Sudan University of Science and Technology

College of Graduate Studies

Extraction and Analysis of Baobab seeds Oil *(Ophelussitularius)*

إستخلاص وتحليل زيت بذور التبلدي (القنقليز)

AThesis Submitted in Partial Fulfillment for the Requirements for Master Degree in Chemistry

By

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(B. Sc. Honors in Chemistry)

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الإستهلال

قَالَ تَعَالَىٰ:

﴿ ٱقۡرَأۡ بِٱسۡمِ رَبِّكَ ٱلَّذِي خَلَقَ ١ خَلَقَ ٱلۡإِنسَٰنَ مِنۡ عَلَقٍ ٢ ٱقۡرَأۡ وَرَبُّكَ ٱلۡأَكۡرَمُ ٣ ٱلَّذِي عَلَّمَ بِٱلۡقَلَمِ ٤ عَلَّمَ ٱلۡإِنسَٰنَ مَا لَمۡ يَعۡلَمۡ ٥ ﴾

صدق الله العظيم
(الاية 1-5) سورة العلق

Dedication

To My.....

Parents

Brothers

Sisters

Family

Friends and

For Being the Pillows, Role Models

Cheerleading Squad and Sounding Boards

I have Needed.

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I must thank Almighty Allah for blessing me and helping me with everything I needed throughout my study of the Master program. I would like to express my deepest appreciation to all those who provided me the possibility to complete this study.

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Abstract

This study aimed to extract and analyses Baobab seeds oil. for some physical properties, chemical properties, anti-biological effect, antioxidant and GC-MS for some essential oils. 98 mL of the oil were extracted from 450 grams of seeds powder with soxhlet apparatus using hexane as a solvent. The solvent was separated by a rotary evaporator and the oil content was found to be 19.94%. The physical analysis carried out are; the density, specific gravity, viscosity, color, flash point and moisture content gave the results; (0.9158g/cm^3, 0.9168, 40.58cSt, 1.5 Yellow , 280.90 C^o, 0.18%) respectively. The chemical properties analysed are; Acidity value, saponification value, iodine number and peroxide number gave the results; (0.49g KoH, 190. 70 mg/ KoH/g , 102.45 and 2.98mg Eq.O_2/g oil), respectively. The results of the biological analysis showed that the oil does not contain antibacterial activity. The baobab seeds oil was found to exhibited an antioxidant and its oxidation value equals ± 46.00 %. The baobab seeds oil was also analysed by GC-MS. The results were interpreted using the data of the NIST library for mass spectrometry, and the compositions were confirmed by studying the disintegration pattern. A match was found up to 5-9% between the spectra of the components and the library data attached to the device. While the results of the gas chromatography analysis showed that the oil contains seven components, the most important of which are:

9,12-Octadecadienoic acid (Z,Z)-, methyl ester (23.71%).

9-Octadecenoic acid, methyl ester, (E) (38.50%) .

Methyl stearate (4.43%)

Hexadecanoic acid, methyl ester(30.16%)

المستخلص

الهدف من هذه الدراسة استخلاص وتحليل زيت بذور التبلدي (القنقليز) ودراسة الخواص الفيزيائية والكيميائية والحيوية له وذلك بغرض الاستفادة منه في المستحضرات التجميلية. تم استخلاص 98مل من الزيت باستخدام 450جرام من مطحون البذور باستخدام الهكسان كمذيب بنسبة استخلاص بلغت 19.94% بعد فصل المذيب باستخدام جهاز المبخر الدوار. اجريت التحاليل الفيزيائية: الكثافة ' الكثافة النوعية ' اللزوجة ' اللون ' نقطة الوميض ومحتوي الرطوبة و وجدت انها تساوي (0,9158ج/سم ^{3}- 0,9168 ^{3} - 40,58 cSt – 280,90 C^{o} 1,5% مصفر -0,18 %) علي التوالي. كما اجريت التحاليل الكيميائية: قيمة الحموضة ،قيمة التصبن ، رقم اليود و رقم البيروكسيد ووجدت انها تساوي (0,49جم/جم - 190,70ملجم/جم – 97,25جم/جم و2,98 ملجم / O_2جم زيت) اوضحت نتائج التحليل الحيوي أن الزيت لايحتوي علي مضاد بكتيري ولكنة يحتوي علي مضاد للأكسدة وقيمة الاكسدة لة تساوي ± 46,00% . كذلك تم تحليل زيت بذور التبلدى بواسطة كروموتوغرافيا الغازية الملحقة بمطياف الكتلة، وفسرت النتائج بالاستعانه ببيانات مكتبة (NIST) لاطياف الكتلة . كما تم تاكيد التراكيب بدراسة نمط التفتت ووجد تطابق يصل الى05-09%بين اطياف المكونات وبيانات المكتبة الملحقة بالجهاز. بينما اوضحت نتائج تحليل كروماتوغرافيا الغاز ان الزيت يحتوي علي سبعة مكونات اهمها:

12,9 - اوكتا ديكادينويك استر (23,71%)

9- حمض اوكتا يكانويك ميثايل استر(38,50%)

ميثايل ايستريت (4’43%)

هيكساديكانويك اسيد ميثايل ايستر (30’16%)

