بسم الله الرحمن الرحيم


**Sudan University of Science and Technology**

**College of Graduate Studies**

# Physicochemical Analysis of *Balanites aegyptica* Fruits

# تحليل فيزيوكيميائي لثمار الهجليج (اللالوب)

A Thesis Submitted in Partial Fulfillment for the Requirements of Master Degree of Science in Chemistry

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**November, 2018**

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Thesis title: Physicochemical Analysis of Balanites aegyptica Fruits

(تحليل فيزيوكيميائي لثمار الحجليج)

Degree Examined for: Master Degree in Chemistry

"M.Sc. Chemistry"

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# إستهلال

قَالَ تَعَالَىٰ: ﴿ وَفِي ٱلۡأَرۡضِ قِطَعٞ مُّتَجَٰوِرَٰتٞ وَجَنَّٰتٞ مِّنۡ أَعۡنَٰبٖ وَزَرۡعٞ وَنَخِيلٞ صِنۡوَانٞ وَغَيۡرُ صِنۡوَانٖ يُسۡقَىٰ بِمَآءٖ وَٰحِدٖ وَنُفَضِّلُ بَعۡضَهَا عَلَىٰ بَعۡضٖ فِي ٱلۡأُكُلِۚ إِنَّ فِي ذَٰلِكَ لَأٓيَٰتٖ لِّقَوۡمٖ يَعۡقِلُونَ ٤ ﴾ الرعد: ٤

صدق الله العظيم

# Dedication

I dedicate this work to my Parents, Husband, son,

Brothers and sister.

# Acknowledgements

First of all, my endless thanks to Allah almighty for blessing and giving me health, strength and patience to accomplish this work.

My thanks and appreciation go to my supervisor Dr. Omer Adam Mohamed Gibla, for his suggestions, guidance and encouragement.

My thanks extend to my parents for their great help, support and encouragement through the time of this research.

I'm deeply indebted to my husband, sister Eman and brothers for their support.

# Abstract

The aim of this work was to study the physicochemical characteristics of *Balanites. aegyptiaca* fruits. The analysis included, determination of fruit layers percentage (epicarp, mesocarp, endocarp and kernels), elemental content of the fleshy pulp and seeds kernels, extraction and analysis of seeds kernels oil as well as antimicrobial activity of methanolic leaves extract. Inductively coupled plasma and GC-MS instrumentation were used for analysis, chemical composition and investigate the effect of *Banalities* leaves extract as antimicrobial agent. Moisture and ash contents were measured. Some of the oil properties were measured using the appropriate techniques. These include viscosity, density, refractive index colour, free fatty acid, peroxide value, saponification value, iodine value and acid value.

The obtained results showed that moisture content as (7.28%), ash content as (3.64%) and oil (46.6%) for seeds kernels. The elemental measurement showed high concentration of calcium (1588.4 ppm), potassium (8439 ppm), sodium (4398 ppm), phosphorus (2460 ppm), zinc (117.8 ppm) and iron (28.29 ppm) in seeds. And high concentrations of potassium (23046 ppm) and calcium (509.8 ppm) in the fleshy pulp. The physicochemical analysis of *Balanites aegyptiaca* oil showed, viscosity 66.014, density 0.9120, refractive index 1.467, colour 35.4 (yellow), free fatty acid 0.7%, peroxide value 6.8 (mgEq/kg), saponification value 266.475 (mgKOH/g), iodine value 99.104 (mg $I_2$/g) and acid value 1.4022 (mgKOH/g). The GC-MS characterization showed good percentage of the main fatty acids including oleic acid (28.35%), linoleic acid (27.45%), palmitic acid (17.35%) and stearic acid (15.97%). The antimicrobial test on four bacteria species, showed the leaves extract inhibits Escherichia coli gram negative bacteria.

The edible, fleshy part, of desert date was found to be rich in K, Ca, Mg and P. The kernels of the seeds were rich in Na, K and Ca.

# المستخلص

هدف هذه الدراسه هو التوصيف الفزيوكيميائي لثمرة الهجليج (اللالوب). شمل التحليل تقدير النسب المئوية لطبقات الثمرة المختلفة (epicarp,mesocarp, endocarp and kernels) وتحديد المحتوي المعدني لكل من لب ونواة الثمرة؛ بالإضافة لإستخلاص زيت نواة الثمره وتحديد خواصه الفزيوكيميائية كما تم إجراء اختبارات فعالية المستخلص الكحولي لأوراق الشجرة كمضاد للبكتريا.

تم إستخدام جهاز بلازما الحث المزدوج لتقدير المحتوى المعدني لكل من لب الثمرة ونواة الثمرة؛ كما استخدم جهاز كوماتوغافيا الغاز ومطيافية الكتله لتوصيف مكونات الزيت.

أظهرت النتائج ان محتوى الرطوبه(7.28%) ومحتوي الرما د (3.64%) و محتوي الزيت 46.6% لنواة الثمرة. قياس المحتوي المعدني اظهر تراكيز عالية لكل من الكالسيوم 1588.4ppm ، البوتاسيوم 8439ppm، الصوديم 4398ppm الفسفور 2460ppm ،الزنك 117.8ppm والحديد 28.29ppmفي نواة الثمرة. وتركيز عالي من البوتاسيوم 23045 ppm والكالسيوم 509.8 ppmفي لب الثمره.

قياس الخواص الفيزيوكيميائية للزيت اظهرإحتوائه علي لزوجه 66.014 الكثافه النسبيه 0.9120 ، معامل الانكسار 1.467 اللون الاصفر35.5، الاحماض الدهنيه الحره 0.7%، رقم البيروكسيد 6.8، رقم التصبن 266.475 ،الرقم اليودى 99.104،رقم الحموضه 1.4022.

التحليل بكروماتوغرافيا الغاز ومطيافية الكتلة اظهر 21 مكونا للزيت وكانت المكونات الاعلي وجوداً هي الاوليك 28.35% ، اللينولك27.45 % ، الاستريك 15.9% و البالمتك 17.35%.

إختبار المضاد الميكروبي علي أربعه أنواع من البكتريا اظهر أن مستخلص أوراق الالوب يثبيط البكتريا سالبه الغرام E.coli.

مقارنة خواص الزيت المقاسه بالزيوت الاخري اظهرت صلاحيته للاستخدام كزيت طعام وهو اشبه بزيت الفول السوداني.

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# Chapter One

## Introduction

# Chapter One

# 1. Introduction

## 1.1 General

In Sudan, and many dry lands of Africa, numerous types of wild plants are exploited as sources of food. They provide an adequate level of nutrition to the inhabitants. Edible wild plants may primary considered as sources of medicines, food, shelters and other purposes used by humans for so many long periods. Their roots, stems, leaves, flowers, fruits and seeds provide food. (Edem and Miranda, 2011). Most of the edible wild plants provide natural products e.g. Shea, Buckthorn, *Baobab*, *Tamarind*, *Balanites*, *Hibiscus*, *Acacia*… and other. These products play critical role in terms of food security, health, income and ecological services. New trend for production of plant oils from untapped and widely available source and replace it with manufactured oils with high value of natural content (Alia, 2016).

Plant oils represent one of the key materials that can be obtained cheaply from biomass and processed readily to supply the appropriate raw material for chemical industries (Akintayo, 2009). Plants oils have both edible and non-edible applications such as: lubricants, soap production, cosmetics, insulating materials and biodiesel (Ogala et.al ,2018).

In Sudan, oils are generally produced from peanut, sesame, sunflower, coconut, Moringa, castor, cotton seeds, etc. But there are other potential sources of oil which are, still not utilized. *Balanites aegyptiaca* plants seed is one of the oil rich sources. that may need to be used for increasing the oil yield to fulfill the people demand of, and to upgrade the oil quality to protect people from health risks (Ogala et.al ,2018).

## 1.2 *Balanites aegyptiaca* Taxonomical profile: -

The taxonomic classification of *Balanites aegyptiaca* as reported by (National Plant Data Center 2017), was:

| | |
|---|---|
| Kingdom | *Plantae* |
| Subkingdom | *Tracheobionta* |
| Super division | *Spermatophyta* |
| Division | *Magnoliophyta* |
| Class | *Magnoliopsida* |
| Subclass | *Rosidae* |
| Order | *Sapindales* |
| Family | *Balanitaceea* |
| Genus | *Balanites* Delile |
| Species | *Balanitesa egyptiacus* (L.) Delile |

## 1.3 Definitions:

*Balanites aegyptiaca.,* also known as 'Desert date' in English and 'Heglieg' in Arabic, is a species of tree, classified either as a member of the *zygophyllaceae* or *Balanitaceae* (Saed*, et al;* 2018). It is multi branched, ever green, with small flowers. The tree is native to the Sudano-Sahielian region of Africa, the Middle East and South Asia. The plant grows in tropical and desert areas. It is found in many kinds of habitats, tolerating a wide variety of soil types from sand to heavy clay and climatic moisture (Ogala et al; 2018).

*Balanites aegyptiaca* seed kernel is considered as an extremely useful edible product. It is used for producing Balanites oil (lalobe oil). Lalobe oil is used for human consumption and making cosmetics. *Balanites aegyptiaca* seed oil has been used in many countries as ingredient and substituent to groundnut oil in the preparation of local food (Ogala et al ,2018).

According to Abu Al-Futuh (1983), *Balanites aegyptiaca* has a wide range of nutraceutical applications. The Fleshy pulp of the fruit is eaten fresh or dried. The pulp contains 64 –72% carbohydrates, plus crude protein, steroidal saponins, vitamin C, ethanol and other essential minerals for human.

Mohammed et al; (2002) reported that, the seed kernel is edible product, containing good quality of oil and high protein content. The most important constituent is the steroidal saponins, which yield diosgenin, as a source of steroidal drugs, such as corticosteriods, contraceptives and sex hormones (Pettit *et al;* 1991). Tesfay *et al;* (2014), reported, *Balanites aegyptiaca* as a multipurpose tree provides food and fuel-wood, valued for subsistence living in arid and semi-arid areas where other options are few.

The potential of *Balanites aegyptiaca* under management remains unexplored and it is a priority to construct a picture of variation within the natural range and to generate the capacity to raise plants with desirable features as described by Chothani and Vaghasiya (2011).

There are so many common Vernacular names for *Balanites aegyptiaca* in Arabic (zachun, zaccone or heglig, In Hausa, it is known as AduwaLozi (mwalabwe); Luganda (musongole). In Amharic, kudkuda, jemo, bedeno, Jericho balsam,lalob tree, heglig. Egyptian myrobalan, desert date, torch wood.In French, dattiersauvage, dattier du desert, myrobalau d' Egypte. In Hindi, engua, ingudi, betu, hingan, hingn, hingot, hongot, hin *Balanites* gota. In Bemba katikayengele, mubambwangoma Bengali (hin). In English soap berry tree, simple-thorned torchwood, simple thorned torch tree, heglig (in sudan), Mandinka (sumpo); Nyanja (nkuyu); Sanskrit (ingudi); Swahili (mjunju, mwambangoma); Tamil (nanjunda); Tigrigna (indrur, mekie).In Tongan (mulyanzovu, mwalabwe); zacon, kuge, lalob (fruit)); Trade name (desert date (dried fruit, egyptianmyrobalan) (Orwa, *etal*;2009).

*Balanites* tree is described as a savannah tree, which is important species in dry areas in Africa. It is a woody plant of fragile ecosystem of the Great Indian. Flowering and fruiting occurs during October. It is attains a height of more than (6-15) m with generally narrow form. It has a spherical crown and angled mass of long thorny branches. *Balanites* tree is a deep-rooted, evergreen or semi-deciduous tree (figure 1-1). This plant `can be listed among the plants, which

are used as famine foods. The yield of this marvelous tree is about 125 Kg/tree/annum. One tree produces 100 - 150 kg/ year. The thorns, up to 8cm long, are soft at first and later become woody ( Debela, *et.al,* 2011).

(a)

(b)

**Figure 1.1**: *Balanites* tree (Heglig) a,b

## 1.4 *Balanites aegyptiaca* Distribution

According to Orwa, *et.al*.(2009), *Balanites aegyptiaca*, or Hegleig tree has a wide range of geographical distribution. It is indigenous to all dry lands south of Sahara and extending southwards. It is distributed all over the drier parts of India, Kanpur to Sikkim, Bihar, Gujarat, Khandesi and the Deccan plateau. Globally it is found in tropical lands, northern Africa, Syria, Asia and Sudan. It is also found growing in neighboring parts of east and west Africa, particularly Nigeria, and Burma. It is also found in Arabian Peninsula, Iran and Pakistan.Natural distribution is obscured by cultivation and naturalization. It is introduced into cultivation in Latin America and South Asia. Hegleig occurs in a wide ecological range, including the Saharan, Sahelian and Sudanian zones, and can also be found further south, down as far as Tanzania. It is commonly seen in Palestine and Jordan. It is cultivated in Cape Verde, the Dominican Republic and Puerto Rico. In addition, it can also grow in many soil types, including sand and heavy clay, with different climatic moisture levels. The tree has a good adaptive mechanism to grow and thrive under combined water and salinity stresses (Saed, *et.al.*2018).

