of chloroform. To 5 ml of the solution, 0.5 ml acetic anhydride was added and then 3 drops of conccentrated. sulphuric acid at the bottom of the test tube. At the contact zone of the two liquids a gradual appearance of green - blue pink to purple color was taken as an evidence of the presence of sterols (green to blue) and or triterpenses (pink to purple) in the sample.

2.7.2.3 Test for Alkaloids:

0.5 g of each extract was heated with 5 ml of 2N HCl in water bath and stirred for about 10 minutes, cooled, filtered and divided into two test tubes. To one test tube few drops of Mayer's reagent was added while to the other tube few drops of Valser's reagent was added. A slight turbidity or heavy precipitate in either of the tow test tubes was tanked as presumptive evidence for the presence of alkaloids.

2.7.2.4 Tests for Flavonoids: -

0.5 g of each extract was washed three times with petroleum ether and dissolved in 30 ml of 80% ethanol.

The filtrate was used for following tests: -

A/ to 3 ml of the filtrate in a test tube 1ml of 1% aluminum chloride solution was in methanol was added. Formation of a yellow color indicated the presence of Flavonoids. Flavones or and chalcone.

C/ to 2 ml of the filtrate 0.5ml of magnesium turnings were added. Producing of defiant color to pink or red was taken as presumptive evidence that flavonenes were present in the plant sample.

2.7.2.5 Test for Saponins: -

0.3 g of each extract was placed in a clean test tube. 10 ml of distilled water was added, the tube stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of foam, which persisted for least an hour, was taken as evidence for presence of saponins.

2.7.2.6 Test for Coumarins: -

0.2 g of each the extract dissolved in 10 ml distilled water in test tube and filter paper attached to the test tube to be saturated with the vapor after a spot of 0.5N KOH put on it. Then the filter paper was inspected under UV light, the presence of coumrins was indicated if the spot have found to be adsorbed the UV light.

2.7.2.7Test for Anthraquinone glycoside: -

0.2 g of each extract was boiled with 10 ml of 0.5N KoH containing 1ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of benzene. 5ml of the benzene solution was shacked with 3ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of anthraquinones was indicated if the alkaline layer was found to have assumed pink or red color.

2.7.2.8Test for cyanogenic glycoside: -

0.2 g of each extract was placed in Erlenmeyer flask and sufficient amount of water was added to moisten the sample, followed by 1ml of chloroform (to enhance every activity). A piece of freshly prepared sodium picrate paper was carefully inserted between a split crock which was used to stopper the flask, a change in color of the sodium picrate paper from yellow to various shades of red was taken as an indication of the presence of cyanogenic glycoside

2.7.3 Physiochemical properties of oil

Physiochemical properties were carried out according to British Pharmacopoeia (2007):

2.7.3.1 Acid value:

The acid value is the number that expresses, in milligrams the quantity of potassium hydroxide required to neutralize the free acids present in 1 g of the substance.

10.00 g (m) of sample were dissolved in 50 ml of a mixture of equal volumes of 96 % ethanol and light petroleum, previously neutralized with 0.1 M potassium hydroxide and the mixture was titrated with 0.1 M potassium hydroxide until the color changed from colorless to pink color using ph.ph indicator.Acid value was calculated as followed.

$$I_A = \frac{5.610n}{m}$$

Where

n= ml of potassium hydroxide

m= g of sample

2.7.3.2 Saponification Number

2 g of sample were weighted into a 200-ml flask. 25.0 ml of ethanolic solution of potassium hydroxide were added and boiled under a reflux condenser for 1 hour and rotated the contents frequently. While the solution is still hot, the excess of alkali was titrated with 0.5M hydrochloric acid using 1 ml of phenolphthalein solution as indicator. Operation was repeated without the substance being examined (blank).

Saponification value was calculated from the expression

$$S_N = \frac{28.05\ V}{W}$$

Where v = the difference in ml between the titrations and w is the weight in g of substance taken.

2.7.3.3 Ester value:

Ester value was calculated as followed:-

Ester value = Saponification value – Acid value

2.7.3.4 Iodine number

0.25 g of each sample were dissolved in 10 ml of dichloromethane in a dry iodine flask. 20 ml of iodine monochloride solution was added and the flask was closed with stopper, solution inserted and allowed to stand in the dark at 15° to 25° for 30 minutes. 15 ml of dilute potassium iodide solution were placed in the top cup carefully when previously moistened with dilute potassium iodide then the stopper was removed. The stopper and the sides of the flask were rinsed with 100 ml of water, shacked and titrated with 0.1M sodium thiosulphate using starch mucilage as indicator.

A blank test carried out under the same condition, but without sample.

Iodine value was calculated from the expression:

$$I_V = \frac{1.269\ V}{W}$$

where v is the difference in ml between the titrations and w is the weight in g of the substance taken.

2.7.3.5 Peroxide value

5.00 g of sample were placed in a 250 ml conical flask fitted with a ground-glass stopper. 30 ml of a mixture of 2 volumes of chloroform and 3 volumes of glacial acetic acid were added. The mixture was shacked to dissolve the sample and 0.5 ml of saturated potassium iodide solution was added. The mixture shacked for exactly 1 min, then 30 ml of water added. Titration was done with 0.01 M sodium thiosulphate, with continuous vigorous shaking, until the yellow color is almost disappeared. 5 ml of starch solution was added and titration continued with vigorously shaking, until the blue color disappeared. A blank test carried out under the same condition, but without sample.

Peroxide value was calculated from the expression:

$$I_p = \frac{10\,(n_1 - n_2)}{m}$$

Where n_1 ml of 0.01 M sodium thiosulphate n2 ml of 0.01 M sodium thiosulphate.

m g of sample

2.7.3.6 Determination of density:

A clean density bottle of 25 ml capacity was weighted (W_1), and then filled with oil, stopper inserted and reweighed to give (W_2). The density was calculated from expression:

$$\text{Density} = W_2 - W_1 / 25 \text{ (g/ml)}$$

2.7.3.7 Determination of specific gravity:

A clean density bottle of 25 ml capacity was weighted (W_0), and then filled with oil, stopper inserted and reweighed to give (W_1). Oil was substituted with water after washing and drying the bottle and weighted to give (W_2).