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Chapter one

Introduction

1. Introduction

1.1 Introduction

Dry lands of africa are popular for a wide range of natural products e.g. Shea, Buckthorn, Baobab, Tamarind, Balanites, Hibiscus, acacia... etc. These play critical role in terms of food security, health, income and ecological services. The development of good immune system in humans contributes extensively to freedom from frequent sickness and illness, resulting in good health. The consumption of high quality food helps in achieving this goal. New trend for the production of vegetable oils from untapped and available widely source is replaced with manufactured oils with high value of natural content. World vegetable oil production increased continuously in the Last decades. A vegetable oil is a triglyceride extracted from a plant; such oils have been part of human culture for millennia. New discovered of vegetable oils like Baobab, Balanites, watermelon, Buckthorn, Hibiscus, Neem tree. In commercial practice, oil is extracted primarily from seeds. It have been used since ancient times and in many cultures. Wild seeds offer a convenient but cheap means of providing adequate supplies of mineral, fat, protein and carbohydrate to people living within the tropics. Chemical screening programs for new oils have not only identified many plant species with new or unusual kinds of oils that will not only compete with the presently used vegetable oils (soybean, cotton seed, peanut and corn), but also those with high industrial promise (Hagir,2017).

1.2 Baobab plant

The Baobab is common to livelihood of people in arid zone. Baobab tree the most common name in the world is Albaoba. The baobab is extremely important to humans and animals in the dry areas of africa because it offers shelter, clothing, medicine and a source of nutrition as well as raw material for many useful items. The flesh of fruit has the taste of acid, it used as juice which is very rich of vitamins C, A, E and calcium. Seeds contain more oil is used in cooking, medicine and cosmetics. Baobab oil aren't as widely known. This oil has a story to tell one of beauty and nutrients for hair and skin can benefit. It is slightly more expensive than vegetable oils, but the price is well worth it. As more and more beauty companies begin to introduce baobab oil into their products, it has easily become a rival of organic oil. Recently western companies began discover the many benefits, formulation of skin care products and nutritional extension. Baobab oil contains omega 3, 6, 9 and fatty acids which contribute towards maintaining a skin Baobab (Hagir,2017).

1.3 Study

The Objectives of this study are:

- Extraction of oil from the Baobab seeds.
- Study of the physical, chemical, biological and antioxidant properties of the extracted oil.

Chapter Two

Literature Review

2.1 Synonyms

Baobab (A. digitata L.) is tropical fruit, it is a large iconic tree indigenous to africa where it is found in many countries. The plant is wide spread throughout the hot and dried regions of tropical africa, certainly able to live up to 1000 years, making it one of the oldest known. It is an emblematic, culturally important and physically majestic sub-tropical tree. The baobab has been referred to as "arbre a palabre", meaning the place in the village where the elders meet to resolve problems. The origin of the vernacular name "baobab" is uncertain. Other common names include Tebaldi, the bottle tree, upside down tree.The scientific name Odansonah named it in honour of French plant Odanson Michel, who described Alodansonah tree digitalis. It can be called as of perennial trees. Botanical genus followed marshmallows rank kpaziat, name for adansoniadigitata, baobab means fruit with many seeds. However, most scientists believe it is derived from the arabic name buhibab meaning fruit with many seeds. Several names are used to describe the baobab depending on its geographical location and include "magic tree", "chemist tree", "symbol of the earth" and "monkey bread of Africa" amongst numerous others. Baobab belongs to the Malvaceae family and is a deciduous tree native to arid central africa (Decaluwe E *et al.*, 2010).

2.2 Baobab Description

The plant is a very massive tree with a very large trunk which can grow up to 23 m in height. Baobab is a very long-lived tree with multi-purpose uses. It has thick, angular, wide spreading branches. The baobab is a massive deciduous tree easily distinguishable by its huge trunk. It is regarded as the largest succulent plant in the world with a

diameter of 10–12 m. Stout trunk which attains 10–14 m or more in girth and often becomes deeply fluted. The form of the trunk varies, it is swollen and stout, usually tapering or cylindrical and abruptly bottle-shaped; often butter seed. In young trees it is conical; in mature trees it may be cylindrical, bottle shaped, or tapering with branching near the base. Branches are distributed irregularly and large. The tree produces an extensive lateral root system and the roots end in tubers. The higher number of seeds produced by an individual baobab. (Chadare F. J. *et al.,* 2009).

2.2. 1 Baobab fruit

The fruit of the baobab tree called Gingilis hangs singly on long stalks with an ovoid, woody shell 20 to 40 cm long and up to 10 cm in diameter. This shell contains numerous hard, brownish seeds, round or ovoid, up to 15 mm long. The ripe fruit pulp appears as naturally dehydrated powdery, whitish colored and with a slightly acidulous taste. It can reach 12 cm and contains many seeds, which is covered on the outside with greenish-brown felted hair (fiber). It is estimated that it takes between eight and twenty-three years before the baobab produces seeds and the mature plant (over 60 years) can produce more than 160–250 fruits per year. Seeds are brown ovoid about 1-2cm and diameter vary to 0.50-0.75cm. (UNCTAD, 2005).

2. 2. 2 Baobab Leaves

Baobab tree leaves zero tree in the rain season, then will appear on the branches white flowers smell good, and the fruits of velvety green leaves and glossy. Leaves are palmate with five sessile leaflets, alternate and hand-shaped with 3–9 tapering leaflets, about 10x5cm at the ends of branches; digital foliate, simple leaves on young plants.

Leaves of young tree are often simple. Overall mature leaf size may reach a diameter of 20 cm, large and showy; some baobab trees bear leaves only for three months per year. During the leaf less period physiological processes such as photosynthesis take place in the trunk and branches, utilizing water stored in the trunk (Gebauer J. *et al*., 2002).