According to (Alia, 2016).

## 1.5 Fruit constituents

*Balanites* fruit (Lalobe), consists of four parts or layers; The crisp easy outer cover shell (Epicarp), the fleshy pulp (Mesocarp), which, contains sweet and saponins stuff, then the wooden layer (Endocarp), and the inner seed or kernel (Fig 1.2). The fruit is an ellipsoid, long narrow drupe, 2.5 to 4 cm long and 1.2 to 1.5cm in diameter. Young fruits are green and tormentors, turning yellow and globous when mature. Pulp is bitter-sweet and edible. The ripe fruit is brown or pale brown to yellow and resembles a small date. Fruits and young shoots are edible. The leaves and fruits are widely consumed by animals (Rathore, and Meena, 2004) Figure (1.3).

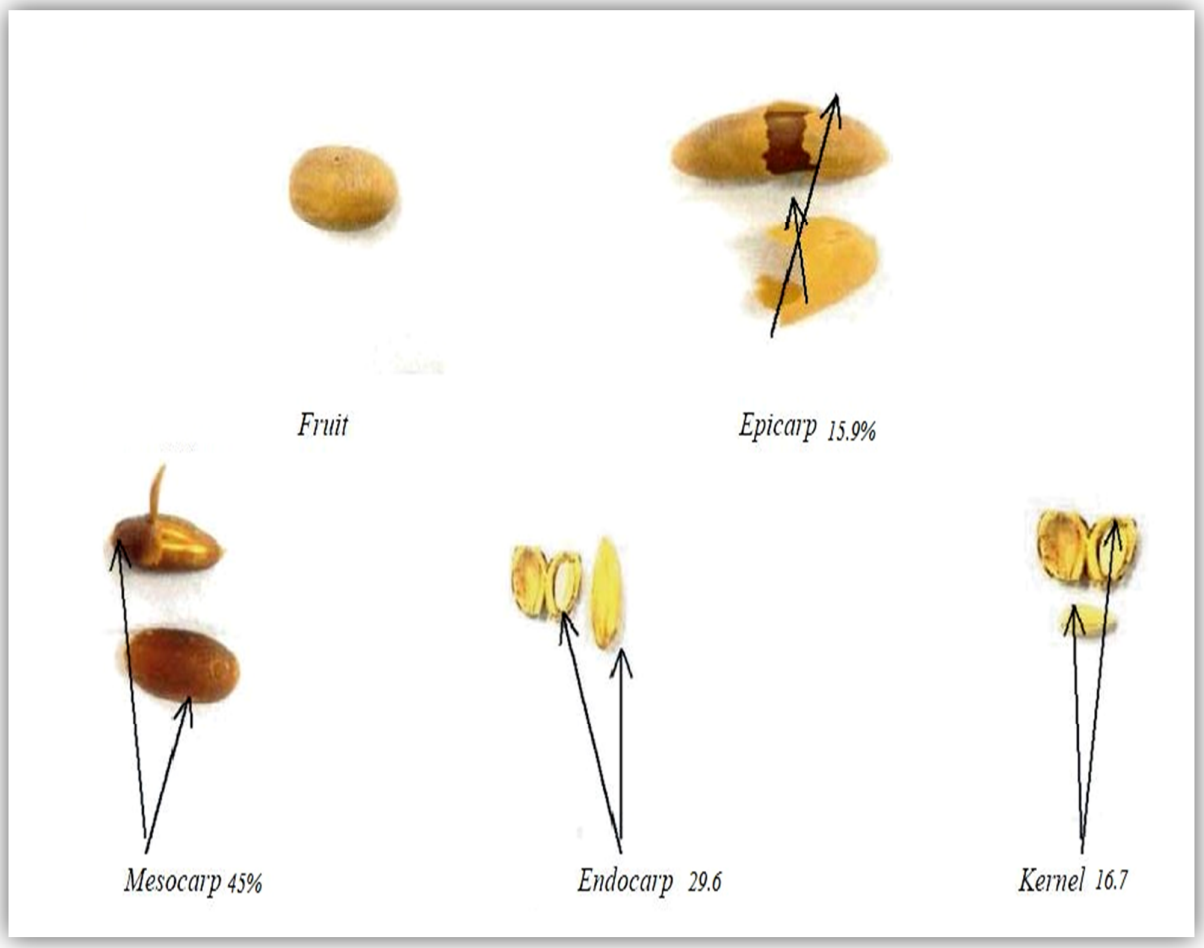



**Figure 1.2:** The four layers of *Balanites* fruit

(a)

(b)

(c)

**Figure 1.3** *Balanites* fruit (a-fruit in tree. b- harvested fruit, c-fruit without cover).

## 1.6 *Balanites* Seeds:

The fruit has thin brittle layer(epicarb), a fleshy layer (mesocarp), woody shell (endocarp) which contain the seed or kernel. The seed is 1.5 to 3 cm long, light brown, fibrous, and extremely hard (Fig 1.4 a). It makes about 50 to 60% of the fruit. There are 500 to 1500 dry, clean seeds per kg. The most important use of the seed is oil extraction from kernels, but the nut is very hard compared to other nuts (Rathore, and Meena, 2004).

(a)

(b)

**Figure 1.4** a-*Balanites* seed. b-*Balanites* seed kernel

## 1.7 *Balanites* Flower, bark and leaves

The Flowers are small, inconspicuous, hermaphroditic, and pollinated by insects. It is in fascicles in the leaf axils, fragrant, yellowish-green, the flowers are yellow-green in colour and up to 4 cm long and 2.5 cm in diameter (Fig 1.5), although flowering most likely takes place in the dry season. The tree begins to flower and fruit at 5 to 7 years of age and maximum seed production is when the trees are 15 to 25 years old. Flowering in Nigeria varies between November and April with ripe fruits becoming available in December. (Vinod and Tarun K,2012).

**Figure 1.5** *Balanites* flowers

The Trunk of the tree is short and often branching from near the base. The Bark is dark brown to grey, deeply fissured. Branches armed with stout yellow or green thorns up to 8 cm long (Vinod and Tarun K,2012).

The Leaves with two separate leaflets. Leaflets obovate, asymmetric, 2.5-6 cm long, bright green, leathery, with fine hairs when young (Figure 1-6). The leaves are sub sessile or shortly petiolate, grey green in colour, orbicular, rhomboid in shape, the apex is acute or rarely obtuse. Leaves are bi-folio late and spirally arranged on the shoots, dark green or grey-green, fleshy succulent with 2 firm leaflets.

(a)

(b)

**Figure 1.6** *Balanites* leaves ( a,b)

## 1.8 Traditional uses

*Balanites aegyptiaca* is used for various needs, such as, firewood, charcoal, poles, timber, tool handle, food, fodder mulch, shade, windbreak and gum The tradition of eating wild plants has not completely disappeared, when they are important as dietary supplements, providing trace elements, vitamins and minerals. The most important parts are fruit pulp and kernel, that, contain saponins, which, have wide industrial and medicinal values (Elfeel, 2010).

*Balanites aegyptiaca* plants are reported to be used in a variety of folk medicines, in Africa and Asia. It has been reported to be used in the treatment of skin diseases, remedy for stomach ache, jaundice and cough treatment, as well as treatment of diarrhea, syphilis and Typoid fever (John et al., 1990; Boulos, 1992; Doughari et al., 2007). The Roots were also reported to be used in treatment of inflammation and antidote for snake bite (Inngerdigen, 2004 and Kubmarawa et al., 2007). Earlier studies have shown, that, Banalites aegyptiaca contains steroidal saponins, with most of them, reporting, the presence of saponins as the main cause of these activities. Besides it is medicinal uses, Balanites trees are widely used as fodder and for timber purposes (Arbonier, 2002).

The chemicals which are referred to as active principles or phytochemical substances, include, terpenses, flavonoid, bioflavonoid, benzophenones, xanthenes as well as some metabolites, such as, tannins, saponins, cyanates, oxalate and anthrax- quinones (Iwu, 1993; Asaolu, 2002).

Fruits can be eaten either fresh or dry or it can be processed to produce different traditional products. Sometimes, the fruits pulp is fermented to make an alcoholic drink or may be macerated in water to make a refreshing beverage (NRC, 1983 and Von- Maydell,1986).

In Kordofan, the fleshy mesocarp is extracted and mixed with gum Arabic to make sort of sweet called "Sernev". Also, the seeds kernels of the fruit are boiled with continuous change of water, to remove the bitterness of the fruit and

then eaten with sorghum. This kind of food is called “Kornaka” (Abdon,2005 and Wildpedia, 2010).

In Kordofan and Darfur the green leaves, young sprouted leaves and green thorns are eaten fresh as vegetable salad or may be cooked (NRC, 1983;Von-Maydell, 1986; and Abdoun, 2005). In Sudan, Senegal, Nigeria, Morocco, Ethiopia, and Chad, fresh twigs are put on the fire in order to keep insects away. Fruits of *Balanites* aegyptiaca are available during two seasons of the year: the cold season (December to February) and shortly before the rainy season (Alia,2016).

Seeds kernel may be eaten after removal of the wooden cover, with water and honey. The seeds kernel contains 30-60% oil. The plant contains high amount of nutritive oil, extracted from *Balanites* seeds which may be used for cooking, hydrated skin and treated for diseases. it contains good quality oil and high protein content, constitute the majority of foodstuffs. They are also used in wide industrial applications, like formulation of soap. The oil used in Sudan to made chaplet. Seeds can be stored with insecticides. Oil cake is used as an animal feed(Alia,2016).

Leaves are used as fodder for livestock. Leaves are also used for cleaning infected wounds. In Sudan and Chad *Balanites* is component of soap. For contraception, in Nigeria, a mixture of dried leaves powder of *Balanites* in water is used. Latex of the plant is used in epilepsy, administered through intranasal route. Used as tooth brush.

Bark is used to produce baseboard to save holy Quran, wooden pot to drink water. Dry bark is use as fuel in villages, it is a good firewood source give strong light with low smoke, it is also used for making furniture. (Alia,2016)

## 1.9 Medicinal uses

Medicinal uses of this plant are numerous. The fruit is mixed into porridge and eaten by nursing mothers. The oil is consumed to cure headaches and to improve lactation. Bark extracts and fruits repel snails and copepod as well as organisms that, host the parasites *schistsome* and *guinea* worm.

The fruit is used to treat liver disease and the bark is effective in the treatment of syphilis, round worm infection and fish poisoning. The aqueous leave extract and saponins isolated, from, the kernel cakes have anti-bacterial effect and potent larricidal activity.

Von-Maydell (1986) and EI-Ghazali, et.al (1994) reported that, *Balanites aegyptiaca* leaves and branches clean malignant wounds and enhance wound healing, while, the bark extract is used for the treatment for toothaches, stomach complaints, heart burn, sore throat and as a remedy for mental diseases, sterility, epilepsy and yellow fever.

*Balanites aegyptiaca* contain steroidal (saponins, sapogenins and diosgenins) which can be used as starting material for the synthesis of certain steroidal drugs for the treatment of stomach complaints, sterility, mental diseases and as sex hormones. In India, it is also used in curing of some skin diseases like Leucodermia. Moreover, the fruits and leaves are used for the treatment of bilharzia and as laxative material (Karlyn and Deboraha, 1993; Abdoun, 2005) In North Kordofan, people usually use *Balanites aegyptiaca* fruits as a drink against' constipation and as anti-diabetic (E1- Ghazali et. al., 1994).

The macerated *Balanites aegyptiaca* fruit pulp is also mixed with millet to make Porridge, which is usually, given to women after child birth or during the lactation

Period, to give them energy and to increase the milk production. Also, the *Balanites aegyptiaca* seeds were mentioned in the treatment of headache, influenza and rheumatism (Abdoun, 2005).

Bark extracts and the fruit repel or destroy freshwater snails and copepods, organisms that act as intermediary hosts the parasites *Schistosoma,* including *Bilharzia,* and *guinea* worm, respectively. Existing worm infections are likewise treated with desert date, as a liver and spleen disorders. A decoction of the bark is also used as an Abortifacient and an antidote for arrow-poison in west African traditional medicine.

## 1.10 Chemical Composition of *Balanites* fruits

Cook, *et. al.* (1998) reported that, *Balanites aegyptiaca* fruit pulp contains high amounts of sugar, protein, lipid, minerals and vitamins. NRC (2008), reported that the total sugar of Lalobe fruits pulp ranges from 40-70 % and it contains about 5% proteins and 0.1 % fat,15% organic acids and 46% other inorganic materials. The meal left after oil extraction is also edible and contains vitamins. Extraction of the kernels yields 20-58% light yellow oil, it consisted of four major fatty acids; palmitic, stearic, oleic and linoleic, constituting 98- 100% of the total fatty acids in the oil of all tested genotypes. Linoleic acid was found to be the most prevalent fatty acid, ranging from 31% to 51% of the fatty acids profile, which is very similar to soybean oil profile. The leaves contain protein, linolein, olein, saturated acid glycosides. The mineral contents of *Balanites aegyptiaca* fruits pulp were investigated by Abdulrazak, *et. a.,* (2010), where the concentrations of calcium, magnesium, phosphorus, sodium, sulpher .and iron were found to be 24.4, 6.33, 1.58, 0.542, 1.81 and 1.23 as g/kg on dry basis respectively. Available reports on the nutritional and antinutritional profile of seeds powder shows that the seed powder contains a relatively high amount of protein and lipid.