Specific gravity was calculated from the expression:

$$SG = (W_1 - W_0) / W_2 - W_0$$

3.7.4 GC-Mas analysis:

GC / MS was carried out for citrus paradisi seeds oil and citrus sinensis seeds oil using GC-MS system consisted of a Shimadzu 17A Gas- Chromatography, a Shimadzu QP5050A Mass Spectrometer and Aclass-5000 data system. The isolated compound was separated on a 30 m × 0.25 mm. Capillary column of fused silica (30x 0.25) was used. The temperature of the column was 150 ºC after injection, then programmed at 5 ºC·min-1 to 250 ºC, and maintained at that temperature for 5 min. Split injection was conducted with a split ratio of 10:1, the flow-rate was 1.0 ml·min-1, helium gas was used as the carrier gas and the injector temperature was 250 ºC. The MS detection conditions were as follows: interface temperature, 230 ºC; ionization mode, EI+; electron energy, 70 V; full scan acquisition mode; mass range, 33-450 amu. The isolated compound was identified by using NIST-library spectra, Library: WILEY7.LIB and published MS data. Retention time, area percentage, matching percentage, formula, and major mass spectral Data (m/z) of each compound were recorded.

2.7.5 Separation of some compounds by thin Layer Chromatography:

Thin layer chromatography was carried out for methanolic extracts of citrus paradisi seeds and citrus senensis seeds, which showed potent antimicrobial activities, according to the method described by Stahl, (1969).

All solutions was prepared by the method according to Stahl, (1969).

3.7.5.1 Stationary phases:

The stationary phases used were the following:

A) Silica gel type G.
B) Silica gel type GF254.

2.7.5.2 Mobile phases

1- Chloroform : Methanol (8: 2)
2- Butanol : Acetic acid : Water (4:1:5) upper layer
3: Butanol : Acetic acid : Water (4:1:1)
4- Toluene : Methanol (84:16)
5- Toluene : Ethyl acetate : formic acid (4:5:1)
6- Benzene : Ethyl acetate (80: 20)
7- Butanol: Ethanol: Water (40: 19: 11)

2.7.5.3 Preparation of thinlyer chromatography plates:

30 g of silica gel were shaken with 60 ml distilled water for two minutes using 250 ml stopper conical flask. The slurry was spread using spreader making 0.25 mm thickness on five glass20×20cm plates.The coated plates were then allowed to dry at room temperature and activated at 105°C for one hour. The plates were cooled and stored till use.

2.7.5.4. TLC analysis for citrus paradisi (grape) and citrus sinensis (orange) seeds crude extraction

0.5 g of each methanol extraction for two plants were dissolved separately in 10 ml methanol and applied to the plated using capillary tube. Samples were spotted at about 2 cm from the bottom of the plate and 2 cm from each edge. Plate was kept to dry by air and then inserted in tank containing the selected solvent system (Toluene : Ethyl acetate : formic acid (4:5:1))After reached the height of 15 to 20 cm, the plate was took out of the tank and allowed to air till solvent was

completely evaporated. The plate was inspected in day light, then examined under UV lamp and finally sprayed with the specific spraying reagent. Rf values of separated compounds which appeared in day light or under UV or after sprayed and heated were calculated as followed:

Distant crossed by spot / distant crossed by solvent front.

2.7.5.5 Preparative Thin layer chromatography of citrus paradisi methanol extraction (crude)

2.7.5.5.1 Preparation of preparative plates:

60 g of Silica gel GF_{254} were added to 120 ml distilled water and the mixture was shaken for two minutes in 250 ml Stoppard conical flask. The slurry was spread using spreader to make 0.5 mm thickness on five glasses 20×20 cm each. The coated plates were then allowed for two hour to dry at room temperature and activated at 105°C for hour. The hot plates were allowed to cool and stored till use.

2.7.5.5.2. Application of citrus paradisi (grape) crude extraction:

Citrus paradise methanolic extraction was applied to the plated as line using capillary tube. Sample was spotted at about 2 cm from the bottom of the plate and 2 cm from each edge and 0.5 cm between each spot. Plate was kept to dry by air and then inserted in tank containing solvent system (Toluene: ethyl acetate: formic acid), (4:5:1). After reached the height of 15 to 20 cm, the plate was took out of the tank and allowed to air till solvent was completely evaporated. The plate was inspected in day light, and then examined under UV lamp. Bands containing the same color were scratched and the scratches of all plates were collected together. Collected scratches were then dissolved in ethyl acetate and filtered using filter paper. Filtrate was exposed` to air till concentrated. Concentrated solution was then tested by thin layer chromatography using the following solvent systems:

Toluene: ethyl acetate: Acetic acid (48: 60: 6).

Chloroform: Methanol (70:30).

Butanol: Ethanol: Water (40: 19: 11).

Plates were inspected in day light, under UV lamp and sprayed with Vanillin-Sulphuric acid.

2.5.7.6 Ethyl acetate fraction

Ethyl acetate fraction was carried out according to method described by Sukhdev et al, 2008).

5.0 g of citrus pardisi was dissolving in distilled water, the solution was transferred to separately funnel, the ethyl acetate was added and shacked well, after the two layer was separated the ethyl acetate layer was separated in a beaker evaporated and analyzed by GC. Mas.

3.7.6 Anti microbial Activity method

Disc diffusion method

Bacterial suspension was diluted with sterile physiological solution to 10*8 cfu/ml (turbidity =McFarland standard 0.5)one hundred micro liters of bacterial suspension were swabbed uniformly on surface of MHA(Mueller Hinton Agar) and the inoculums was allowed to dry for 5 minutes sterilized filter paper discs (WhatmanNo1,6mm in diameter)were placed on the surface of MHA and soaked with 20 micro liter of solution of each planet extract .the inoculated plates were incubated at 37 c for 24 hour in the inverted position .the diameters (mm) of the inhibition zones were measured .

Result and discussion

3.1 Extraction of crude and oil of *Citrus paradisi* (grape) and *Citrus sinensis* (orange) seeds.

Table (3.1) shows the percentage yield of crude and oil extractedfrom grape and orange seeds. The percentage yield of crude extracted from the grape seeds gave 8.65% while that extracted from orange seeds is 7.8% .The two types of seeds contain approximately the same content of crude. The percentage yield of oil in grape seeds was found to be 36.24% while the oil content in orange seeds gave 27.7%. The grape seeds oil gave the higher extraction %yield. These results agree with the results obtained by (Oikeh,2013)
The value of the percentage yield of oil makes the industrial practice of the oil recovery a Profitable venture, and will reduce the level of waste that is obtained from lab extraction method. The extraction %yield of the two types of plants compared with sesame oil lab % yield extraction is good according to the results obtained by (Ahmed yacine et al, 2012) (sesame oil yield obtained using different solvents ranged from 28.86-52.83)
The extraction oil yield is sufficient yield compare with sesame oil extraction yield done by (Abubakr Elkhaleefa, *etal* (2015).