2. 2. 3 Baobab flower

Flowers are pendulous, solitary or paired in leaf axils, white, 12 cm across; cup-shaped, 5 cleft, hairy;5 petals, leathery and ultimately re-flexed, hairy inside; stamens many stamina columns dividing into many filaments, flower bud is globes sometimes ovoid, the baobab produces large pendulous white flowers from October to December.(Watson R. 2007).

2.2. 4 Baobab bark

The bark is smooth, reddish brown to grey, soft and fibrous with a purplish tinge or rough and wrinkled like an elephant's skin, and contains a yellow or green inner layer, which is composed of thick, tough, longitudinal fibers (Sidibe M. and Williams J. T., 2002)**.**

2.3 Baobab Taxonomical profile

Kingdom:-plantae.

Phylum:-Tracheophyat.

Class:-Malvaceae.

Order:-Malvales.

Family:-Malvaceae.

Genus:-Adansonia.

Species: -digitata

Botanical name:-A. Digitata

English name: -Baobab (en.M.wikipedia.org, 2016).

2.4 Environment Impact

Baobab trees have a positive environmental impact; it helps in maintaining soil and water.

They can reduce soil erosion, the roots prevent soil erosion with heavy rains provides cover, the ability to withstand extreme stress from drought allows the tree to be grown on degraded or marginal lands where other species would not survive. The large white baobab flowers, which open at night, are and many types of trees have helped to stop the spread of deserts. Canopy pollinated by bats and other small mammals. The protection of the pollinators is important for the production of fruits. Tree leaves absorbed carbon dioxide from air (BAOBAB Adansonia digitata, 2006).

2.5 Distribution

The baobab is found in many african countries. Ninth baobab species have been identified globally and seven species found on the island of Madagascar are endemic to that region. It is postulated that the center of evolutionary origin of the genus Adansonia is Madagascar. The African species A. Digitata is indigenous to and widely distributed throughout the savannah‟ s and savannah woodlands of sub-Saharan Africa. The only species which is not endemic to the African continent

is A. gibbosa native to Australia. In southern Africa, A. Digitata is commonly found in Malawi, Zimbabwe, Mozambique and South Africa especially in the warm parts, while in west africa, it is found in Mali, Benin, Senegal, the Ivory Coast, Cameroon and Burkina Faso. In east africa, the plant is found in countries such as Kenya, Uganda and Tanzania and the remaining seven species are endemic to Madagascar. A. digital is widespread throughout the hot, drier regions of tropical africa, it extends from northern Transvaal and Namibia to Ethiopia, Sudan and the southern fringes of the Sahara. In Sudan, the baobab is most frequently found on sandy soils and by seasonal streams " khorsÌ in short grass savannahs (Wickens G. E. and Lowe P., 2008).

2.6 Traditional use

The baobab tree is extremely important for humans and animals in the dry areas of africa. It offers protection and food, clothing and medicine as well as raw material for many useful items. The tree has been known to be used for shelter. The hollowed tree will continue to thrive, bearing fruit every season. Its leaves bark and fruit are used as food and for medicinal purposes in many parts of africa. Every part of the baobab tree is reported to be useful**. (** WYK, B. and N. GERICKE, 2000)**.**

2.7 Part uses

Baobab is used for several purposes and these include: fruit for food; oil from the seeds; rope, cordage and cloth from the bark fiber; tannin for curing leather from the tree bark; glue from the pollen grain of the flowers (Brady O., 2011).

2. 7.1 Fruit

During acute seasonal food supply fluctuations or famine periods, the leaves and fruit of baobab are of particular importance as supplementary and emergency food. Baobab is a popular food source, the fruit pulp is used in preparing cool and hot drinks in rural areas and has recently become a popular ingredient in ice and jam products in urban areas. The fruit pulp is commonly sucked, chewed or made into a drink when mixed with water or milk, either with or without sugar, or as a supplement to mix with staple food such as corn meal and cassava. Pulp for making paper from the harvested tree (although of low quality) and seasoning. Other uses for baobab pulp include sauces for food, hair rinse, milk curdling agent and a substitute for cream of tartar, among other things. Ripped fruit pulps are removed from the fibers and seeds by kneading in cold water: the resulting emulsion is sieved. This is then added to thick grain preparations to make thinner gruels. The dry pulp is either eaten fresh or used to add to gruels on cooling after cooking and that is also a good way of preserving the vitamin content. The pulp is also ground to make a refreshing drink with a pleasing wine-gum flavor. More so, it is added to aid fermentation of sugar cane for beer making. Pulp can be stored for fairly long period of time for use in soft drink production but it needs airtight containers. The fruit pods are also good for burning and a potash-rich vegetable salt may be obtained from this ash for making soap. The pulp is also eaten fresh in Sudan. Whole fruits or just the fruit pulp can be stored for months under dry conditions. The pulp powder is extracted and stored in polyethylene bags which protect it against ambient moisture.

2.7.2 Bark

The bark, which produces strong fiber, is used in making ropes, mats, beautiful bags, and hats. The smooth fibers of the inner side of the bark are more important than the outer bark for weaving. The wood is whitish, spongy, and light, it is used mainly for fuel. Inner bark is strong and widely used for making basket nets, snares, fishing lines and is even used for weaving. It is also available from disintegrated wood and have been used for packing. Leaves of the baobab tree are a staple food source for rural populations in many parts of africa. During the rainy season when baobab leaves is tender, people harvest the leaves fresh. Leaves are typically sun-dried and either stored as whole leaves or pounded and sieved into a fine powder. In markets, the powder is the most common form. Young leaves are commonly used as a vegetable in soups or cooked and eaten as spinach, also used for sauces over porridges, thick gruels of grains, or boiled rice. Dried green leaves are used throughout the year, mostly in soups served with the staple dish of millet. The leaves, for instance, are used in the preparation of soup “miyankuka” in northern part of Nigeria, the Hausas use it. Balanites they provide fresh vegetables that are substituted for the commercially grown leafy vegetables such as cabbages and lettuce. In Mali, the leaves are called Lalo and they are used in making sauce and they usually mix it with seeds of Parkiabiglobosa, onion, okra, pepper, ginger, sometimes meat, but more often fish.