In addition to the nutrients, the kernels contain high level of antinutritional factors; tannins, oxalate and phytic acid. Tannins are secondary plant metabolites that are rich in phenolic hydroxyl groups and have been implicated in the inhibition of non-heme iron absorption, by complexing with iron in the gastro intestinal lumen. Tannins are also known to inhibit oxidation of alkaloids

and morphine and form colored complexes with iron, thus reducing the bio-availability of this important mineral.

Oxalic acid has the ability to form a strong bond with various minerals such as sodium, potassium, magnesium and calcium. The compounds formed are usually referred to as oxalate salts. Some of these salts are practically insoluble in water; such as calcium oxalate. If consumed in significant quantity, calcium oxalate has the propensity to precipitate in the kidneys or in the urinary tract to form calcium oxalatecrystals leading to diseases such as kidney stone. Binding of oxalates to calcium renders such calcium metabolically unusable.

Phytate is a major storage form of phosphorus Phytate is normally found in form of complexes with cations like iron, zinc, magnesium calcium. Phytate inhibits non heme absorption thus affecting cation bio-availability. Since there is limited information on the potential effects of various processing methods and techniques on the level of nutritional and antinutritional components present in *Balanites aegyptiaca,* it becomes necessary to examine how ethanolextraction as a processing technique, reduces these antinutritional components (Lohlum *et.al* ,2012).

## 1.11 Plant based fats and oils

Fats and oils are fundamental components of human diet. They have more caloric values than proteins and carbohydrates. This make them good energy sources. They contain essential fatty acids, which are not synthesized by human body and should be obtained only from food sources. Fatty acids serve to carry fat-soluble vitamins A, D, E, and K, as well as, an array of phytonutrients.

Fats and oils are water-insoluble compounds consisting mainly of triacylglycerols. “Three fatty acids esterified to aglycerol molecule”. Products are generally called fats, when they are solid at room temperature, and known as “oils” when they are liquid at room temperature. The two terms are often used interchangeably. Edible fats and oils contribute to the flavor, texture, aroma, and mouthfeel of foods, while providing nutritive value. Their origin may be

animals, plants, or marines. Plant-based fats and oils are obtained from seeds. While used mainly for human consumption, they also find use in animal feed, biodiesel, and industrial applications. They are integral components in a wide range of products such as margarines, shortenings, dressings, confectionery products, baked goods, snack foods, infant formulas, and non-dairy creamers (Clark *et.al*, 2014).

### 1.11.1 Sources, composition, and uses of plant-based fats and oils

Oils may be obtained from hundreds of plant species. While some are produced on a large scale and internationally traded as commodities, most are considered minor oils and find specialized use. The worldwide predominant four oils, are palm, soybean, rapeseed/canola, and sunflower seed. They are now in decreasing order of production. Within the last decade, palm has overtaken soybean as the top commodity plant oil. Total production of commodity plant oils has steadily increased over time, and further increases in four productions are projected. In Sudan the most famous edible oils are cotton seed, ground nut, sesame and sun flowers (FAS, 2011).

The properties of fats and oils are highly dependent on their fatty acid and triacylglycerol make-up. In addition to the length of the carbon chain and degree of saturation in a particular fatty acid, the position of fatty acids on the glycerol molecule affects the physiochemical properties and functional characteristics of the fat or oil. Fatty acid composition varies widely between different oil sources. Some variability exists within the species based on factors such as climate, soil quality, growing season, and plant maturity.

### 1.11.2 Physical properties

Physical properties of fats and oils include melting point, boiling point, smoke point, density, solid fat index, viscosity, refractive index, and color. Melting point is a function of many variations, e.g. melting point increases with increasing fatty acid chain length, complexity of triacylglycerol components, degree of saturation, and content of trans fatty acids. Since they are composed of mixtures of triacylglycerols, fats possess a melting range rather than a true melting point; thus, the term "melting point" refers to the end of the melting range. Solid fat index is a measure of the solid content of a fat at various temperatures, which is an important predictor of melting and crystallization behavior. The solid content of a fat influences its plastic range, the temperature ranges over which the fat is moldable. Plastic fats possess properties of both solids and liquids, as the liquid portion is trapped in the solid crystalline network (Hidalgo and Zamora,2006). Various processing procedures are applied to fats to extend their plastic range, leading to a wider range of use in food applications (Clark *et.al*, 2014).

### 1.11.3 Chemical properties

This include iodine value, saponification value, peroxide value, and acid value. Iodine value is a measure of the average degree of unsaturation of a fat and is a predictor of its oxidative stability. Saponification value measures the average chain length of fatty acids in a fat. Peroxide value and acid value measure peroxides and free fatty acids present in fat/oil, respectively. Standard methods for the determination of physicochemical properties of fats and oils are available (Horwitz and Lattimer, 2005; Firestone, 2009).

### 1.11.4 Degradation of plant-based fats and oils

Crude oils are highly susceptible to degradation via lipolysis and oxidation. Lipolysis, is the hydrolysis of free fatty acids from the glycerol molecule, It decreases the stability of the oil. (Lipolysis may be catalyzed by enzymes,

particularly from microbial sources, or water and heat. Oxidation occurs in the presence of atmospheric oxygen and yields low molecular weight compounds responsible for development of off-flavors and odors. Care must be taken to protect oils from these reactions during refining, modification, and storage (Clark *et.al*, 2014).

According to Choe and Min (2006), Plant-based fats and oils are subject to both autoxidation and photo-oxidation. Both mechanisms are selective, acting at points of unsaturation. Thus, susceptibility of fats and oils to oxidation increases as degree of saturation decreases. Oxidation may be promoted by environmental factors e.g. temperature, light or components, naturally, present in the crude oil, such as free fatty acids and trace metals.

### 1.11.5 Handling considerations

Degradation of edible fats and oils cannot be stopped, but can be slowed by taking certain precautions during processing and storage. where appropriate, care should be taken, e.g.

-Equipment surfaces should be cleaned and sanitized regularly to eliminate spoilage by microorganisms and other adulterants, and to avoid build-up of oxidation products

-Some processing steps require an inert atmosphere and may be conducted under vacuum or nitrogen blanket to avoid the effect of atmospheric dioxygen.

-Trace metals (e.g. Cu) act as prooxidants, and their levels may be minimized by the use of stainless steel equipment's during processing

-Oils should be stored in a cool, dry, dark locations, because direct light and heat promote reactions that lead to oxidative rancidity and in the case of water, hydrolytic rancidity, take place (Clark *et.al*, 2014).

## 1.12 Classification of oils

Depending on the source, there are two types of oils, organic oils and mineral oils. Organic oils are produced by plants, animals and other organisms through

natural metabolic processes. Organic oils which are generally laid down by plants and animals are called triglycerides and are mainly energy reserves (Albert *et al*., 2002). Mineral oil is a type of oil which includes crude oil or petroleum and its refined components collectively termed petrochemicals (Dinardo, 2005).

Oils are heterogeneous biochemical substances, which, have in common, the property of being soluble in most organic solvents) and insoluble in water. Oils are often divided into three categories according to their qualities and as non-drying oils, semi drying oils and drying oils (Gunstone, 2002). Non-drying oils are slow to become oxidized, they remain liquid for a long time, and this quality makes them particularly useful as lubricants and as a fuel for lamps. Drying oils are quite quick to become oxidized and turn to solid after their oxidation, so there for are often used in paints and varnishes. A good example of a drying oil is linseed oil. Semi-drying oils have qualities intermediate between non-drying oils and drying oils (Zang*,et.al.,*2017).

Plant oils represent one of the key materials, that can be obtained cheaply from Biomass, and readily processed to supply the appropriate raw material for chemical industries. Plants oils have both edible and non-edible applications such as: lubricants, soap production, cosmetics, insulating materials and biodiesel (ogla *et. al,*2018).

## 1.13 Fixed oils Extraction

The goal of extraction is to recover a maximum amount of crude oil, with, highest purity. There are three main types of extraction processes; which are mechanical pressing, pre-press solvent extraction, and direct solvent extraction. The extraction procedure is normally selected based on some variables such as oil content of the starting material, size of the extraction facility, and desired specifications for the resulting oil. During the extraction process, it is critical to

control moisture and temperature in order to avoid damage to the oil while achieving maximum yield (Clark *et.al*, 2014).

## 1.14 *Balanites* seed kernel oil

The seeds oil of *Balanites aegyptiaca* is a triglyceride organic oil normally obtained from *Balanites aegyptica* seed kernel. It is classified as a vegetable oil. The seed contains 30-48% of fixed oil. These oils have been part of the human culture for millennia (zang, *et.al.2017*). The oil was reported to be rich in saturated fatty acids and is used as cooking oil (Hall and Walker, 1991; NRC, 2008). Hussain *et al, (*1949), Cook *et al*, (1998) and Mohamed *et al, (*2002) Reported, that, the seed oil consists of four major fatty acids; linoleic, oleic, stearic and palmitic acid, but in varying proportions across study sites. Some studies have demonstrated and recommended use of *Balanites* oil for biodiesel production. There is, therefore, a growing interest in understanding the development potential of *Banalites aegyptiaca* as, a resource, for improving livelihoods of dryland communities. Natural vegetable oil and fats are increasingly becoming important worldwide in nutrition and commerce because they are sources of dietary energy, antioxidants, biofuels and raw material for the manufacture of industrial products. (Sara Mohamed, 2016).

According to FAO (2007), vegetable oils account for 80% of the world's natural oils and fat supply. Nutritional information of *Balanites* oil will prove useful to nutritionists, policymakers, development agencies and the general public in Uganda and elsewhere, where, nutrition and health benefits would be most beneficial. Most of the reported biological activities included using *Balanites* seed extract as anticancer and fruit mesocarp extract as fasciolicidal related to polar constituents. Unsaturated fatty acids where reported to have anticancer and antimutagenic activity; in addition, fatty acids have antimicrobial activity (Sara Mohamed, 2015).

## 1.15 Physicochemical characteristics of *Balanites. aegyptiaca* seeds oil

Physicochemical characteristics of any oil are important for determining its nutritional quality and commercial value (Omujal, 2008; Chapagain *et al.,* 2009).

### 1.15.1 Colour

Colour in *Balanites* oil may be due to presence of carotene. According to FAO/WHO (1994) and WHO (2004), carotenoids and their derivatives are responsible for the yellow colour of fruits, vegetables, cereals and some crude oils. The presence of carotene makes *Balanites* oil nutritionally important because carotenoids are highly unsaturated polyisoprene hydrocarbons that are lipid and are precursors for vitamin A (WHO, 2004). The light yellow colour of the oil also makes it visually attractive thus, along with other good attributes; this could make *Balanites* oil a viable and competitive market commodity. Okia (2013), Babagana *et.al*., (2012) and Babeker (2013) reported that *Balanites aegyptiaca* oil colour was light yellow. (7,633R.y. b).

### 1.15.2 Refractive index

Sara, (2016), reported that, refractive index is an important attribute of oil quality. Of light in vacuum to the velocity of light in the medium being measured. The RI of oils and fats were closely related to oxidation products and development of rancidity. It is useful for identification purpose and for establishing purity, and also for observing the progress of reactions, such as catalytic, hydrogenation and isomerization. Babeker (2013) Okia (2013) and Manji (2013) reported refractive index of *Balanites aegyptiaca* oil were 1.46 ,1.47 and 1.48 at 40℃ respectively.

### 1.15.3 Viscosity

Eugene *et.al*;(1991) defined the viscosity as the measure of resistance to flow. Viscosity is also defined as the measure of the internal fractions in the oil, and is an important index of the study of oils and their intermolecular forces. It's a

useful criterion for degradation or depolymeraization, such as that, occur in initial stage of hydrolysis of fat and oil during storage (Sara,2016).

Babagana *et.al*., (2011), Okia (2013) and Babeker (2013) found viscosity of *Balanites aegyptiaca* oil were 34.00 22.60 ,34.00 and 37 at 40℃ respectively.

Viscosity equal the ratio of viscosity of solution to the viscosity of solvent used.

$\mu=\eta_1/\eta_2$

Where;

$\eta_1$= Relative viscosity of oil.

$\eta_2$= Relative viscosity of water=1002 pois (Pascal. Sec).

$\eta_1= \frac{d1\times t1}{d2\times t2}$

d1= density of oil.t1=time of oil

d2= density of water=1000m3/sec. t2=time of water=30 sec.

## 1.15.4 Density

Relative density or specific gravity, is the ratio of the density of substance to the density of a given reference material. Specific gravity usually means relative density with respect to water. It is defined as a ratio of density of particular substance with that of water. The specific density of the oil should be calculated as follows;

Specific density = $\frac{w1-w0}{w2-w0}$

Where;

W0 = Weight of empty density bottle (g)

W1 = weight of density bottle filled with water (g)

W2 =weight of density bottle filled with oil (g)

Babagana *et.al*., (2011), Babeker (2013) and Manji (2013) found density of *Balanites aegyptiaca* oil to be 0.277, 0.92 and 1.001 respectively

## 1.15.5 Free fatty acid

The low FFA% reduces the tendency of the oil to undergo hydrolytic activities. Babagana *et.al*., (2011) and Babeker (2013), reported that FFA values of *Balanites aegyptiaca* oil as 1.84 and 2.8% respectively.