According to the lab technique and varieties of plants species can investigate the lowest of orange extractions compare with grape extractions.

Table (3.1)

Extraction of crude and oil from citrus paradisi (grape) and citrus sinensis (orange) seeds.

Sample	**Weight of sample**	**Petroleum ether (oil)**		**Methanol (crude)**	
		Weight of Extract	**Yield %**	**Weight of Extract**	**Yield %**
Citrus pardisi	152.6 g	55.315 g	36.248 %	13.2 g	8.65 %
Citrus senensis	147.9 g	40.98 g	27.71 %	11.557 g	7.81 %

3.2 Phytochemical screening observations of *Citrus paradisi* (grape) seeds crude extraction

Table (3.2) shows Phytochemical observations of citrus paradisi. Higher compound components as screening showed are sapponins, alkaloids, tannins and falvonoids traces of cumarins, sterols and triterpenses. The plants have not any components of glycosides or anthraquinone.

Table (3.2)

Phytochemical screening observations of *Citrus paradisi* (grape) seeds crude extraction

Test	**Observation**	**Result**
Saponins	Foam	+++
Cumarins	UV absorption	+
Alkaloids	Turbidity	+++
Anthraquinones	No observation	_
Tannins	Turbidity	+++
Flavonoids	Yellow color	+++
Sterols	Colored ring	+
Triterpenes	Pink color	+
Glycoside	No observation	_

Key:

+ **Trace**
++ **Moderate**
+ ++ **High**
- **Negative**

3.3 phytochemical screening observation of citrus sinensis seeds crude extraction

Table (3.3) shows Phytochemical observations of citrus sinensis (orange). Higher compound components as screening showed are sapponins, alkaloids, tannins and falvonoids traces of cumarins, sterols and triterpenses. The plants have not any components of glycosides or anthraquinone.

This result of plant seed screening compare with same plant pulp did by (Chede PS, 2012) considered that the seeds are rich in secondary metabolite like sapponins and alkaloids falvonoids and tannins, so it can used in medicinal purpose

Table (4.2) and table (4.3) are show same observation screening for two plants.

Table (3.3)

phytochemical screening observations of *Citrus sinensis*(orange) seeds crude extraction

Test	**Observation**	**Result**
Saponins	Foam	+++
Cumarins	UV absorption	+
Alkaloids	Turbidity	+++
Anthraquinones	No observation	_
Tannins	Turbidity	+++
Flavonoids	Yellow color	+++
Sterols	Colored ring	+
Triterpenes	Pink color	+
Glycoside	No observation	_

3.4 physicochemical properties of *Citrus paradisi* (grape) seeds oil

Table (3 .4) shows the physicochemical properties of grape seeds oil. Grape fruits seeds oil physicochemical properties are: acid value 9.46, saponification number 270, iodine number 66.49 , peroxide value 20 value, ester value 56.85, density 0.974 and specific gravity 0.912 .The physical properties of grape classified grape seeds oil as normal vegetable oil according to Harry Lawson vegetable oils reference value, but the iodine number for grape seeds oil is lower compared to vegetable oil stander as grape seeds oil more saturated than vegetable oil and this consider the highest specific gravity. The highest saponification number and lowest iodine number which gave the oil behavior like fat. Oil can use in a cosmetic industrial manufacturing for it is saturated fatty acid as moisture agent and soap produce.

Table (3.4)

physiochemical properties of *Citrus paradisi* (grape) seeds oil

Analysis	Result
Acid value	9.46 mg KOH/g
Saponification number	270 mg KOH/g
Iodine number	66.49 gI_2/100g oil
Peroxide value	20 mEq/Kg
Ester value	56.85 mg KOH/g
Density	0.974g/ml
Specific gravity	0.912g/ml

3.5 physicochemical properties of *Citrus sinensis* (orange) seeds oil

Table (3.5) shows physicochemical properties of orange seeds oil the acid value was found 9.36, saponification number 312.7, iodine number 60.9, peroxide value 20, ester value 56.85, density0.974 and specific gravity 0.912

This values semi to grape seeds oil but as economic value we found orange more sufficient in cosmetics industrial because grape seeds oil has less saponification number and high iodine number made the grape seeds oil under the orange seeds oil in saturated class. The Physicochemical properties values of orange seeds oil showed good result compare with result of analysis did by (Nwobi et al, 2006) he found acid value 82, saponification number 192,iodine value 108 and peroxide number 92.84 this refer to the different in countries climate and soil and plant growing circumstances also the lab techniques and material collection method . Generally the physical properties for two type plants seeds oil semi same and sufficient economic in cosmetics industrial.

Table (3.5)

physiochemical properties of *Citrus sinensis*(orange) seeds oil

Analysis	Result
Acid value	9.36 mg KOH/g
Saponification number	312.7 mg KOH/g
Iodine number	60.9 gI_2/100g oil
Peroxide value	19 mEq/Kg
Ester value	51.54 mg KOH/g
Density	0.973 g/ml
Specific gravity	0.911 g/ml

3.6 Chemical composition of *Citrus paradisi* (grape) seeds oil

Table (3.6) shows GC- Mas analysis results for grape seeds oil. The traces compounds are cyclo hexane, 1,3dimethylcyclo hexane, α pinine, ethyl cyclo hexane, oxylene and decane.

The compound n-oktan has a sufficient percentage 26.28% in grape seeds oil.The less compounds found in grape seeds oil are palmatin 1, 2 diaminoethyl hydrogen phosphinyl 4.15%, lionleinc acid chloride 3.18% and linoleic acid 4.82%. grape seeds oil rich in many types of fatty acids and sterols this agree with study research did by (Nwobi et al, 2006) and (Bhide et al, 2013). Myristic and arashidic acid are absence in grape seeds oil. The economic value of oil depends mainly to the storage condition. The values of iodine number (60 and 66) make the oil to be considered as or fat. By implication, the oil can be easily converted into solid by hydrogenation of the oils in presence of oleic stearic and lienoleic to be used in the production of paints and vanish, while it may also be used as oil lubricant industry. The golden-yellowish color in the two oils may be an indication of the presence of carotene which bears the important vitamin A, giving the oil a medicinal value. The presence of α pinine also gives the two oils economic properties as commercial product to introduce Camphor (which used to protect clothes from mouth) from α pinine. Orange seeds oil consists of valuable components which used in cosmetics range such as linolein 1- mono. Grape seeds oil has nitrogen and phosphours compounds not present in orange seeds oil. Stearic acid which present in orange seeds oil can increase vanishing agent to the oil.