2.7. 3 Flowers

Flowers can be eaten raw or used to flavor drinks.

2.7. 4 Seeds

Baobab seeds can be eaten fresh, or they may be dried and ground into flour which can either be added to soups and stews as a thickener, used as a flavoring agent, or roasted and eaten as snacks. Seeds are ground into a paste, or boiled for a long time, fermented and then dried for use. In Sudan they are pounded whole into a coarse meal and added to soups and other dishes like „Burma" , in some areas roasted seeds are used as a coffee substitute. Seeds are ground with peanuts, water and sugar to make a sauce used with porridge.

2.7. 5 Root

Shoots and roots of germinating seeds are also edible; the roots may also be cooked and eaten. An infusion of roots is used in Zimbabwe to bathe babies to promote smooth skin. Other fibers used for rope are obtained from root bark.

2.8 Medicinal applications of Baobab

Medicinally, baobab fruit pulp is used as a febrifuge and as an anti-dysenteric and in the treatment of small box measles as an eye instillation, and fevers. Traditional medical uses of Digitata tree in Indian medicine, the aqueous extract of baobab fruit pulp exhibited significant heap to protective activity and consumption pulp may play an important part in human resistance to liver damage in areas where baobab is consumed (Kamatou, I *et al*.,2011).

In Mali, it is reported that swollen joints are treated by rubbing a paste made with baobab fruit into the affected area. Baobab pulp is used internally with butter milk in cases of diarrhea. Pulp could be used to treat sickle cell anemia, as it showed considerable anti-sickling

activity. Antioxidants could help prevent oxidative stress related diseases such as cancer, aging, inflammation and cardiovascular diseases as they may eliminate free radicals which contribute to these chronic diseases (Kamatou, I *et al*.,2011).

Baobab fruit pulp has traditionally been used as an immune stimulant, analgesic and antipyretic. Water-soluble fraction of the fruit pulp has stimulating effects on the bacteria in vitro. In fact, soluble dietary fibers, such as those in the pulp (about 25%), are known to have 16 prebiotic effects, which means they stimulate the growth and/or the metabolic activity of beneficial organisms. Extracts from the fruit, seeds and leaves are antimicrobial against: bacillus subtilis, escherichia coli, mycobacterium leprosy, and antifungal against penicillium crus to-sum, Candida albinos, and saccharomyces cerevisiae. Baobab fruit pulp improves the iron status of children with low iron levels in their blood.

Baobab bark is used in Europe as a febrifuge (antipyretic) and treatment of fever, especially that caused by malaria. In the Gold Coast (Ghana), the bark is used instead of quinine for curing fever. In Indian medicine, baobab bark is used internally as a refrigerant, antipyretic and anti-periodic. Barkis certainly used for the treatment of fever in Nigeria. Moreover, bark contains a white, semi-fluid gum that can be obtained from bark wounds and is used for cleansing sores, baobab extract is poured onto the wound of an animal killed in this way to neutralize the poison before the meat is eaten**(** Kamatou, I *et al*.,2011).

In Congo Brazzaville bark is used to bathe rickety children and in Tanzania as a mouthwash for toothache. Sufferers of malaria in africa, India, Sri Lanka and the West Indies are said to consume a mash

containing dried baobab bark as a febrifuge in order to treat the fever associated with this illness. A digitata leaves, fruit-pulp and seeds have shown antiviral activity against influenza virus, herpes simplex virus and respiratory virus and polio. Leaf infusions are used as treatment for diarrhea, fever, inflammation, kidney and bladder diseases, blood clearing and asthma. Leaves of baobab are used to treat various conditions including internal pains, disease of the urinary tract, otitis, as a tonic and for insect bites and Guinea worms **(** Kamatou, I *et al.*,2011).

The leaves form a component of many herbal remedies and ash prepared from the dried powdered roots is given to malarial patients as a tonic. A semi-fluid gum, obtained from baobab bark, is used to treat sores. The antioxidant capacity of baobab fruit pulp was compared with antioxidant properties of other fruits including kiwi, orange, apple and strawberry. Citric acid and other typical constituents of the fruit pulp may be responsible for its effect against diarrhea. It also shows analgesic (pain killing) and antipyretic (temperature reducing) activities. In Indian medicine, powdered leaves are similarly used to check excessive perspiration. Baobab leaves are as a diaphoretic, an astringent. Leaves also have hyposensitive and antihistamine properties. The powdered leaves can be used as anti-stress properties. In Senegal baobab leaves and the fruit pulp are used for external bleeding **(** Kamatou, I *et al*.,2011).

Lalo (baobab leaf) is taken for anemia and also claimed to lower blood pressure. A drink made from leaves and pulp are also used in the treatment of hemorrhoids. Seeds are used in cases of hiccough. Seed also burned in used to treat wounds. Oil extracted from seeds is used for inflamed gums and to ease diseased teeth. The witch doctors in

Senegal treat endue/edema (numbness of the limbs) with incantations and a salve of A. digitata. In west africa the sap, or a paste from roasted crushed seeds, is applied to the diseased teeth and gums. Preparation of the baobab seed is taken to relieve stomach ache in adults. In Somalia, fresh or dried roots are boiled in two to four glasses of water and two cups are taken in the morning as a remedy for urine retention. **(** Kamatou, I *et al*.,2011).