## 1.15.6 Peroxide value

Hydro peroxides are the primary products of lipid oxidation; therefore, determination of peroxide value can be used as an oxidation index for the early stages of lipid oxidation. Amany *et al., (*2012), Mohammed *et al*., (2013) noticed that, peroxides are formed as a result of oxidation, under normal conditions. These peroxides can break down in to secondary oxidation products usually containing carbonyl group. Oxidation of lipid to hydroxide, referred to as the peroxide value. The change peroxide value of oils and fats during storage under controlled conditions is an important parameter for detecting their quality. Standards showed that the peroxide value of oil should not be more than 10 milliequivalents of peroxide. Manji (2013) and Babeker (2013) reported peroxide value of *Balanites aegyptiaca* oil as 6.0 and 8.0 (mgEq/kg) respectively. The peroxide value is calculated as;

$$\text{PV}= \frac{(va-vp)\times M\times 1000}{W}$$

Where:

Va= Volume of sodium thiosulphite solution used in titration

Vb= Volume of sodium thiosulphite solution used in blank test

W= Weight of sample in grams

M= molarity of sodium thiosulphate solution

## 1.15.7 Acid value

Acid value is a common parameter in specifications of fats and oils. It is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids in one gram of the sample. And its measure of the free fatty acids presents in fat or oil. The acid value is calculated by using the expression

$$AV = \frac{56.1 \times V \times M}{W}$$

Where;

V = Volume in ml of the standard potassium hydroxide or Sodium hydroxide used

M = molarity of the potassium hydroxide solution or sodium hydroxide solution

W = Weight in g of the sample.

56.1 = molar mass of KOH.

Standards showed that the acid value should not be more than 0.8 mg KOH/g. The acid value is measure of the extent, to which, the glycerides in the oil have been decomposed by lipase action. Babagana *et.al*., (2011), Okia (2013) and Babeker (2013) reported acid value of *Balanites aegyptiaca* as 0.57, 1.41 and 2.08% respectively.

## 1.15.8 Saponification value

Is the number of milligram of potassium hydroxide required to neutralize the free acids and to saponify the esters in one gram of fat or oil sample. Babagana *et.al*., (2011), Babeker (2013), Manji (2013), and Okia (2013) founded saponification value of *Balanites aegyptiaca* were 174.5,168.3 ,168.80, and 182.80 mgKOHg respectively.

The saponification value (Sap.V) is calculated as;

$$\text{Sap.V} = 28.05 \frac{(b-a)}{W}$$

where;

SV = Saponification Value (mgKOH/g)

a = ml of HCL from sample.

b = ml of HCL from blank.

W = weight of oil in gram

## 1.15.9 Iodine number

This is the number of milligrams of iodine absorbed by one-gram fat or oil sample. It is a measure of proportion of unsaturated constituents present in fat sample (Hartley,1967). The iodine number gives an indication of the number of double bonds in any particular oil or fat, Babagana *et.al*., (2011), Manji (2013) and Okia *et. al.*, (2013) reported iodine number of *Balanities eagyptiaca* were 56.4 ,76.8 and 98.28 mg $I_2$/g respectively. The iodine number is calculated as follows;

Iodine number = $\frac{M(b-a)\times 126.9\times(100/1000)}{W}$

Where;

126.9 = Molar mass of iodine.

M = Molarity of Sodium thiosulphate.

a = Volume of Sodium thiosulphate used for blank.

b = Volume of thiosulphate used for the test.

100/1000 = Multiplication factor as define for iodine number.

W = Weight of oil sample

### 1.15.10 Fatty acid composition of oils

The fatty acid profile is important for determining the nutritional value of oils (WHO, 2004; NAS, 2005). The fatty acid composition of *Balanites* oil revealed linoleic acid as the predominant fatty acid. Four major fatty acids in the order linoleic>oleic> stearic>palmitic were found in oils. linoleic acid derivatives serve as structural components of the plasma membrane and as precursors of some metabolic regulatory compounds Gottenbos, the presence of one of the four essential fatty acids in *Balanites* oil makes it nutritionally valuable and highly recommended for human consumption. (sara,2016)

## 1.16 Minerals content of *Balanites aegyptiaca* seed

Macronutrients are important in human diet because of their various functions in the body (Christian and Ukhun, 2006). Sodium is a vital mineral for

maintaining fluid volume, osmotic equilibrium and acid-base balance. It's deficiency during hot weather is attributed to heavy work in hot climate. Christian and Ukhun, (2006). Elfeel (2010) and Lohlum (2012) reported *Balanites aegyptiaca* seed sodium content as 0.02, 0.90 and 0.93 mg/l00g respectively. Magnesium Functions as a cofactor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis as well as maintenance of electrical potential of nervous tissues and cell membranes.

Potassium is very important in human body where, along with sodium, it regulates the water balance and the acid-base balance in the blood and tissues. In the nerve cells. Lohlum (2012), and Elfeel (2010) reported that *Balanites aegyptiaca* seeds content as 1.09, and 1.95 mg/l00g respectively. Calcium is required for proper bone and tooth growth; during adolescence, as the bones develop. Lohlum (2012) and Elfeel (2010) measured calcium content of 0.19 and 0.415 mg/l00g respectively. phosphorus is required by the body for bone and teeth formation. New research shows, that calcium needs phosphorus to maximize its bone-strengthening benefits. So taking a lot of calcium supplements, without, enough phosphorus may not be goods (sara 2016).

## 1.17 Biological effects of balanites

### 1.17.1 Antidiabetic effects

Different extracts of *Balanites aegyptiaca* show antidiabetic and hypoglycemic effects as reported by many studies done to prove and understand the possible mechanisms involved. Water extract of the mesocarp of fruits of *Balanites aegyptiaca* was studied to possess lowering sugar level effect in STZ- induced diabetic mice. Ethyl acetate extract from *Balanites aegyptiaca* has a defensive effect against oxidative stress induced by streptozocine with reduction in blood glucose levels, (Al-Malki, *et.al* 2015).The fruit extracts decreased the level of blood glucose by 24%, with decreasing liverglucose-6-phosphatase activity extensively in diabetic infected rats. The aqueous and ethanolic extracts of

*Balanites aegyptiac* fruit induce significant reduction in every component of diabetes which include serum glucagon, total lipids, total cholesterol, triglycerides level (Baragob, *et.al* 2014). It is reported that the antidiabetic activity was due to the presence of steroidal saponins in the extracts (Gad, *et.al* ,2006).

## 1.17.2 Antibacterial effects

The aqueous and organic leaves extracts of *Balanites aegyptiaca* and *Moringa oleifera* were reported to have antibacterial effect against *Salmonella typhi* isolated from blood clot culture using the disc diffusion method. The extracts of *Balanites aegyptiaca* leaves demonstrated the highest activity than *Moringa oleifera*. The ethanolic extracts of both plants demonstrated the highest activity whereas the aqueous extracts of both leaves showed the least activity at 100 mg/ml as compared with ethanolic extracts. The activities of these plant extracts were comparable with those of antibiotics, ciprofloxacin, cotrimoxazole, and chloramphenicol, commonly used for treating typhoid fever. The antibacterial activity appears to increase when extracts of the two plants were used in combination at 100 mg/ml each. (Ezzat,*et.al*. 2017).

## 1.17.3Anti-cancer effects

According to two studies –conducted in mice, Saponin extracted from *Blanities aegyptiaca* fruit showed anti-tumor activity and it reduced the number of ehrlich ascites carcinoma in both therapeutic group and preventive groups with an increase in life span compared to controls (Al-Ghannam, *et.al*.2013).In the same context, it showed anti-proliferative and cytotoxic activity using various extracts as ethylacetae extract, ethanol extract and chloroform with ethylacetae extract being the most effective among them (Al-Malki, *et.al*.2016).

Methanol extract of *Blanities aegyptiaca* (L.) Del stem bark acted as anti-tumor agent in mice injected with HCT-116 cells with significant reduction in cancer cell growth (Hassan, *et.al*.2016).

### 1.17.4 Anti-oxidant effects

Different parts of *Blanities aegyptiaca* extracts has been reported to have an anti-oxidant effects. In addition, a raise in antioxidant enzymes as superoxide dismutase and catalase in mice, treated with these extracts was an evident in comparison to control group (Issa, *et.al* 2015). Another study showed that methanol extract of *Blanities aegyptiaca* revealed the highest antioxidant ctivities while, hexane and water extracts were showed unimportant activity. It revealed a strong positive relation between total flavonoid and total phenoliccontents and ferric reducing anti-oxidant power, although a negative relation was found between both against Di(pheny)-(2,4,6-trinitrophenyl) iminoazanium (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (Hassan, *et.al*.2016).

Phenolic and flavonoid contents of *Blanities aegyptiaca* were found to be responsible for their anti-oxidant effect; both have redox properties that allow them to act as hydrogen donors, single oxygen quenchers and reducing agents (Hassan, *et.al*,2016).

It was reported that what prevents lipid oxidation in food thus inhibiting many diseases as cancer and atherosclerosis. According to some reports, the anti-oxidant activity of desert date extract is dose dependent with safety dose up to 1000 mg/kg (Balakrishnan, *et.al*,2014).

### 1.17.5 Anti-viral Activity

Aqueous Extract of *Blanities aegyptiaca* bark is used to treat both acquired immune deficiency syndrome and leukemia. When this extract was orally administrated for a month to patients, it showed good results. The same was shown with leukemia patients. An increase in the stem bark extract was tested against Herpes Simplex Virus, CoxsackieB2, Semliki forestA7 and Vesicular Stomatitis Virus, it gave negative results with no activity on them (Maregesi, *et.al*,2008).

### 1.17.6 Anti-inflammatory Activity

It has been reported, that, both methanol and butanol extracts of *Balanites aegyptica* have a significant anti-inflammatory effect on the rat paw edema with respect to controls. Furthermore, methanol extract had showed no dose-response relation, as both the lowest (200mg/kg) and the highest (400 mg/kg) doses showing the same effect on edema reduction. Although, butanol extract showed a significant dose-response relation (Speroni, *et.al*.2005). A study conducted by whom on rats indicated that petroleum and ethanolic extracts of aerial parts of desert dates have a significant effect on carrageenan-induced hind paw edema in comparison to the effect of the standard drugs as control group, indomethacin and diclofenac sodium, respectively. The same study reported that ethanol extract had more has significant effect on treating inflammatory related pains (Gaur, *et.al*.2008).

## 1.18 Objectives of the study

The objectives of this study are:

1-To determine the mass percentage of the four layers of *Balanites aegyptica* fruits.

2-To investigate the elemental content of the edible fleshy pulp and kernel of the

Fruit, using inductive coupled plasma instrumentation.

3- To extract and measure the yield percentage of *Balanites aegyptica* seeds oil.

4-To measure the physicochemical properties of the *Balanites aegyptica* seeds oil.

5-To determine the fatty acids constituent of the extracted oil as edible oil by GC-MS analysis.

6-To investigate the antimicrobial effects of the methanolic leaves extract of *Balanites aegyptica* on some bacteria species.

# Chapter Two

## Materials and Methods

# Chapter Two
# 2. Materials and Methods

## 2.1 Samples collection

Two kilograms of *Balanites aegyptiaca* (lalobe*)* fruits were purchased from local market in Omdurman (Sudan). *Balanites aegyptiaca* leaves were collected from the heglieg trees area near Omdurman abu-seid.

## 2.2 Chemicals

-Normal hexane -assay: n-isomer95%, all isomer 99.5%, Bp 67-70°C, d=0.66, water < 0.02%) – Chevron philipes chemical company.

-Methanol -assay by GC 99.5% -CH4O .M.wt 23.04.

-Sulphuric acid- assay 99.5% - d=1.84g/$cm^{3-}$ ALPHA CHEMIK- India.

-Nitric acid - 99.9% - d= 1.5129g/$cm^3$ ALPHA CHEMIK- India.

-Hydrochloric acid -35% - d= 1.200 g/$cm^3$ ALPHA CHEMIK- India.

-Ethanol (absolute-extra pure, assay GC 99.9%) African Modern Distillation for ethanol.

- Petroleum ether (60-80CLR, min assay 95%, wt permal=0.67g) –SD fine chem limited- India.

-Potassium hydroxide (min assay 85.0%, KOH 56.11, max impurities K2CO3 2%-CL 0.03% -SO4 0.02%) BDH chemical ltd –England.

-Glacial acetic acid-99% - d=1.040 g/$cm^3$- ALPHA CHEMIK- India.

-Chloroform (assay 99.5%, M.wt=119.35 ,nonvolatile matter 0.002%, acidity 0.01ml N%).

-Potassium iodide- 66% - SD fine chem limited- India.