Orange and grape seeds oil contains palmatic acid oliec acid and linoleic acids, palmatic acid facilitates long term storage of energy in human cells. Oliec acid must be present in cells in adequate amount to produce other important fatty acids and fatty acids derivatives which lower the risk of heart disease. Linoleic acid is a type of omega$_3$ fatty acid found in the plants when found in highly concentration in the brain it helps in the normal growth.

Table (3.6)

Chemical composition of *Citrus paradisi* (grape) seeds oil

Peak No.	**Compound**	**Retention Time (RT)**	**Area %**	**Formula**	**Molecular weight**
1	n-octane	6.50	26.28	C_8H_{18}	**114**
2	Hexadecanoic acid (palmatic)	37.67	21.0	$C_{16}H_{32}O_2$	**256**
3	O-xylene	8.77	7.55	C_8H_{10}	**106**
4	Cyclo hexane 1,3 dimethyl	6.40	6.19	C_8H_{16}	**112**
5	Cis,cis-9,12-octadecadienoic (linoleic)	40.86	4.82	$C_{18}H_{32}O_2$	**280**
6	1,3 propanediyl ester –(CAS) glycerol	43.52	4.62	$C_{35}H_{68}O_5$	**586**
7	Alpha pinene	10.87	4.36	$C_{10}H_{16}$	**136**
8	Palmitin,1,2-diaminoethyl hydrogen phosphinyl	42.75	4.15	$C_{37}H_{74}NO_8P$	**691**
9	Ethyl cyclohexane	7.52	4.03	C_8H_{16}	**112**
10	Linoleic acid choloride	45.69	3.81	$C_{18}H_{31}ClO$	**298**
11	9-Octadecanoic acid,1,2,3propanetriyl ester	46.52	3.34	$C_{57}H_{104}O_6$	**844**
12	Octadecanoic acid (oleic)	40.94	3.15	$C_{18}H_{34}O_2$	**282**
13	1,3dimethyl cyclo hexane	6.63	2.91	C_8H_{16}	**112**
14	Decane,2-methyl	7.37	2.35	$C_{11}H_{24}$	**156**
15	Oleic acid chloride	45.75	0.82	$C_{18}H_{33}ClO$	**300**
16	Di-(9-octadecenol) glycerol	46.58	0.62	$C_{39}H_{72}O_5$	**621**
			100%		

3.7 chemical composition of *Citrus sinensis* (orange) seeds oil

Table (3.7) shows GC- Mas analysis results for grape seeds oil. The traces compounds are cyclo hexane, 1,3dimethylcyclo hexane, α pinine, ethyl cyclo hexane, oxylene and decane.

The compound n-oktan has a sufficient percentage 26.28% in grape seeds oil.The less compounds found in grape seeds oil are palmatin 1, 2 diaminoethyl hydrogen phosphinyl 4.15%, lionleinc acid chloride 3.18% and linoleic acid 4.82%. Grape seeds oil rich in many types of fatty acids and sterols this agree with study research did by (Nwobi et al, 2006) and (Bhide et al, 2013). Myristic and arashidic acid are absence in grape seeds oil. orange seeds oil gave lower values than grape seeds oil Compounds found in orange seeds oil are stearic acid 16.25%, 2,4 decadienal1 36%, palmatic acid chloride 1.27%, oleica acid anhydride 0.77%, 1-mono liolein 3.64% and decanol l0.69.%

Table (3.7)

Chemical composition of *Citrus sinsensis* seeds(orange) oil

Peak No.	**Compound**	**Retention Time (RT)**	**Area %**	**Formula**	**Molecular weight**
1	Palmatic acid	37.75	28.8	$C_{16}H_{32}\ O_2$	256
2	Octadecanoic acid(oleic) chloride	41.02	16.25	$C_{18}H_{36}O_2$	284
3	Oleic acid (oliene)	40.93	15.45	$C_{18}H_{34}O_2$	282
4	n- octane	6.50	11.17	C_8H_{18}	114
5	1- mono linolein	43.53	3.64	$C_{21}H_{38}O_4$	354
6	O-xylene	8.77	3.37	C_8H_{10}	100
7	1,2 dipalmatin	42.76	2.89	$C_{35}H_{68}O_5$	569
8	Cyclo hexane	9.39	2.76	C_8H_{16}	112
9	Oleoyl chloride	45.69	2.55	$C_{18}H_{33}ClO$	300
10	Alpha pinine	10.88	2.14	$C_{10}H_{16}$	136
11	Octadecenoic acid1,2,3propanetriyl ester	46.53	2.12	$C_{57}H_{104}O_6$	844
12	Ethylcyclohexan	7.51	1.74	C_8H_{16}	112
13	1,3- cyclo hexane	6.63	1.41	C_8H_{16}	112
14	2-4 decadienal	22.77	1.36	$C_{10}H_{16}O$	152
15	Palmatic acid chloride	41.34	1.27	$C_{16}H_{31}ClO$	274
16	Decane,2-methyl	7.37	1.05	$C_{11}H_{24}$	156
17	Oleic anhydride	45.76	0.77	$C_{36}H_{66}O_3$	546
18	3-decanol	7.62	0.69	$C_{10}H_{22}O$	158
19	Di (9-octadecenoyl)-glycerol	46.59	0.57	$C_{39}H_{72}O_5$	621
			100%		

3.8 TLC analysis for *Citrus paradisi* (grape) and *Citrus sinensis* (orange) seeds crude extraction

Tables (3.8) and (3.9) show the TLC results. For grape crude extract three compounds were isolated spot No.(1), spot No (2) and spot No (3) with RF values 0.564, 0.717 and 0.865 respectively.

The orange crude extract gave the same compounds with the same RF values. This proves that the two extracts for grape and orange are containing the same chemical compounds.