In west african a solution of the baobab fruit matrix and water, or preferably rice water in which iron rust has been boiled, was used to treat small pox. In Tanzania people who are HIV positive drink the liquid obtained by boiling baobab roots, bark and fruit pulp. In south africa the venda use a baobab bark decoction together with the root to treat sexually transmissible diseases. Pregnant women in Malawi drank baobab juice made from fruit pulp mixed with water. Australian Aboriginal mothers with new born babies also used to drink baobab fruit pulp crushed into water. In Benin the flowers are used to speed the ejection of the fetus. In Tanzania the Maasai use the bark and leaves for treating afterbirth retention. Pregnant women in Zimbabwe use the bark from mature baobabs to enlarge their birth canals in order to reduce pain during delivery. In India, to relieve delivery pains, pregnant women bathe in water in which baobab bark has been boiled **(** Kamatou, I *et al*.,2011).

2.9 Cosmetic uses

Baobab seeds oil is an important commodity in market of cosmetic products. Baobab oil is an excellent moisturizing agent and ideal for numerous cosmetic applications. It is one of the most promising oils in africa. The oil is quite viscous, with a rich, silky feel and mild aroma.

Seed oil is used to treat skin complaints. It "naturally great for chapped lips, hair conditioner and nail care. The market for natural cosmetics will grow by 10-20% yearly. For most customers, the biologically certified origin of the plant material is an important factor. Natural cosmetics are sold in health food and natural product stores. A current trend in cosmetics is toward health and wellness products. Consumers are prepared to pay more for cosmetic products with health claims. Substances that are added to health food and botanical remedies are also often used as additives in the cosmetic sector (Eurostat, 2004).

2.10 Oil uses

The oils have been used for centuries by local communities for the purpose of food, medicine, cosmetic applications, production of lubricants, soaps and personal care products. Oil golden yellow, was used in topical treatment of various conditions such as hair dandruff, muscle spasms, varicose veins and wounds. It contains high proportions of linoleic and oleic acid. Baobab seed oil is an excellent source of mono and poly unsaturated fatty acids. Poly-unsaturated fatty acid plays an important role in modulating human metabolism. Therefore, the high linoleic acid content is of nutritive significance because of the ability of some unsaturated vegetable oils to reduce cholesterol levels. This high content of mono-and poly-unsaturated fatty acids suggests that baobab seed oil would be useful as food oil. The saponification value is high, suggesting that baobab oil may be suitable for soap. Oil extracted is used for inflamed gums and to ease diseased teeth. Since seed oil is used to also treat skin complaints, it can be considered to have cosmetic applications as well. In Madagascar, baobab seeds (including A. digitata) have been used for the production of vegetable oil. It is suitable for use on the skin as it is

non-irritating and non-allergenic. Other properties of pharmaceutical/cosmetic importance include that it is excellent for restoring and remoisturising the skin due to its high penetrability and nourishing properties. It can also be used to treat eczema and psoriasis. Baobab oil or fruit pulp contains several vitamins that are essential for skin care. These include vitamins A (rejuvenation and cell renewal); vitamin E (anti-oxidant and anti-ageing effects) and vitamin D which increases calcium absorption and decreases blood pressure in the elderly. The oil is said to alleviate pain from burns and regenerates the epithelial tissues in a short time, thereby improving skin tone and elasticity. The oil can be used as a protecting, nourishing, moisturizing, soothing and regenerating agent. It's also contains anti-oxidants, can protect the skin against premature ageing and prevents the appearance of wrinkles. Alone or combined with other ingredients, used to aid in skin healing (small cuts, chapping) or as a mask for hair care (dry, brittle hair, split ends) (Abdullahi, N., 2010).

2.11 Chemical composition

Chemical analyses have reported the presence of various potentially bioactive ingredients compounds. Baobab kernel is rich in energy, protein and mineral content and also has a potential usefulness as a food protein source in tropical and subtropical region. The fruit pulp serves as a calcium supplement due to its high calcium content. The baobab fruit was found to have the highest content of vitamin C at 280 to 300 mg/100 g, when compared with vitamin C content of 46 mg/100 g in oranges, which is seven to ten times more than oranges. The pulp is very nutritious; an average of 8.7% moisture, the pulp contains about 74% carbohydrates, 3% proteins, 9% fibers, 6% ash and only 0.2% fat(Parkouda *et .al*, 2012).

The content of pectin is approximately56%, which is why the pulp is traditionally used as a 30base for jam making, the energy value of pulp is similar to that of baobab leaves. The acidulous taste is attributed to the presence of organic acids, such as citric acid, tartaric acid, malic acid and succinic acid. Natural anti-oxidants, including poly phenolic compounds from plants, vitamins A and C are believed to be effective nutrients in the prevention of these oxidative stress-related diseases. The major interest in baobab products is as a result of its ascorbic acid and dietary fiber content. One study demonstrated that the consumption of 40 g of baobab pulp provides 100% of the recommended daily intake of vitamin C in pregnant women (19–30 years).The pulp sweetness is provided by fructose, sucrose and glucose contents (Parkouda *et .al*, 2012).