-Sodium thiosuphat e(Na2S2O3.2H2O) 98% -SD fine chem limited- India

-Diethyl ether (min assay GC 99.0%, wt. pre malate 20C=0.713-0.717g) Bp (34-36) acidity 0.003%.

## 2.3 Instruments

GC-MS-QP2010-Ultra.japans'Simadzu Company, serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25 mm×0.25µm).

Inductively Coupled Argon Plasma - Atomic Emission spectrometer (ICP-AES),

Lovibonod Tinto meter – Type D.

Ostwald-U-tube viscometer.

Refrectometer (Switzerland).

Moisture Analyzer device (Dsh-50-10 Auto).

Rotary Evaporator (Buchi Switzerland).

Electric muffle furnace 575 (TAPP T211 om-39).

## 2.4 Methods of Analysis

### 2.4.1 Samples treatment

After collection, the fruits of *Balanites aegyptica* were screened to remove the defective ones. Those in good condition were soaked in clean water (overnight), to remove the glycoside pulp from seed coats. The washed seeds were air dried and, then, crushed by a metal hammer to remove the woody cover and obtain seed kernels. The resulting kernels were oven-dried at 60°C for 2 hours and ground manually with Pestle to fine powder. The sample was divided into three portions for proximate analysis, chemical analysis and n-hexane oil extraction. The extraction was repeated two times and all the analysis were performed in triplicates.

The leaves were air-dried under shade. After shade drying the plant material was ground into small particles using pestle and mortar. Exposure to sunlight was avoided to prevent the loss of active components, and finally taken to the Biochemistry research laboratory for further processing.

### 2.4.2 Determination of layers Percentage for *Balanites aegyptiaca* fruits

30 pieces of *Balanites aegyptiaca* fruits were weighed first, then, the outer shell or curst was removed and weighed. The fleshy pulp (mesocarp) was removed with water to obtain seeds, which were left until complete air drying. The dried. the dried seeds were crushed by a metal hammer to remove the woody cover (endocarp) to obtain inner seeds (kernels). The kernels were weighed. Then the percentage of each component was calculated according to equation:

Layer% = W.t of the layers (g) /W.t of the whole sample(g) ×100

### 2.4.3 Moisture content

The moisture content was determined by Moisture analyzer device.

### 2.4.4 Ash content

Ash content was determined according to the method described by Pearson (1981). Five grams of sample were weighed in porcelain crucible and ignited in a Muffle furnace at 600°C until a white gray ash was obtained. The crucible was transferred to a desiccator and allowed to cool to room temperature and weighed. Ignition, cooling and weighing was repeated to constant weight. The ash content was then calculated as a percentage based on the initial weight of the sample as:

Ash % = [(Wt of crucible +Ash) - (Wt of empty crucible]/ wt. of sample x 100

### 2.4.5 ICP analysis of the fleshy pulp and seed kernel

Minerals content in fleshy pulp and seed kernels were measured using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-AES). The composition of the elements was determined after the sample microwave digestion 1200w at temperature 230°C, with mineral acids. 0.5g of powdered seed kernels sample was transferred to 100 mL beaker.

6 Ml of conc. sulphuric and 6 ml of conc. nitric acid were added. 1 mL of hydrogen peroxide was also added and the mixture was heated until a clear solution was obtained. The mixture was allowed to cool down to room temperature and filtered. The resulting solution was transferred to a 50 mL volumetric flask and made up to the mark with distilled water for elemental analysis. The fleshy pulp sample was treatment similarly and the concentration of each element was determined.

### 2.4.6 Extraction of the *Balanites* oil

The *Balanites aegyptica* seeds kernels oil was extracted by continuous extraction method using soxhlet extractor in n-hexane at 65°C. 300 g of the crushed seeds kernels were packed into a porous thimble and placed in a soxhlet extractor. 500 mL of n-hexane (60 – 80°C) was used as extracting solvent, for 8 hours. A rotary evaporator was then used to remove the excess solvent from the extracted oil. The oil was weighed and the percentage of oil yield was calculated as follows:

$$Yield \text{ percentage} = W1/W2 \times 100$$

Where; W1= weight of oil extracted; $W2$= weight of sample (g).

### 2.4.7 Maceration extraction of active constituents from *Balanites* leaves

500 ml of extraction fluid was mixed with 100 g of dry leave powder were added to 500 ml of methanol 80 % and The mixture was kept for 2 days in tightly sealed flask at room temperature and shake several times daily. The mixture was then filtered through a filter paper. The process was repeated four times until a clear colorless supernatant liquid was obtained. The different filtrates were collected and kept under a ceiling fan to evaporate the solvent and to dryness of the isolated constituents as a semi-solid material, which was then used for antimicrobial test.

## 2.4.8 Measurement of oil Colour intensity

The colour intensity was measured using a Lovibond tintometer, units of red, yellow and blue were recorded according to the AOAC (2008) .2inches tintometer cell was filled with oil sample and introduced to the instrument. The instrument was switched on and adjusted until colour matching was obtained. The readings of the filters, used to make the match (red, yellow, and blue) were recorded.

## 2.4.9 Determination of refractive index

Refractive index was determined by Abbe-60 refractometer as described by AOAC (200) where a double prism was opened by means of screw head and few drops of sample were placed on the prism. The prism was closed firmly by lighting screw head. The instrument was left to stand for few minutes before reading in order to equilibrate the sample temperature with that of the instrument. The prism was cleaned between readings wiping off oil with soft cloth, then with cotton moistened with petroleum ether and left to dry. The refractive indices of all samples were determined at 28.9°C.

## 2.4.10 Determination of viscosity

The viscosity of the oil samples under investigation were recorded using Ostwald-U-tube viscometer according to Cocks and Van Rede (1966). The viscometer was suspended in the constant temperature water bath so that the capillary was vertical.

The instrument was exactly filled to the mark at the top of the lower reservoir with the oil by means of a pipette inserted in the side arm, so that the tube wall above the mark is not wetted. The instrument was then left to stand for few minutes before reading in order to equilibrate the sample temperature with that of the instrument (35°C).

By means of pressure on the respective aim of the tube, the oil moved into the other arm so that the meniscus is (1 cm) above the mark at the top of upper

reservoir. The liquid was then allowed to flow freely through the tube and the time required for the meniscus to pass from the mark above the upper reservoir to that at

the bottom of the upper reserve was recorded and the viscosity was determined.

### 2.4.11 Determination of the specific density

The specific gravity of the oil was determined using a density bottle according to the methods de-scribed by (AOAC, 2000). The weight of 50 mL empty density bottle (w0) was recorded and the density bottle filled with water recorded (w1). Equivalent quantity of oil was replaced with the water in the same bottle (w2) and weighed. The specific density of the oil was determined.

### 2.4.12 Determination of acid value

The acid value was determined using titration and the method adopted was described by the British Pharmacopeia (2007 Version 11). 10.00 g of oil to be examined were dissolved, in 50 ml of a mixture of equal volumes of ethanol (98%) and petroleum ether (1:1), then was neutralized with 0.1 M potassium hydroxide, 0.5 ml of phenolphthalein solution was used as indicator. After the mixture dissolved, was titrated with 0.1 M potassium hydroxide until the pink colour persists for at least 15s (n ml of titrant). The acid value was then calculated

### 2.4.13 Determination of peroxide value

The peroxide value (PV) of oils was determined using titration and the method adopted was described by the British Pharmacopeia (2007 Version 11). Peroxide value is the most widely used. It gives a measure of the extent to which an oil sample has undergone primary oxidation, extent of secondary oxidation. It is defined as amount of peroxide oxygen per 1 kilogram of fat or oil.5.00g of oil was placed 250 ml conical flask, 30 ml of mixture a glacial acetic acid/chloroform solution (3:2) were added, and the flask was' swirled until the sample was dissolved 0.5 ml of saturated potassium iodide was added.

The solution was again swirled for one minute, 30 ml of distilled water were added and 0.5 ml of 1% starch solution were also added. The contents of the flask were then titrated with 0.1 N sodium thiosuphate added gradually with constant and vigorous shaking and the titration was continued until the blue colour just disappeared. A blank test was carried out. The number of 0.1 N sodium thiosulphate required was recorded. The peroxide value was calculated by;

### 2.4.14 Determination of saponification value

The determination of saponification number was carried according to the British Pharmacopeia (2007 Version 11). About 2.00 gram of oil sample was weighed accurately in 200 ml conical flask. 25 ml of 0.1N alcoholic KOH solution was added, the contents of the flask were boiled under reflux for one hour with frequent rotation. Three of phenolphthalein indicator was added, while the solution was still hot, and the excess alkali was titrated against 0.5N HCL. Blank titration was carried in the same way. The saponification value was then calculated.

### 2.4.15 Determination of iodine number

The iodine number of the oil sample was determined according to AOCS recommended practice (AOCS, 1998). 0.2 g of oil sample was dissolved in 15 mL carbon tetrachloride in a conical flask and 25 mL WIJ'S solution was added. The contents were mixed vigorously and 20 mL of 10 % potassium iodide solution and 15 Ml of distilled water were added. A blank was also prepared. The two flask were kept in a dark cupboard and allowed to stand for 1 hour. Then few drops of starch solution were added and the mixture was titrated against standard 0.1N Sodium thiosulphate to the blue end point. iodine value was then calculated.

### 2.4.16 Determination of ester value

Ester value was obtained by subtracting the measured acid value from Saponification value.

## 2.4.17 GC-MS Analysis of *Balanites* oil

The qualitative and quantitative analysis of the sample was carried out by using GM/MS technique. The sample was prepared by taking 2ml of oil in test tube, then 7 ml of alcoholic NaOH and 7 ml of alcoholic $H_2SO_4$ were added. The mixture was shaken by vortex for 3 minutes and left overnight. 2ml of Add 2ml from supersaturated NaCl Was added to the mixture and with continuous shaking 2ml normal hexane was added and was shake for three minutes and then was collected the hexane layer, 5µL of collected hexane was taken and was diluted with 5ml diethyl ether, then 1gram from sodium sulphate was added as drying agent, finally the sample was filtered through syringe filter 0.45 µm, the filtrate was Transferred and 1µL directly was injected to the GC-MS. injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60c with rate 10c/min to 300c as final temperature degree with 3 minutes hold time , the injection port temperature was 300c, the ion source temperature was 200c and the interface temperature was 250c.The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 26 minutes .Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patents with those available in the library ,the National Institute of Standards and Technology (NIST). , results were recorded.

## 2.4.18 Biological activity tests

- **Preparation of bacterial suspensions:**

One ml aliquots of a 24 hours' broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24

hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about $10^8$- $10^9$ C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used.

The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (**Miles and Misra, 1938**). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

- **Testing of antibacterial susceptibility (Disc diffusion method)**

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to $10^8$cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each

*Balanite*s extracts. The inoculated plates were incubated at 37 °C for 24 hours in the inverted position. The diameters (mm) of the inhibition zones were measured.

# Chapter Three

## Results and Discussions

# Chapter Three
# 3. Results and Discussions

## 3.1 *Balanites aegyptica* fruit layers percentages

The percentage of the *Balanites aegyptica* fruit layers were shown by Table (3.1), Epicarp layer percentage was lower than the other constituents (13.5) The higher percentage was shown by the mesocarp layer (38.7), compared with the endocarp (29.0) and the kernel (46.6). Oil content was found to be 46.6 %, which was close to that reported by Jock (2017) as (45.3%), but it was lower than the yield that reported by Elfeel,AA., (2010) which was (50%). The oil content was found to be above the limit for most oil seed crops (Pritchard, 1991). The oil yield percentage may indicate the feasibility of lalobe oil production from the seeds, but to produce large amounts of oil *Balanites* trees should be cultivated and treated as one of the oil production sources.

**Table 3.1: percentage of the *Balanites aegyptica* fruit layers and oil yield**

| The component | Percentage% |
|---|---|
| outer skin (epicarp) | 13.5 |
| fleshy pulp (mesocarp) | 38.7 |
| Woody part (endocarp) | 29.0 |
| seed kernel | 18.5 |
| Oil yield | 46.6 |

## 3.2 Moisture and Ash contents of *Balanites* seed kernel

Moisture content of seeds kernels was found to be (7.28%). This is similar to that reported by Ajayi and Folorunsho (2013) as (7.23 %) , but higher than that reported by Sara and Mahdi (2016) as (3.1%) . Moisture content gives an indication of food life and nutritive value, since low moisture content is a

requirement for long storage life (Aurand et al., 1987). Seeds may deteriorate as a result of high moisture content.

Ash content of seeds kernels was found to be (3.46%) which is close to that reported by Lohlum (2010) as (3.98%) but its higher than the (3.19%) reported by Sara and Mahdi (2016). The mineral content of a food is a measure of the amount of specific inorganic constituents present and ash is the inorganic residue remaining after the water and organic matter have been removed. *Balanites aegyptica* seed kernel ash content was lower when compared, with, cotton seed (4.56%) as reported by Muhammad et al., (2012). In this study ash content was measured for the pure seeds kernels, whereas cotton seeds may be ashed as a whole (coated kernel) in Muhammad et al., (2012)

## 3.3 ICP Analysis of *Balanites,* fleshy pulp and seed kernel

Concentrations of minerals in seeds kernels and fleshy pulp of *Balanites aegyptica* were shown by Tables (3.2) (3.3) (3.4), The metal with highest concentration in the seed kernel was potassium (8439ppm), followed by sodium (4398ppm), phosphor (2460ppm), calcium (1588ppm), magnesium (997ppm) while the highest in the fleshy pulp was potassium (23046ppm), calcium (509.8ppm) and followed by magnesium (590.4ppm).