Table (3.8)

TlC analysis for *Citrus paradisi* (grape) crude extraction

Spot No	Distance crossed by Solvent front(cm)	Distance crossed by Spot(cm)	RF value	Color of peaks
1	15.6	8.8	0.564	Gray
2	15.6	11.2	0.717	Violet
3	15.6	13.5	0.865	Violet

Table (3.9)

TLC analysis for *Citrus sinensis* (orange) crude extraction

Spot No	Distance crossed by Solvent front(cm)	Distance crossed by Spot(cm)	RF value	Color of peaks
1	15.6	8.8	0.564	Gray
2	15.6	11.2	0.717	Violet
3	15.6	13.5	0.865	Violet

3.9 Chemical composition of *Citrus paradisi* (grape) seeds ethyl acetate fraction

Table (3.10) shows GC. Mas analysis of crude extracted using ethyl acetate. Grape fruit seeds are found to be rich in butlyed hydroxyl toluene (BHT) which known as strong anti oxidant compound. This anti oxidant gave 19.48%. This compound is used in small amount in food additive in addition to this use it is also used to prevent oxidation in fluids and other materials where free radicals must be controlled. The seeds crude extraction also contain an important compound (1H- benzimidazole, 5-methoxy)42.61%. 1H benzimidzole, 5 methoxy is important in pharmacological activity and has broad spectrum of uses. This compound also acts as intermediate subunits for the development of molecules of pharmaceutical or biological interest. All compounds like albendazole, mebendazole, thiabenazole (antihelmentic) and omeprazol(anti-ulcer) are derivatives of benzimidazole.

The GC.Mas results show other chemical compound with different % composition. Such as 4-((IE)-3 hydroxy-1-propenyl)-2-methoxy,2-propenoic acid, tridecyl ester,1-nonadecene and palmitaldehyde with percentage 6.08%, 5.79%, 5.50% and 3.20%. grape fruits extract also contains other chemical compounds with low percentage.

Table (3.10)

Chemical composition of *Citrus paradisi* (grape) seeds ethyl acetate fraction

Peak No.	**Compound**	**Retention Time (RT)**	**Area %**	**Formula**	**Molecular weight**
1	IH-benzimidazole, 5-methoxy	25.586	42.61	$C_8H_8N_2O$	**148**
2	Butylated hydroxyl toluene(antioxidant DBP)	16.540	19.48	$C_{15}H_{24}O$	**220**
3	4-((IE)-3hydroxy-1-propenyl)-2-methoxy	20.511	6.08	$C_{10}H_{12}O_3$	**180**
4	2- propenoic acid ,tridecyl ester	19.529	5.79	$C_{16}H_{30}O_2$	
5	1-nonadecene	23.440	5.50	$C_{19}H_{38}$	**266**
6	Plamitaldehyde	27.745	3.20	$C_{16}H_{32}O$	**240**
7	Linoleic acid methyl ester	24.601	2.91	$C_{19}H_{34}O_2$	**294**
8	Hexadecanoic acid, methyl ester	22.683 22.6	2.79	$C_{17}H_{34}O_2$	**270** **270**
9	1,3-Isobenzofurandione,3a,4,7,7a-teterahydro-4,4,7-trimethyl	23.864	2.55	$C_{11}H_{14}O_3$	**194**
10	2-methoxy-4-vinylphenol	12.780	2.09	$C_9H_{10}O_2$	**150**
11	1-tetradecane	14.114	1.89	$C_{14}H_{28}$	**196**
12	1 -deodecanol	10.036	1.32	$C_{12}H_{26}O$	**186**
13	2-Propenoic acid,3-(4-hydroxy-3methoxyphenyl)-methyl ester	21.837	1.29	$C_{11}H_{12}O_4$	**208**
14	Pentadecanal	19.879	0.93	$C_{15}H_{30}O$	**226**
15	Cinamic acid ,3,4,5-trimethoxy	24.725	0.86	$C_{12}H_{14}O_5$	**238**
16	10-octadecanoic acid methyl ester	24.653	0.69	$C_{19}H_{36}O_2$	**296**
			100%		

3.10 Anti microbial activity of citrus paradisi (grape) and citrus sinensis (orange) crude and oil extraction

Table (3.11) shows anti microbial activity in many concentration of sample the four extracted sample of plant are active towards antibacterial. the activity range from partial active in low concentration to very active in high concentration .the activity towards fungal was found in grape partially in high concentration only but in orange seeds oil found active in high concentration and partially in low concentration.

The activity of orange seeds oil towards organism Ec, Sa and Bs is active in low concentration to very active in high concentration, partially active to active towards Ps organism.

Orange seeds oil and crude extraction is more active than grape seeds oil.

The activity of two plants support the idea of using plant extractions as substituent traditional drug, the traditional uses of seeds in treat of injuring infections may be profitable.

Table (3.11)

Anti microbial activity of *Citrus paradisi* (grape) and *Citrus sinensis* (orange) seeds crude and oil extractions

Grape	**Orange**	Anti microbial				
Methanol extraction		**Ec**	**Ps**	**Sa**	**Bs**	**Ca**
100mg/ml		**18**	**12**	**17**	**16**	**-**
50mg/ml		**17**	**11**	**16**	**15**	**-**
25mg/ml		**16**	**10**	**15**	**14**	**-**
12.5mg/ml		**13**	**9**	**13**	**13**	**-**
6.25mg/ml		**12**	**8**	**12**	**9**	**-**
Oil extraction						
100mg/ml		**15**	**15**	**12**	**18**	**11**
50mg/ml		**14**	**12**	**11**	**15**	**10**
25mg/ml		**13**	**10**	**10**	**15**	**-**
12.5mg/ml		**11**	**10**	**10**	**12**	**-**
6.25mg/ml		**10**	**8**	**8**	**10**	**-**
	Methanol extraction					
	100mg/ml	**17**	**17**	**16**	**16**	**12**
	50mg/ml	**15**	**15**	**15**	**12**	**10**
	25mg/ml	**14**	**14**	**14**	**10**	**10**
	12.5mg/ml	**14**	**13**	**-**	**-**	**10**
	6.25mg/ml	**13**	**12**	**-**	**-**	**-**
	Oil extraction					
	100mg/ml	**15**	**17**	**18**	**14**	**-**
	50mg/ml	**14**	**16**	**17**	**13**	**-**
	25mg/ml	**13**	**15**	**15**	**12**	**-**
	12.5mg/ml	**12**	**12**	**12**	**10**	**-**
	6.25mg/ml	**10**	**8**	**7**	**8**	**-**

The results were expressed in terms of diameter of inhibition zone

< 9 mm inactive

9-12 mm partially active

13-18mm active

>18 very active

3.11 Conclusion

- The study considers that grape fruit and orange seeds are important part of the plant and they can use in traditional medicine use and industrial manufacturing use.
- The phytochemical analysis of grape fruits and orange seeds oil consider that plants seeds oil has physical behavior put the oil with in other healthy and economic oil the component appear in GC.Mas and physiochemical properties expressed the benefits of the oils and also can make oils edible oils.
- Grape fruit and orange seed oilhave many benefits, they can be used as edible oils, as cosmetics oils, lubricant oils and medicine oils.
- The crude extractions of two plants have anti oxidant and anti microbial compounds, which made the seeds important in traditional medicine and industrial uses.