Simple sugars in baobab pulp account for about 35.6% of the total carbohydrate content. This explains the noticeable sweet taste of the pulp. However, the sweetness may vary for different types of pulp. The low water content, strong acidity and high sugar content. Baobab contains ascorbic acid with PH 3.3. The ascorbic acid content was evaluated in the fruit of A. digitata and it was found to contain 337 mg/100 g of ascorbic acid. The pectin is mainly water soluble and has a low degree of esterification and a low intrinsic viscosity. The absence of starch in the pulp, Protein accounts for about one-fifth of dry matter in baobab fruit pulp (17%), thus can be considered a rich source of amino acids. Most fatty acids in the pulp do not reach detectable levels (Parkouda *et .al,* 2012).

Total lipid content of 155 mg/g dry weight, and stated that significant linoleic acid is present. Also point" baobab fruit out as a rich source of linoleic acid, 27 mg/g dry weight. Baobab fruit pulp contains very

little iron and is a relatively poor source of manganese, but contains exceptionally high calcium content. The seeds have an energy value of 1803 kJ/100g approximately 50% higher than leaves, moisture 8.1%, protein 33.7%, and fat 30.6%, carbohydrates 4.8%, fiber 16.9%and ash 5.9%. Seeds contain appreciable quantities of oil (29.7%, expressed on a dry weight basis). Fermentation of baobab seeds decreases protein and carbohydrate but increases fat levels. Fermentation has varied effects on the mineral concentrations of the baobab seeds. The principal fatty acids in baobab oil are linoleic and oleic acid, 39.42% and 26.07% respectively. Of the total fatty acids 73.11% is unsaturated while 26.89% is saturated. Polyunsaturated fatty acids play an important role in modulating human metabolism; therefore, the high linoleic acid content is of nutritive significance. The saponification value is high, suggesting that baobab oil may be 31suitable for soap making. At the same time, the seeds are a poor source of iron, zinc and copper. The leaves contain 13-15% protein, 60-70% carbohydrate, 4-10% fat and around 11% fiber and 16% ash. Energy value varies from 1180-1900kJ/100g of which 80% is metabolized energy(Parkouda *et .al*, 2012).

The leaves are rich in pro-vitamins A and C. baobab leaves have a high content of iron compared to numerous other wild-gathered foods, and area rich source of calcium. A. digitata23The oils have been used for centuries by local communities for the purpose of food, medicine, cosmetic applications and production of lubricants, soaps and personal care products. Oil golden yellow, was used in topical treatment of various conditions such as hair dandruff, muscle spasms, varicose veins and wounds. It "contained high proportions of linoleic and oleic acid, baobab seed oil is an excellent source of mono and poly

unsaturated fatty acids. Poly unsaturated fatty acid plays an important role in modulating human metabolism(Parkouda *et .al*, 2012).

Therefore, the high linoleic acid content is of nutritive significance because of the ability of some unsaturated vegetable oils to reduce cholesterol levels. This high content of mono-and poly unsaturated fatty acids suggests that baobab seed oil would be useful as food oil. The saponification value is high, suggesting that baobab oil may be suitable for soap. Oil extracted is used for inflamed gums and to ease diseased teeth. Since seed oil is used to also treat skin complaints, it can be considered to have cosmetic applications as well. In Madagascar, baobab seeds (including A. digitata) have been used for the production of vegetable oil. It is suitable for use on the skin as it is non-irritatingand non-allergenic. Other properties of pharmaceutical /cosmetic importance include that it is excellent for restoring and remoisturising the skin due to its high penetrability and nourishing properties. It can also be used to treat eczema and psoriasis (Parkouda *et .al*, 2012).

Baobab oil or fruit pulp contains several vitamins that are essential for skin care. These include vitamins A (rejuvenation and cell renewal); vitamin E (anti-oxidant and anti-ageing effects) and vitamin D which increases calcium absorption and decreases blood pressure in the elderly. The oil is said to alleviate pain from burns and regenerates the epithelial tissues in a short time, thereby improving skin tone and elasticity. The oil can be used as a protecting, nourishing, moisturizing, soothing and regenerating agent. It's also contains anti-oxidants, can protect the skin against premature ageing and prevents the appearance of wrinkles. Alone or combined with other ingredients, used to aid in skin healing (small cuts, chapping) or as a mask for hair care (dry,

brittle hair, split ends). leaf could serve as a significant protein and mineral source in the staple food of the local population (Parkouda *et .al*, 2012).

2.12 Extraction of essential oils

Extraction of essential oils can be carried out by various means, as Follows:

2.12.1 Steam distillation

Steam distillation is the most widely used method for plant essential oil extraction (Reverchon E. *et al.*, 1992).

The **2**proportion of essential oils extracted by steam distillation is 93% and the remaining 7% can be further extracted by other methods. Basically the plant sample is placed in boiling water or heated by steam. The heat applied is the main cause of burst and break down of cell structure of plant material. As a consequence, the aromatic compounds or essential oils from plant material are released (Perineau F. *et al*., 1992) , (Babu K. G. D., *et al*. Al., 2005).

The temperature of heating must be enough to break down the plant material and release aromatic compound or essential oil. A new process design and operation for steam distillation of essential oils to increase oil yield and reduce the loss of polar compounds in wastewater was developed. The system consists of a packed bed of the plant materials, which sits above the steam source. Only steam passes through it and the boiling water is not mixed with plant material. Thus, the process requires the minimum amount of steam in the process and the amount of water in the distillate is reduced. Also, water-soluble

compounds are dissolved into the aqueous fraction of the condensate at a lower extent (Masango, P.,J and Cleaner 2005).