**Table (3.2) Macronutrients contents of *Balanites aegyptica* fruit**

| Element | Concentration in fleshy pulp (ppm) | Element | Concentration in seed kernel (ppm) |
|---|---|---|---|
| K | 23046 | K | 8439 |
| Mg | 590.4 | Na | 4398 |
| Ca | 509.8 | P | 2460 |
| P | 366.6 | Ca | 1588 |
| Na | <0.1421 | Mg | 997.6 |

## Table (3.3) Micronutrients contents of *Balanites aegyptica*

| Element | Concentration in fleshy pulp(ppm) | Concentration in Seed kernel (ppm) |
|---|---|---|
| Co | $<0.198\times10^{-3}$ | $<0.198\times10^{-3}$ |
| Cu | 2.838 | 5.2505 |
| Fe | 25.90 | 28.29 |
| Mn | $<0.75\times10^{-4}$ | $<0.75\times10^{-4}$ |
| Mo | $<0.277\times10^{-3}$ | $<0.277\times10^{-3}$ |
| Ni | $<0.674\times10^{-4}$ | $<0.674\times10^{-4}$ |
| Se | $<0.4993\times10^{-2}$ | 11.120 |
| Zn | $<0.113\times10^{-3}$ | 117.8 |

## Table (3.4) Some other minerals in *Balanites aegyptica*

| Element | Concentration in fleshy pulp(ppm) | Concentration in Seed kernel (ppm) |
|---|---|---|
| Al | 23.206 | 6.668 |
| As | $<0.2547\times10^{-2}$ | $<0.2547\times10^{-2}$ |
| Ba | $<0.57\times10^{-3}$ | $<0.57\times10^{-3}$ |
| Be | $<0.30\times10^{-4}$ | $<0.30\times10^{-4}$ |
| Cd | $<0.198\times10^{-3}$ | $<0.198\times10^{-3}$ |
| Cr | $<0.583\times10^{-3}$ | $<0.583\times10^{-3}$ |
| Li | $<0.127\times10^{-2}$ | $<0.127\times10^{-2}$ |
| Pb | $<0.4727\times10^{-2}$ | $<0.4727\times10^{-2}$ |
| Sb | $<0.6078\times10^{-2}$ | $<0.6078\times10^{-2}$ |
| Si | 55.05 | 208.4 |
| Sn | $<0.9724\times10^{-2}$ | $<0.9724\times10^{-2}$ |
| Sr | $<0.23\times10^{-4}$ | $<0.23\times10^{-4}$ |
| Ti | $<0.147\times10^{-3}$ | $<0.147\times10^{-3}$ |
| V | $<0.99\times10^{-4}$ | $<0.99\times10^{-4}$ |

From the results above the seeds kernels and fleshy pulp of the lalobe were found to be very rich with some macro and micro minerals including K, Ca, Mg, P, Cu, Mn and Zn which required in the human body for certain metabolic function, there for *Balanites* tree may be considered as useful source of these minerals for the indigenous African community where the trees are found, and may be recommended as food supplement due to the high content of calcium, magnesium and potassium.

Some toxic elements such as aluminum and silicon, were showed considerable concentration, due to presence of wedges fungus that increase the absorption of these elements by *Balanites* tree.

## 3.4 Physicochemical properties of *Balanites* oil

The quality assessment of *Balanites aegyptica* seeds kernels oil was analyzed by evaluating physicochemical properties presented in Table (3.5).

**Table 3.5 Properties of *Balanites* oil and Standard.**

| Parameter | *Balanites aegyptica* oil | FAO/WHO Standard |
|---|---|---|
| Viscosity | 66.014 | - |
| Specific Density | 0.9120 | 0.9-1.16 |
| Refractive index | 1.467 | 1.4677-1.4705 |
| Colour | Yellow 35.4- red 0.7 | - |
| Free fatty acid | 0.70 mg KOH/g | 5.78-7.28 |
| Acid value | 1.4022 mgKOH/g | 4 |
| Peroxide value | 8.6 mgEq/kg | <10 |

Viscosity was recorded to be (66.014 pas.s) which is higher than that reported by Alia,M.A.,(2016) as (56.349 pas.s).

Density was found to be (0.9120 g/cm$^3$) which is close to that reported by Sara, M.E. F (2016) as (0.9109) but higher than that reported by Babagana *et.al* (2011) as (0.277) but lower than that reported by Manji *et al*. (2013) as (1.001).

Refractive index was found to be 1.467 which is similar to that reported by both Okia *et al*. (2013) as (1.46) but higher than that reported by Jock (2017) as (1.45) and lower than 1.483 reported by Sara and Mahdi (2016).

Colour of *Balanites aegyptiaca* oil was found to be light yellow, similar to that reported by Okia *et.al* (2013), Babagana *et.al*., (2012) and Babeker (2013) as light yellow. (7,633R.y. b).

Free fatty acid was found to be 0.70 mg KOH/g which is close to that reported by Jock *et al*. (2017) as (0.82 mg KOH/g). and lower than (2.8) and (1.84) were reported by both Babagana *et.al* (2011) and Manj (2013) respectively.

Acid value was found to be (1.4022mgKOH/g) which is close to (1.53mgKOH/g) reported by Sara and Mahdi (2016). But its lower than the (2.08 mgKOH/g) reported by Babeker and Fatmah (2013). The acid value was low and this shows that the oil is stable Haftu (2015). Oils with high acid value, also implied high % free fatty acid and will undergo rancidity due to the hydrolysis of the free fatty acids on storage. The acid value and (% FFA) of *Balanites aegyptica* seeds kernels oil are lower than (FAO/WHO) standard for edible oils (2016). (Table 3.3). The low (%FFA) reduces the tendency of the oil to undergo hydrolytic activities. In most oils, the level of free fatty acids, which, causes deterioration is noticed when the (%FFA) calculated as oleic acid falls within the range of 0.5 - 1.5% (Manji *et al.,* 2013).

Peroxide value was found to be (8.6 mgEq/kg) which is similar to that reported by Babeker (2013) as (8.0 mgEq/kg) and higer than that reported by Manji *et al.*, (2013) as (6.0 mgEq/kg). Peroxide value is used as a measure of the extent, to which, rancidity reactions have occurred during storage, and it could be used as an indication of the quality and stability of fats and Oils. A high peroxide

value in the present study decreases the suitability of the oil for a long storage due to high level of oxidative and lipolytic activities Zang *et al.*, (2017).

Saponification value was found to be (266.475 mgKOH/g) which is higher than the 168.80, 174.5, 168.3 and 182.80 (mgKOH/g) values that reported by Manji *et al*., (2013).

Babagana *et al.,* (2011), Babeker (2013) and Okia *et al.,* (2013) respectively. These high values of oil serve as an important parameter in determining the suitability of the oil for soap making.

Iodine value was found to be 99.104 mg I2/g which is higher than the 76.8 , 56.4 and 98.28 mg I2/g reported by Manji *et al.,(*2013), Babagana *et al.,* (2011) and Okia *et al.,* (2013) respectively.

Iodine value measures the degree of unsaturation in fats or vegetable oils. It determines the stability of oils to oxidation, and allows the overall unsaturation of the fat to be determined qualitatively. Generally, oils having iodine value below 100 are non-drying, "which does not harden when it is exposed to air".

All These values are in close agreement with the FAO/WHO international standards for edible oil Zang*, et al.,* (2017) shown in Table (3.5).

## 3.5 Physicochemical properties of *Balanites* oil compared with other Sudanese vegetable oils

According to Esrra Mhomed (2014), vegetable oils produced in Sudan from cotton seed, sesame. Peanut and sunflower, the comparison of the physiochemical properties of *Balanites* oil with these oils was recorded by table (3.6).

**Table 3.6 Physical properties of *Balanites* oil with other Sudanese vegetable oils**

| Parameter | | Lalobe oil | Sesame oil | Sun flower | Peanut |
|---|---|---|---|---|---|
| Density (g/cm)3 | | 0.9120 | 0.913 | 0.914 | 0.910 |
| Viscosity (poise) | | 66.01 | 21.78 | 22.95 | 22.99 |
| Refractive index | | 1.467 | 1.471 | 1.475 | 1.471 |
| Colour | Yellow | 35.4 | 25.0 | 7.9 | 25.0 |
| | Red | 0.7 | 6.1 | 1.0 | 4.5 |
| | Blue | 0.0 | 4.8 | 0.0 | 2.2 |

From table (3.6), the density of lalobe oil is more close to the density of sesame and peanut oils. The viscosity of lalobe oil was higher than the other oils. The refractive index and the color of lalobe oil were similar to the others oils**.**

**Table 3.7 Chemical properties of *Balanites* oil and other Sudanese vegetable oils.**

| Parameter | | Lalobe oil | Sesame oil | Sun flower | Peanut |
|---|---|---|---|---|---|
| Peroxide value | | 8.6 | 5.2 | 4.443 | 5.57 |
| Saponification value | | 266.4 | 195.0 | 188.2 | 193.20 |
| Iodine value | | 194.21 | 155.0 | 136.0 | 94.00 |
| Free fatty acid | | 0.7 | 1.04 | 1.020 | 0.809 |
| Fatty acids | Palmatic | 17.35 | 12.85 | 11.59 | 15.37 |
| | Oliec | 28.35 | 44.08 | 41.85 | 42.23 |
| | Linolic | 27.45 | 37.72 | 40.18 | 35.2 |

Table (3.7) showed peroxide value of *Balanites* oil (lalobe) was higher than other oils, the saponification value and iodine values are higher for the lalobe oil than other oils, the free fatty acid of lalobe oil was relatively close to the other oils. Lalobe oil contain fatty acid with close values to that of sesame, peanut and sunflower oils.

According, to the physical and chemical properties of lalobe oil may described as nearly similar to the main edible oils consumed by Sudanese people. (Esrra, M.E,2014).

## 3.6 GC-MS Analysis of *Balanites aegyptica* oil

Table (3.8) shows GCMS and identities of the fatty acids present in the oil. The most prominent of the fatty acids are palmitic acid (17.35%), oleic acid (28.35%), linoleic acid (27.45%) and stearic acid (15.97%). The unsaturated fatty acid makes *Balanites* oil a good edible oil. However, oils with high degree of unsaturation are considered good for the heart because it decreases total cholesterol and low density lipoproteins. A high dietary intake of saturated fatty

acids (SFAs) is a risk factor for development of obesity and cardiovascular disease (Gillian *et al*., 2008)

**Table 3. 8**: **GC-MS Analysis of the seed kernel oil of *Balanites aegyptica.***

| ID# | Name | Ret.Time | Area | Area% |
|---|---|---|---|---|
| 1 | 9-Octadecenoic acid (Z)-, methyl ester (Oleic acid) | 18.388 | 139085180 | 28.53 |
| 2 | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (linoleic acid) | 18.315 | 133799479 | 27.45 |
| 3 | Hexadecanoic acid, methyl ester(palmitic acid) | 16.503 | 85520896 | 17.54 |
| 4 | Methyl stearate (stearic acid) | 18.554 | 84597575 | 17.35 |
| 5 | 7-Hexadecenoic acid, methyl ester, (Z)- | 16.197 | 498174 | 0.10 |
| 6 | 9-Hexadecenoic acid, methyl ester, (Z)- | 16.245 | 4326719 | 0.89 |
| 7 | cis-10-Heptadecenoic acid, methyl ester | 17.264 | 1586576 | 0.33 |
| 8 | Heptadecanoic acid, methyl ester | 17.479 | 3229158 | 0.66 |
| 9 | Pentadecanoic acid, methyl ester | 15.362 | 88175 | 0.02 |
| 10 | Methyl tetradecanoate | 14.225 | 1335031 | 0.27 |
| 11 | Nonadecanoic aci d, methyl ester | 19.411 | 300288 | 0.06 |
| 12 | 8,11-Eicosadienoic acid, methyl ester | 19.918 | 10995675 | 2.26 |
| 13 | cis-11-Eicosenoic acid, methyl ester | 20.113 | 3781593 | 0.78 |
| 14 | Eicosanoic acid, methyl ester | 20.317 | 9799228 | 2.01 |
| 15 | PGH1, methyl ester | 20.400 | 896804 | 0.18 |
| 16 | 8,11,14-Docosatrienoic acid, methyl ester | 20.514 | 818279 | 0.17 |
| 17 | Docosanoic acid, methyl ester | 22.017 | 1746626 | 0.36 |
| 18 | Tricosanoic acid, methyl ester | 22.821 | 439787 | 0.09 |
| 19 | Tetracosanoic acid, methyl ester | 23.596 | 2438367 | 0.50 |
| 20 | Pentacosanoic acid, methyl ester | 24.355 | 789465 | 0.16 |
| 21 | Hexacosanoic acid, methyl ester | 25.175 | 1419295 | 0.29 |
| | | | 487492370 | 100.00 |