3.2 Recommendation

- Further studies are needed to the following:
- Characterization of *Citrus sinensis* (orange) seeds crude extract.
- Isolation of anti oxidants in *Citrus paradisi* (grape fruit) crude seeds extract and seeds oil.
- Determination of volatile compounds in both plants sample *Citrus paradisi* (grape) and *Citrus sinensis* (orange) seeds oil.
- Study the stability of *Citrus paradisi (*grape) and *Citrus sinensis* (orange) seeds oil.
- Isolation of pure active compound from *Citrus paradisi* (grape) and *Citrus sinensis* (orange) crude extract.
- Make a real application for both *Citrus pardisi* (grape) and *Citrus sinensis* (orange) seeds oil.

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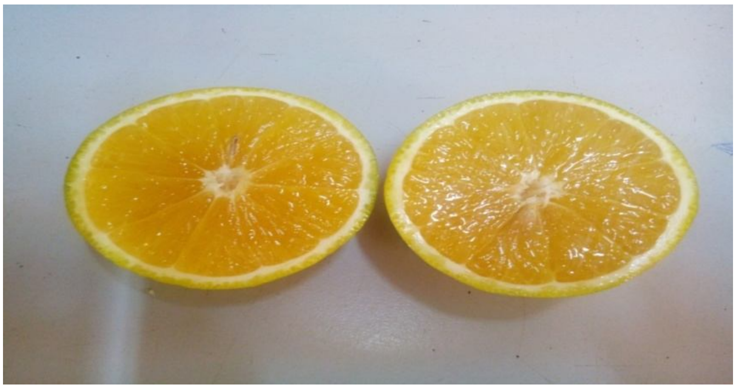
Appendix A