2.12.2 Hydro distillation (HD)

(HD) has become the standard method of essential oil extraction from plant material such as wood or flower, which is often used to isolate non-water soluble natural products with high boiling point. The process involves the complete immersion of plant materials in water, followed by boiling. This method protects the oils extracted to a certain degree since the surrounding water acts as a barrier to prevent it from overheating. The steam and essential oil vapors are condensed to an aqueous fraction. The advantage of this technique is that the required material can be distilled at a temperature below 100 °C. Ohmic-assisted HD (OAHD) is another advanced HD technique (Gavahian M. *et al.,* (2012).

OAHD method had the extraction time of 24-75 min, while HD took one hour for extraction of essential oil from thyme. No changes in the compounds of the essential oils obtained by OAHD were found in comparison with HD.

212.3 Hydro diffusion

Hydro diffusion extraction is a type of steam distillation, which is only different in the inlet way of steam into the container of still. This method is used when the plant material has been dried and is not damaged at boiling temperature. (Vian M. A., *et al*., 2008). For hydro diffusion, steam is applied from the top of plant material, whereas steam is entered from the bottom for steam distillation method. The process can also be operated under low pressure or vacuum and reduces the steam temperature to below 100 °C. Hydro diffusion

method is superior to steam distillation because of a shorter processing time and a higher oil yield with less steam is used (Pizzale L., *et al*., 2002). Solvent extraction conventional solvent extraction has been implemented for fragile or delicate flower materials. Different solvents including acetone, hexane, petroleum ether, methanol, or ethanol can be used for extraction (Areias F., *et. al*, 2000), (Kosar M., *et al*., 2005).

For general practice the solvent is mixed with the plant material and then heated to extract the essential oil, followed by filtration. Subsequently the filtrate is concentrated by solvent evaporation. The concentrate is then retinoid or concrete (a combination of wax, fragrance, an essential oil). The concentrate, is then mixed with pure alcohol to extract the oil and distilled at low temperatures. The alcohol absorbs the fragrance and when the alcohol is evaporated, the aromatic absolute oil is remained. However, this method is a relatively time-consuming process, thus making the oils more expensive than other methods) Li X. M., Tian *et al*., 2009).

2.12 .4 Supercritical carbon dioxide

Conventional methods including solvent extraction and steam distillation have some shortcomings such as long preparation time and large amount of organic solvents (Deng, C. *et al*., 2005). Moreover, the losses of some volatile compounds, low extraction efficiency, degradation of unsaturated compounds, and toxic solvent residue in the extract may be encountered (Gliˇsi´ca, S.B *et al*.,2007), (Gironi, F., and Maschietti, M., 2008). Therefore, supercritical fluids have been considered as an alternative medium for essential oil extraction. Carbon dioxide (CO_2) is the most commonly used supercritical fluid

because of its modest critical conditions).(Hawthorne S. B., *et al*., 1993), (Senorans F. J., *et al*., 2000). Under high-pressure condition CO_2 turns into liquid, which can be used as an inert and safe medium to extract the aromatic molecules from raw material. No solvent residue remains in the final finished product since the liquid CO_2 simply reverts to a gas and evaporates under normal atmospheric pressure and temperature subcritical water. The subcritical water or pressurized hot water has been introduced as an extractant under dynamic conditions (pressure is high enough to maintain water under liquid state and temperature in the range of 100 to 374 C^o). The efficiency (in terms of volume of essential oil/1 g of plant) of continuous subcritical water extraction was 5.1 times higher than HD method (Jimenez Carmona M. M., *et al*., 1999). This method is quicker (needs only 15 min compared with 3 h) and provides a more valuable essential oil with higher amounts of oxygenated compounds and no significant presence of terpenes and allows substantial savings of costs in terms of both energy and plant material (Kubatova A., *et al*., 2001).

Chapter Three

Materials And Methods

3.1 Chemicals

N-hexane , carbon tetrachloride, Bacterial and fungal suspension 2،2 di(4-tert-octylphenyl)-1-picrl-hydrazyl, ethanol, alcoholic sodium hydroxide, diethyl ether , H_2SO_4 1%, supersaturated NaCl, phenolphthalein indicator solution, standard sodium hydroxide, potassium hydroxide ethanol, hydrochloric acid, acetic acid, chloroform, saturated potassium iodide, sodium thiosulphate, carbon tetrachloride, hanus solution.

3.2 Instruments & apparatus

Soxhlet Duran (UK), pycnometer, rotary evaporator (Buchi Switzerland), viscometer, oven, conical flask, volumetric flasks , burets, pipettes, funnel, GC–MS (QP2010-Ultra).

3.3 Methods

3.3.1 Sample preparation and extraction of oil

About 2.3 kg of Baobab fruit were purchased from the super market. 450g of seeds were separated manually from the pods and cleaned with diluted water then dried at 70 C^o for 1 hour, then smoothed by blender and, crushed with hummer to removed woody cover and crashed manually with pestle. Oil extraction was carried out according to method described by (Sukhdev *et al.,* 2008).

Coarsely sample was extracted with n-hexane using soxhlet extractor apparatus. The extraction was carried out for about five hours till the color of solvents at the last siphoning time returned colorless. The solvent was evaporated under reduced pressure using rotary evaporator apparatus. Finally the extract was evaporated at room

temperature in a petri dish till complete dryness. Then the yield percentage was calculated.