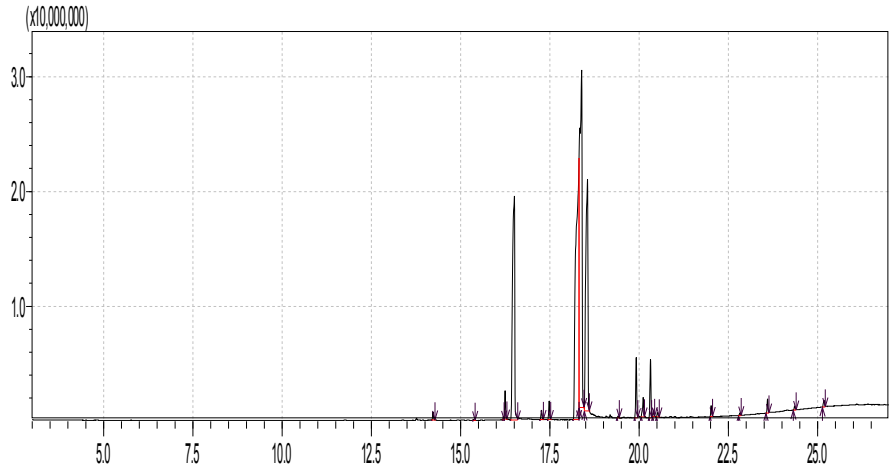



**Figure 3.1 GC-MS spectra of *Balanites* oil**

## 3.7 The major fatty acids in *Balanites aegyptica* oil

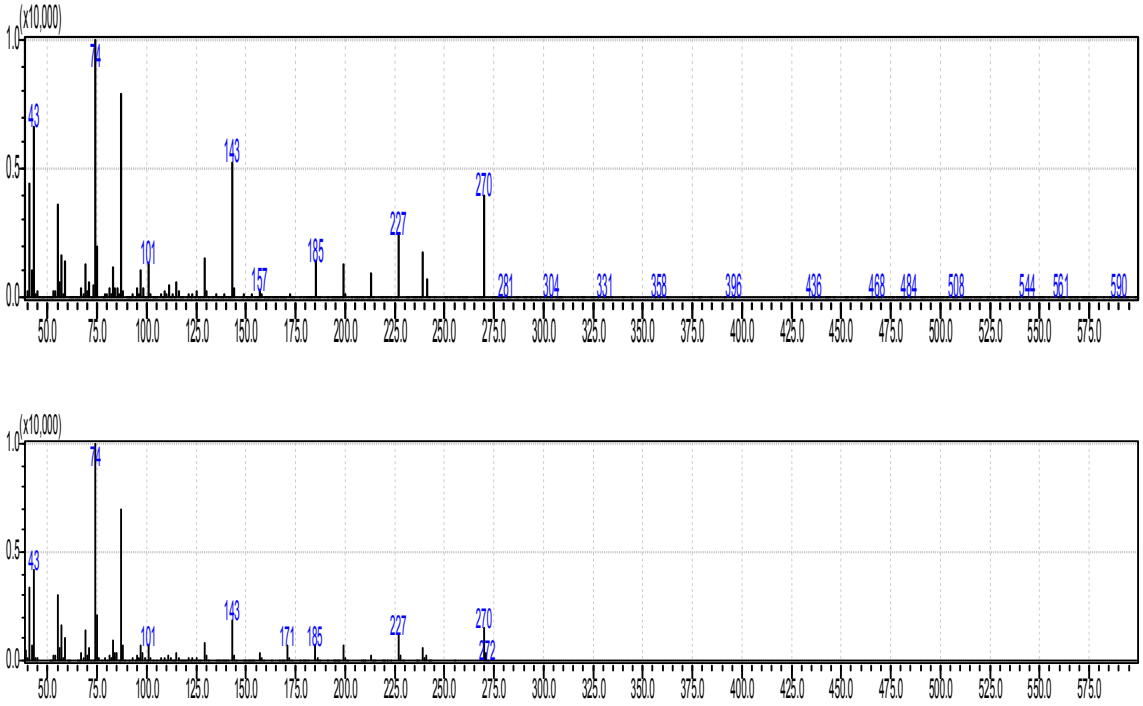
**3.7.1 Palmitic acid (hexadecanoic acid)** in IUPAC nomenclature,( Fig.3.2) is the most common saturated fatty acid found in animals, plants and microorganisms. Its chemical formula is $CH_3(CH_2)_{14}COOH$.As its name indicates, it is a major component of the oil from the fruit of oil palms (palm oil). Palmitates are the salts and esters of palmitic acid. The palmitate anion is the observed form of palmitic acid at physiologic pH (7.4).

Palmitic acid is naturally produced by a wide range of other plants and organisms, typically at low levels. It is naturally present in butter, cheese, milk, and meat, as well as cocoa butter, soybean oil, and sunflower oil.

Biochemistry: Excess carbohydrates in the body are converted to palmitic acid. Palmitic acid is the first fatty acid produced during fatty acid synthesis and is the precursor to longer fatty acids. As a consequence, palmitic acid is a major body component of animals. In humans, one analysis found it to make up 21–30% (molar) of human depot fat, and it is a major, but highly variable, lipid component of human breast milk.

Applications: Palmitic acid is used to produce a soaps, cosmetics, and industrial mold release agents. These applications use sodium palmitate, which is commonly obtained by saponification of palm oil. Hydrogenation of palmitic acid yields cetyl alcohol, which is used to produce detergents and cosmetics.

Health effects: According to the World Health Organization, evidence is "convincing" that consumption of palmitic acid increases the risk of developing cardiovascular disease, based on studies indicating that it may increase LDL levels in the blood. Retinyl palmitate is an antioxidant and a source of vitamin A added to low fat milk to replace the vitamin content lost through the removal of milk fat. Palmitate is attached to the alcohol form of vitamin A, retinol, to make vitamin A stable in milk.



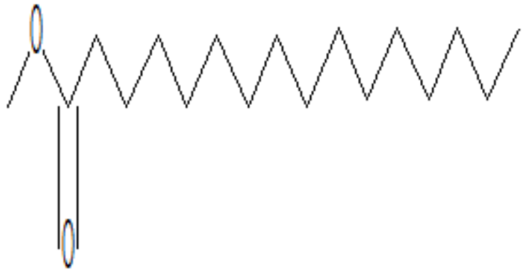


**Figure 3.2 Palmitic acid profile**

**3.7.2 Linoleic acid (9,12-Octadecadienoic acid**) (Fig. 3.3) the two main fatty acids essential in the diet are linoleic (or omega-6) fatty acid and alpha-linolenic (or omega-3) acid. Both of them are polyunsaturated fatty acid, which means that they possess two or more double bonds and lack several hydrogen atoms that are found in saturated fatty acids. Linoleic acid keeps the skin impermeable to water, but to exert other effects the compound must undergo specific metabolism. First step is conversion to gama-linolenic acid by delta-6-desaturation. Gama-linolenic acid is subsequently converted to dihomo-gamma-linolenic acid, which is in turn converted to arachidonic acid. Arachidonic acid can form prostaglandins and thromboxanes – hormone-like lipids that promote blood clotting, induce inflammation and cause smooth muscle contraction. In alternative pathway it can also form leukotrienes, which are one of the most potent inflammatory agents in the human organism.

Health benefits of conjugated linoleic acids: Conjugated linoleic acids (CLA) refers to a heterogeneous group of constitutional and geometric isomers of linoleic acid, which are predominantly found in milk, milk products, meat and meat products of ruminants.

Research has shown that (CLA) has a significant inhibitory effect on the establishment and progression of atherosclerosis in animal models. Both (LDL)

cholesterol to (HDL) cholesterol and total cholesterol to (HDL) cholesterol ratios are reduced when (CLA) is fed to the test animals. There is also proof of (CLA) blocking the growth and spread of malignant tumors, primarily by influencing cell replication and mechanisms of carcinogenesis. The increase in mineralized bone formation was demonstrated in several experiments as well.

On the other hand, it has been shown that (CLA) can induce insulin resistance and fatty liver. Recommended (CLA) daily intake is currently between 0.35 and 1 g per day.

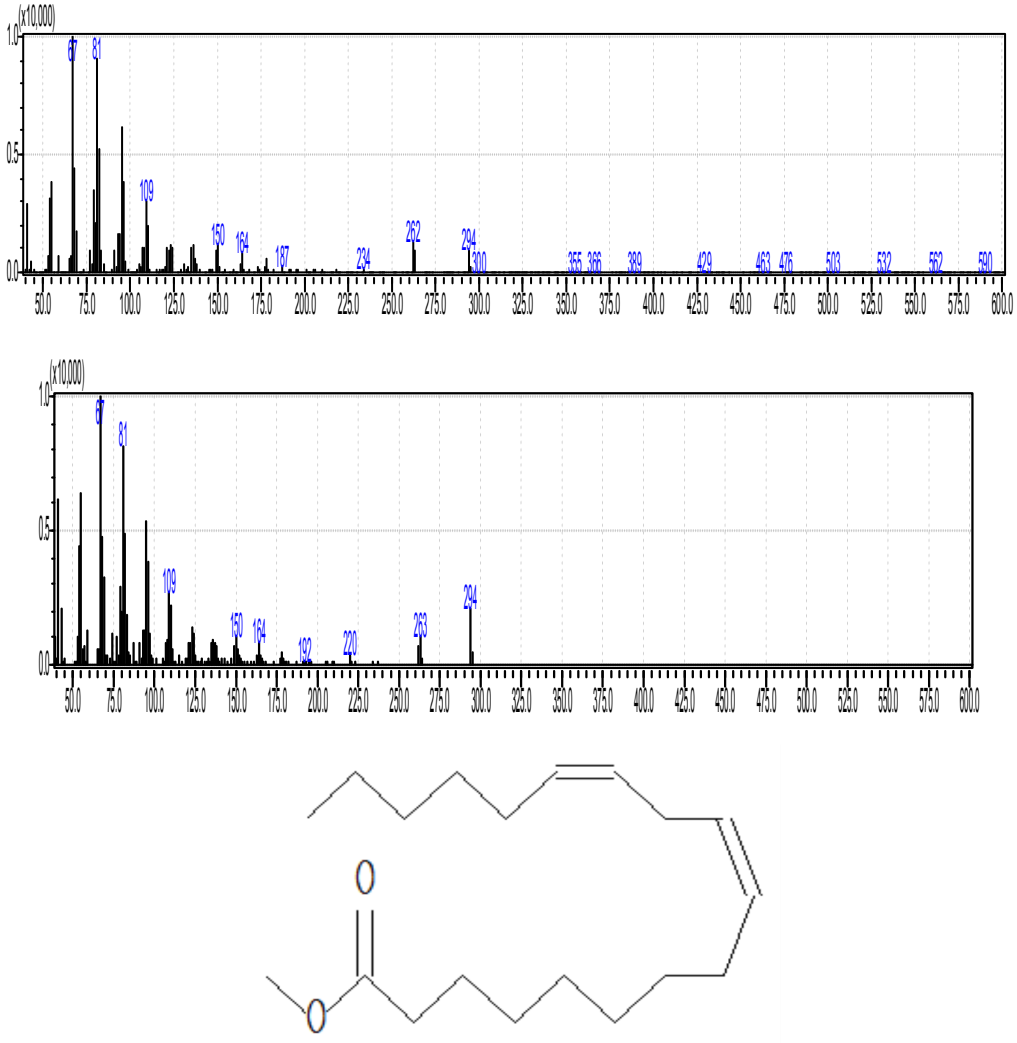


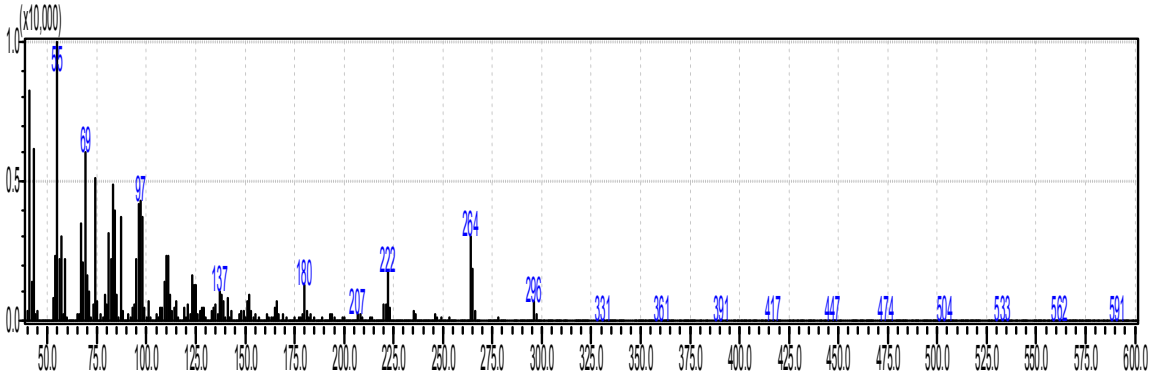

**Figure 3.3 linoleic acid profile**

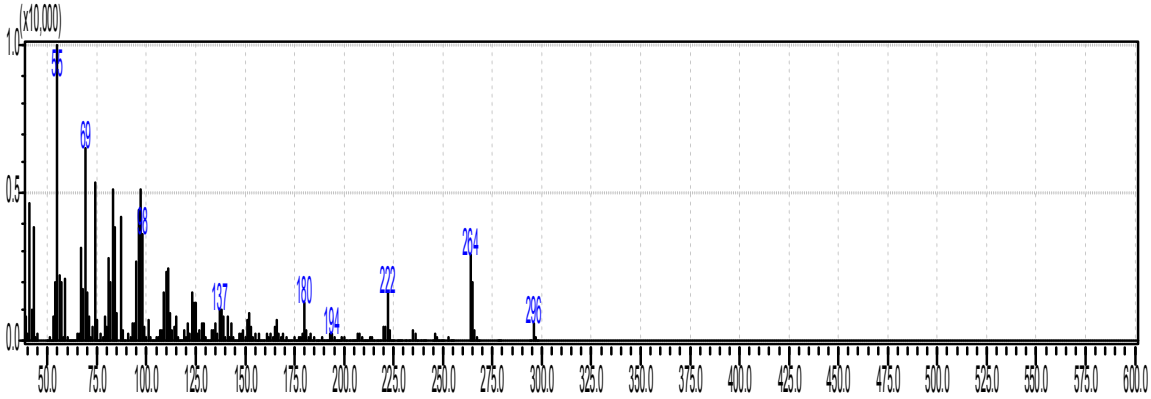
**3.7.3 Oleic acid, (9-Octadecenoic acid)** or Omega-9, (Fig. 3.4) It is a monounsaturated fatty acid,Which are less susceptible to spoilage than some other fats, which makes them useful in food preservation.