Citrus paradisi (grape) fruits and seeds photos

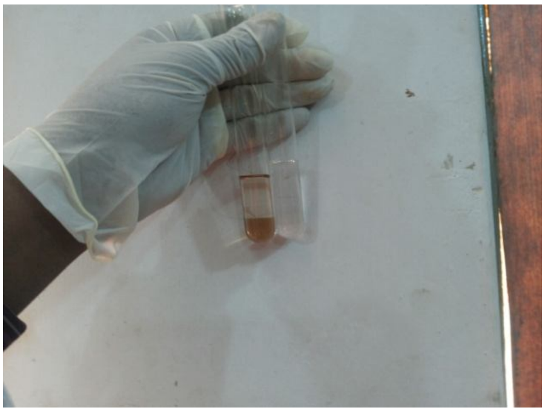
Appendix B

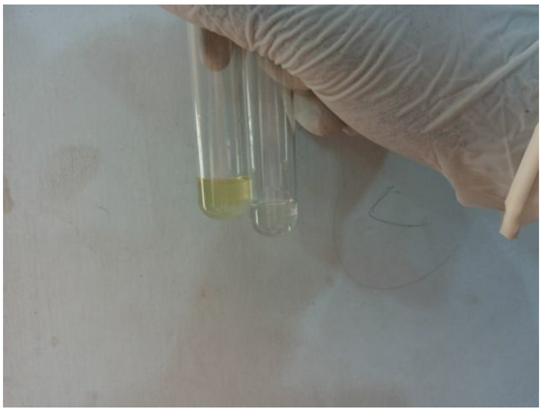
Citrus sinensis (orange) photos

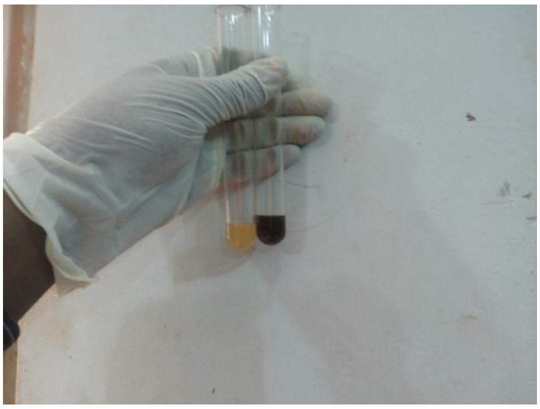


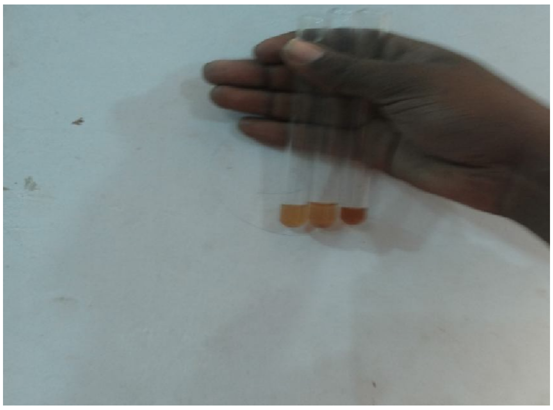
Appendix C

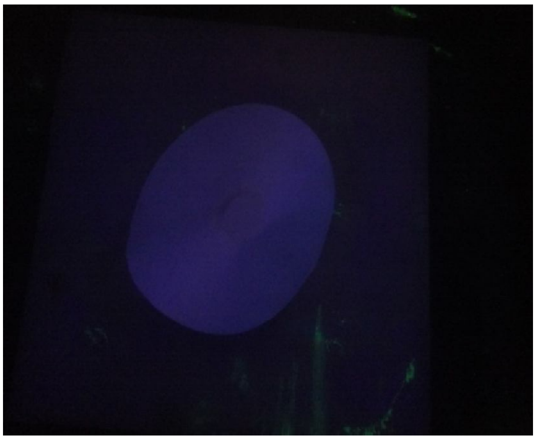
Phytochemical screening photos





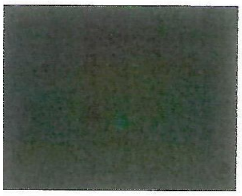




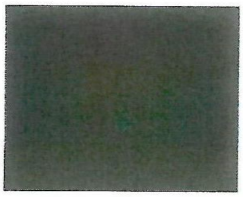


Appendix D

Citrus paradisi (grape) TLC analysis photos

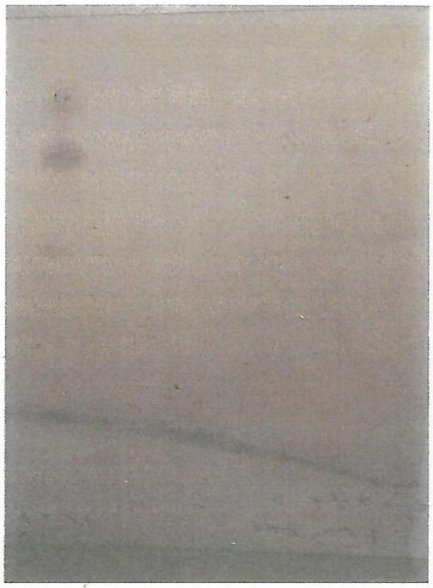


Citrus paradisi (grape) seeds oil TLC photo



Citrus paradisi (grape)seeds oil TLC photo

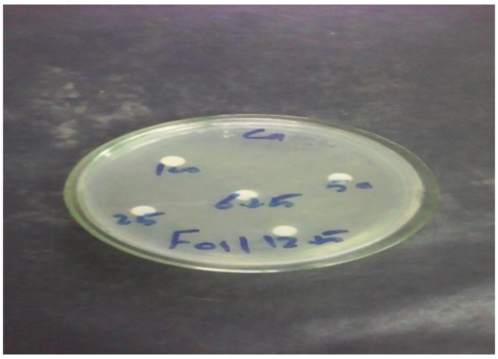
Prep TLC isolation for grape seeds crude extract

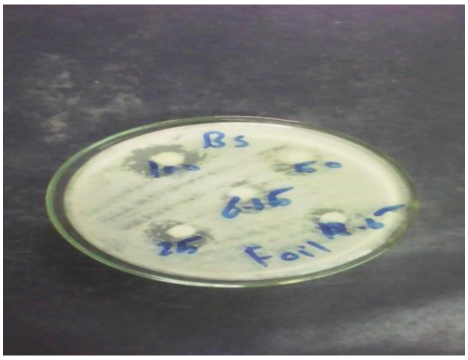


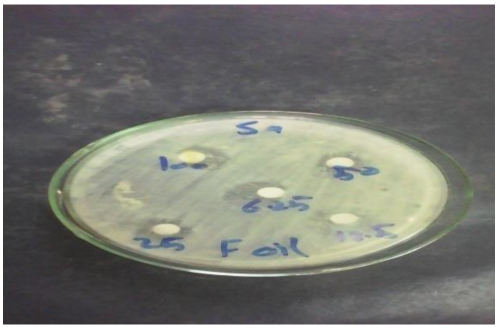
Prep TLC isolation for grape seeds crude extract

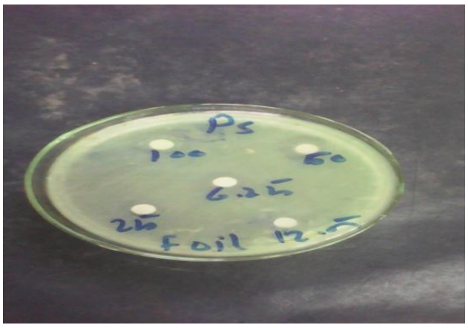
Appendix E

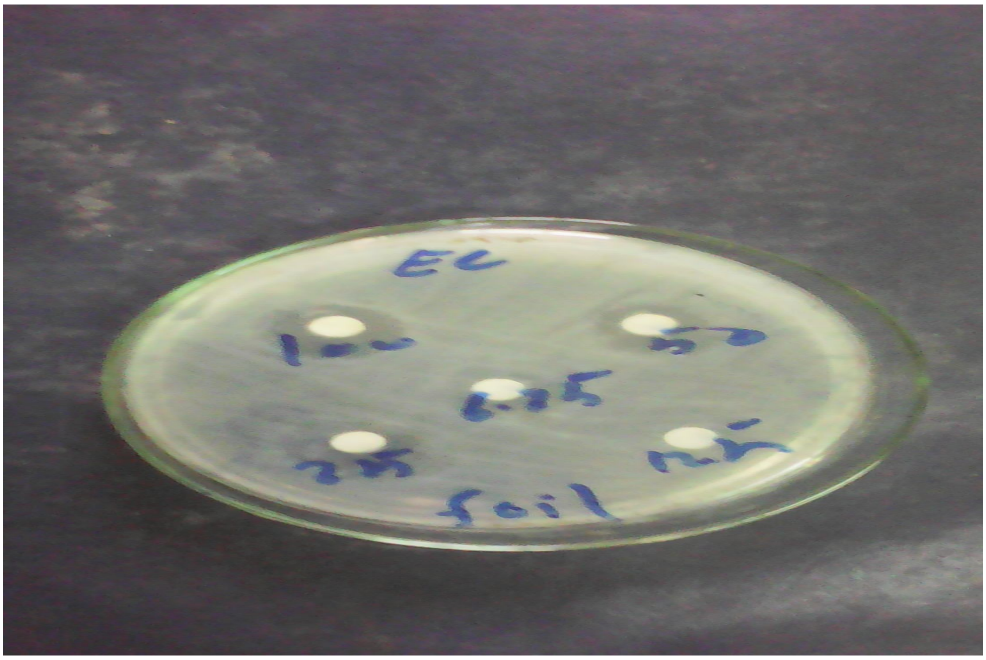
Citrus paradisi (grape) seeds oil antimicrobial activity photos