Sources: Oleic acid is widely distributed in nature. The highest sources of oleic acid are avocados, olive oil, table olives and canola oil. The second-best sources are beef tallow, peanut oil, lard and palm oil. Corn oil, butterfat, soybean oil and sunflower

Uses: If you have recently eaten any bakery product or used soap or skin cream, chances are you used oleic acid. Foods prepared with oleic acid will remain safe to eat for longer periods, even without refrigeration. Such foods include bakery goods such as breads, cakes and pies. Oleic acid is also used as a cleaning agent in the manufacturing of soaps and detergents and as an emollient, or softening agent, in creams, lotions, lipsticks and skin products.

Benefits: In a study published in February 2000 in the medical journal "QJM," researchers in Ireland found that diets rich in oleic acid improved the participants' fasting plasma glucose, insulin sensitivity and blood circulation. Lower fasting glucose and insulin levels, along with enhanced blood flow, suggest better diabetes control and less risk for other diseases. For millions of people with diagnosed diabetes and prediabetes, consuming foods rich in oleic acid may be beneficial in controlling the disease.





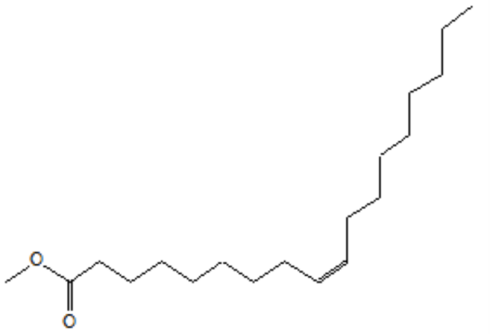



**Figure3.4 Oleic acid profile**

**3.7.4 Stearic acid methyl ester ,(Octadecanoic acid)**, stearic acid methyl (Fig.3.5)ester is a saturated fatty acid with an 18-carbon chain It is a waxy solid and its chemical formula is $C_{17}H_{35}CO_2H$. The salts and esters of stearic acid are called stearates. As its ester, stearic acid is one of the most common saturated fatty acids found in nature following palmitic acid. The triglyceride derived from three molecules of stearic acid is called stearin.

Production: Stearic acid is obtained from fats and oils by the saponification of the triglycerides using hot water (about 100 °C). The resulting mixture is then distilled. Commercial stearic acid is often a mixture of stearic and palmitic acids, although purified stearic acid is available.

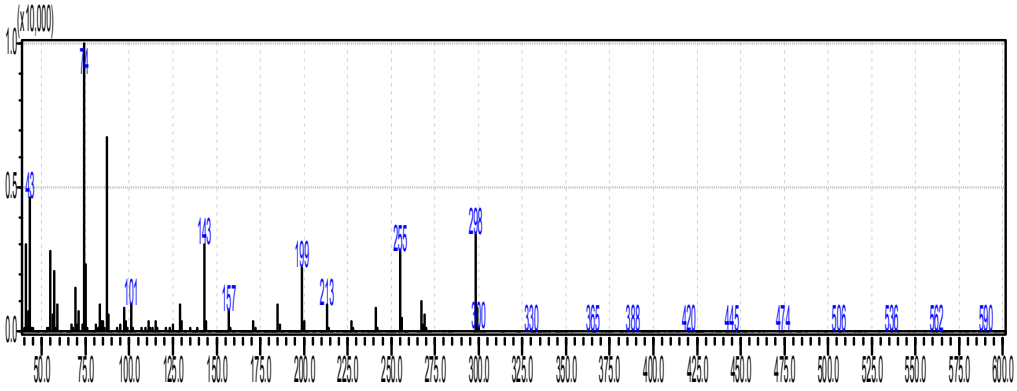
Fats and oils rich in stearic acid are more abundant in animal fat (up to 30%) than in vegetable fat (typically <5%). The important exceptions are cocoa butter and shea butter, where the stearic acid content (as a triglyceride) is 28–45%.

Uses: In general, the applications of stearic acid exploit its bifunctional character, with a polar head group that can be attached to metal cations and a nonpolar chain that confers solubility in organic solvents. The combination leads to uses as a surfactant and softening agent. Stearic acid undergoes the typical reactions of saturated carboxylic acids, a notable one being reduction to stearyl alcohol, and esterification with a range of alcohols. This is used in a large range of manufactures, from simple to complex electronic devices.

Stearic acid is mainly used in the production of detergents, soaps, and cosmetics such as shampoos and shaving cream products. Soaps are not made directly from stearic acid, but indirectly by saponification of triglycerides consisting of stearic acid esters. Esters of stearic acid with ethylene glycol, glycol stearate, and glycol distearate are used to produce a pearly effect in shampoos, soaps, and other cosmetic products. They are added to the product in molten form and allowed to crystallize under controlled conditions. Detergents are obtained from amides and quaternary alkylammonium derivatives of stearic acid.

Lubricants, softening and release agents**:** In view of the soft texture of the sodium salt, which is the main component of soap, other salts are also useful for their lubricating properties. Lithium stearate is an important component of grease. The stearate salts of zinc, calcium, cadmium, and lead are used to soften PVC.

Stearic acid is used along with castor oil for preparing softeners in textile sizing. They are heated and mixed with caustic potash or caustic soda. Related salts are also commonly used as release agents, e.g. in the production of automobile tires. Niche uses: Being inexpensive and chemically benign, stearic acid finds many niche applications. As an example, it can be used to make castings from a plaster piece mold or waste mold, and to make a mold from a shellacked clay original. Stearic acid is used as a negative plate additive in the manufacture of lead-acid batteries. Fatty acids are classic components of candle-making. Stearic acid is used along with simple sugar or corn syrup as a hardener in candies. In fireworks, stearic acid is often used to coat metal powders such as aluminum and iron. This prevents oxidation, allowing compositions to be stored for a longer period of time.



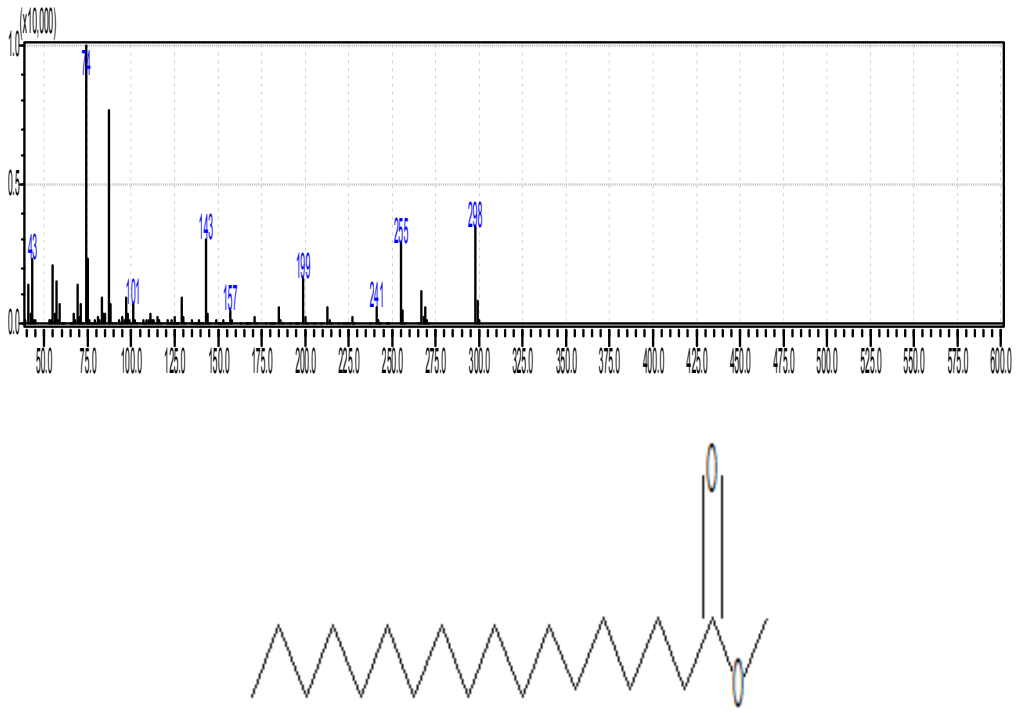



**Figure 3.5 Steric acid profile**

Fatty acids represent a substantial part of lipids in human body and are important sources of energy. They have several benefits for health. Lalobe oil contains considerable amounts of these main fatty acids, and may be considers as good source for producing these fatty acid.

### 3.8 Antimicrobial effect of *Balanites aegyptica* methanolic leaves extract

**Table (3.9) Shows the activity of *Balanites aegyptica* methanolic leaves extract against some Bacterial species.**

| Bacteria species | Zones of inhibition in mm |
|---|---|
| *Bacillus subtilis* | - |
| *Escherichia coli* | 14 |
| *Staphylococcus aureus* | - |
| *Pseudomonas aeruginosa.* | - |

The results were expressed in terms of the diameter of the inhibition zone: < 9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active; >18 mm, very active.

The bacteria sensitivity test carried on the four bacteria species. The leaves extract is only partially active aganist *Escherichia coli* gram negative bacteria with 14mm zone, which may be considered as active inhibiter. There was no inhibition of *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa,* by the leaves extract of B*alanites aegyptica*, according to Elfeel *et al.* (2006) the lack of inhibition shown in the analysis could be as a result of the difference in the method of extraction and variation in the properties of the leaves of *Balanites aegyptica* within different geographical areas in Sudan.
B*alanites aegyptica*. leaves extract showed reasonable suitable sensitivity to gram negative bacteria and this may agree with the use of this plant in traditional medicine practices. There for more work should be conducted to help optimally extract all the bioactive compounds in this plant and formulated into appropriate dosage for the treatment of infectious diseases.

## Conclusions

* Lalobe fruits, fleshy pulp and seeds kernels of *Balanites aegyptica* were rich in Na, K, Ca, Mg and P.

* The fleshy pulp sodium content (<0.1421) was very low compared to sodium content of the seeds kernels (4398).

* With exception of zinc, Iron, copper; Aluminum and selenium in *Balanites aegyptica* fruits showed trace concentration of micronutrients and undesired minerals. Therefore, desert date may be described as one of the most useful and safe popular foods from elemental content sight of view.

*The oil yield content of *Balanites aegyptica* oil was found to be significantly high compared to the other edible oils.

* Compered to sesame, sunflower and peanut oil, lalobe oil showed relatively higher peroxide value, saponification value and iodine value, but significantly low free fatty acid value. The free fatty acid value was near to that of peanut oil.

* Palmatic acid content of lalobe oil (17.35) was higher than that of sesame oil (12.85), sun flower oil (11.59) and peanut oil (15.37).

* Oleic and linoleic acid content of lalobe oil were lower than that of the other edible three oils.

* According to its physicochemical properties, lalobe oil may be good for human consumption as edible oil.

* It may also be good for soap and shampoo production, as well as possibility of use as biodiesel and as lubricant.

## Recommendations

* Heglieg tree is a wild evergreen tree which grows in all parts of Sudan regardless of the climatic conditions and soil type. So it may be cultivated in organized way as good source of oil production for so many years, when compared with seasonal growing plants, sesame, sunflower and peanut.

*The part of the tree may need further study for its nutritional and medical values.

* If the tree is cultivated in organized way it may be good source of food, medicinal

ingredients and oil production.

*If the tree is widely cultivated it may be good for overcoming desertification.

* Further studies may be required for determination of protein, carbohydrates and vitamins content of the fruit.

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