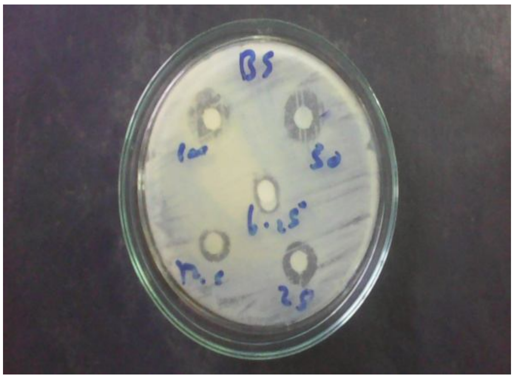


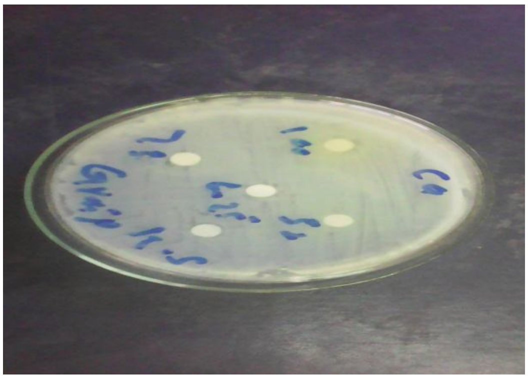


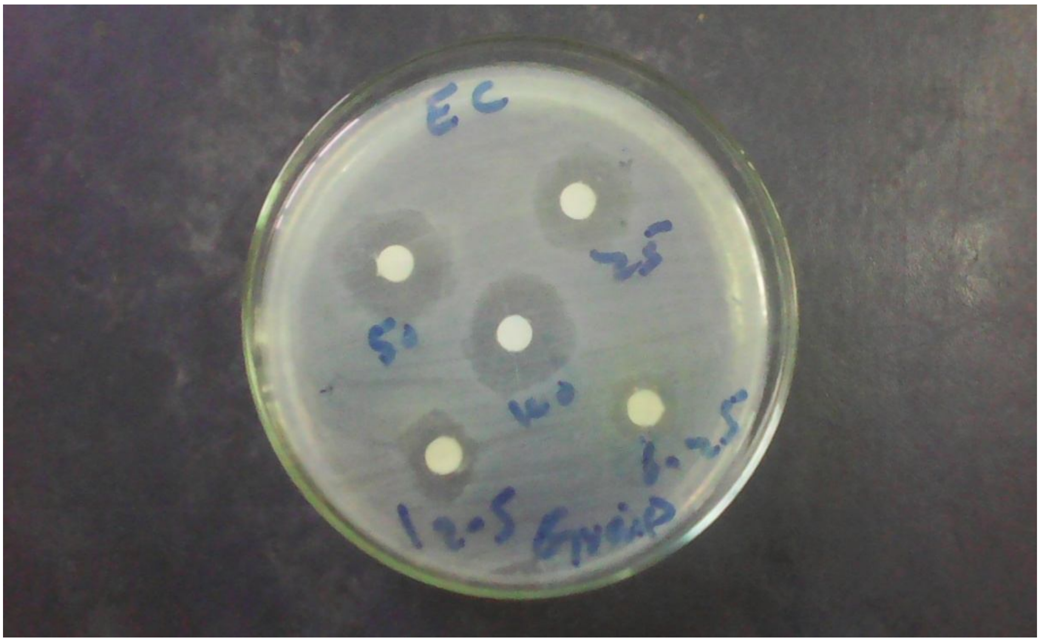




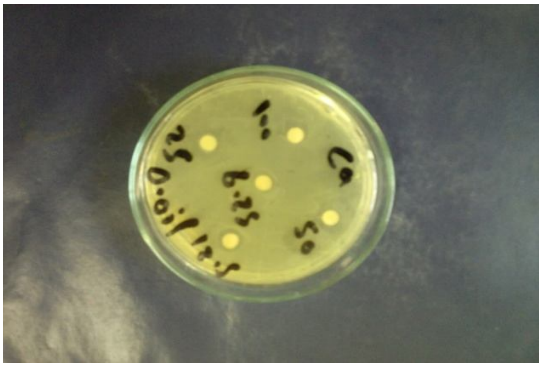
Citrus paradisi (grape) seeds crude antimicrobial activity photos

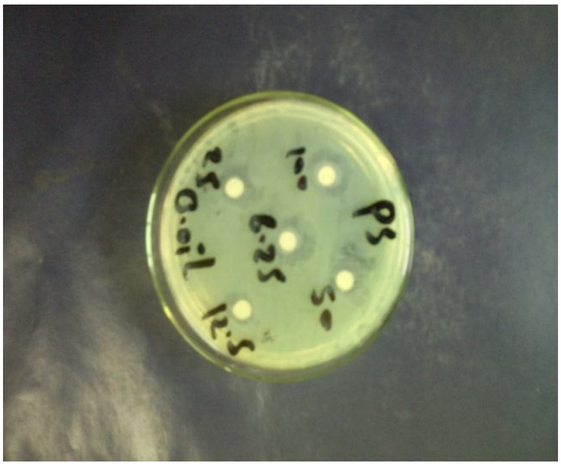


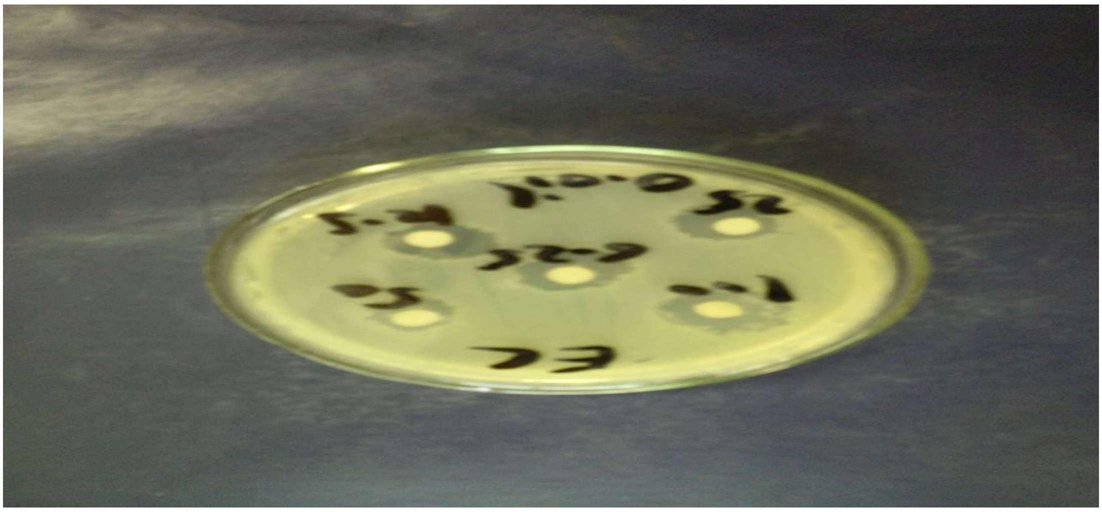




Citrus sinensis (orange) seeds oil antimicrobial activity photos







Citrus sinensis (orange) seeds crude antimicrobial activity photos