3.3.2 Physical properties

3.3.2.1 Density

Density is a physical parameter that plays a vital and important role in all material state whether solid, liquid or gaseous state. It is measured throughout industry to gain insight into material; for example their purity, concentration of component and composition. The density and concentration of liquid product has great impact on their quality behavior and use. Standard test method for density, relative density and API gravity of liquids were determined by digital density meter. The sample is introduced into a U-shape tube that is electronically excited to oscillate at its characteristic frequency. The characteristic frequency change depending on the density of the filled sample. By a precise measurement of the characteristic frequency the density of the sample is determined. (ASTM D 96, 2002).

3.3.2.2 Specific gravity

immersed Is determined by the dry pycnometer filled with prepared sample in such a manner to prevent trap of air bubble after removing the cap of the side arm. The stop was inserted in the pycnometer immediately in water bath 30.0 $\pm$ 0.2 and holded for 30 minutes. Any oil came out of the capillary opening of the pycnometer stopper was wiped out carefully. The bottle removed from the bath, cleaned and dried thoroughly. The cap of the side arm removed and quickly the bottle weighed while ensuring the temperature did not failed below 30 C^o (ASTM D 96, 2002).

$$\text{Specific gravity at } 30^0\,\text{C} = \frac{A-B}{C-B}$$

Where:

A: weight in gm of specific gravity bottle with oil at 30 C^o.

B: weight in gm of specific gravity bottle at 30 C^o.

C: weight in gm of specific gravity bottle with water 30 C^o.

3.3.2.3 Viscosity

The most common method of determining kinetic viscosity in the lab utilizes the capillary tube viscometer. In this method the oil sample is placed into a glass capillary U-tube and the sample is drawn through the tube using section until it reaches the start position indicated on the tube's side. The section is then released allowing the sample to flow backs through the tube under gravity. The narrow capillary section of the tube controls the oil's flow rate; more viscos grades of oil take longer time to flow than thinner grades of oil.

New call to action because the flow rate is governed by resistance of the oil flowing under gravity through the capillary tube. This test is actually measured and oil's kinetic viscosity. The viscosity is typically reported in centistokes (cst); equivalent to mm^2/s in SI units and is calculated from the time it takes oil to flow from the starting point to the stopping point using a calibration constant supplied for each tube. In most commercially oil analysis labs the capillary tube viscometer method is described in (ISO3104) is modified and automated using a number of commercial evaluable automatic viscometer. When used correctly, this viscometer is capable of reproducing a similar level of accuracy by the capillary tube manual viscometer method. The oil's

viscosity will be meaningless unless temperature at which the viscosity was measured is defined. Typically, the viscosity is reported at one of two temperatures either 40 C^o (100^o F) or 100 C^o (212^o F). In most industrial oils, it is common to measure kinetic viscosity at 40 C^o because this based on the ISO viscosity grading system (ISO 3448) D445-03 .

3.3.2.4 Determination of color

Color was determined according to handbook of food analysis. The sample liquid is filtered through a filter paper to remove any impurities and traces of moisture till is sure that the sample was absolutely clear and free from turbidity. The glass cell of desired size is cleaned with carbon tetrachloride and allowed to dry. The cell is filled with the oil and placed in its position in the tintometer. The color then matched with sliding red, yellow and blue colors.

The color of the oil is reported in terms of Lovibond units as follows: Color reading = (aY + 5 bR) or (aY + 10 bR).

Where:

a : sum total of the various yellow slides (Y) used.

b : sum total of the various red (R) slides used.

Y + 5R: is the mode of expressing the color of light colored oils.

Y + 10 R: are for the dark-colored oils (Garrard, Illinois, 1960) .

3.3.2.5 Moisture determination

Moisture content was determined according to the Association of Official's Analytical Chemists as follows: 2.0 g of oil sample were weighed in clean dry and pre-weighed crucible and then placed in an oven at 105C° and left overnight. The crucible was transferred to desiccators and allowed to cool and then weighed. Further placement in the oven was carried out until constant weight was obtained. Moisture content was calculated using the following formula .(ASTM D 56, (1990).

$$\text{Moisture content \%} = \frac{(W_2 - W_1) - (W_3 - W_1)}{W_2 - W_1} \times 100$$

Where:

W_1: weight of empty crucible.

W_2: weight of crucible with the sample before drying.

W_3: weight of crucible with the sample after drying.

3.3.2.6 Flash point

Using a graduated cylinder, 50 mL of oil sample is placed in the cup of the tester. Both cylinder and sample being precooled, the sample temperature start the testing at 27 ± 5C^o or at least 10 C^o bellow the expected flash point, whichever is the lowest. The sample is heated at a slow constant rate. The ignition source is applied at specified intervals and the flash point is the lowest temperature at which application of the ignitions source causes the vapor above the specimen to ignite. The sample is deemed to have flashed when a large flame appears and instantaneously propagates itself over the entire surface of the test

specimen. The observed flash point shall be corrected for barometric pressure.

3.3.3 Biological properties

3.3.3.1 Antimicrobial

Disc diffusion method: The paper disc diffusion method was used to screen the antimicrobial activity of plant extracts and performed by using Mueller Hinton agar (MHA) and sabouraud dextrose agar. The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines. Bacterial and fungal suspension were diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = Mc Farland standard 0.5). One hundred microliters of bacterial and fungal suspension were swabbed uniformly on surface of MHA and SDA the inoculums were 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 SDA and soaked with 20 µl of a the inverted position. The diameters (mm) of the inhibition zones were measured. (Miles and Misra, 1938).

3.3.3.2 Antioxidant (DPPH radical scavenging assay)

The DPPH radical scavenging was determined according to the method of Shimada et. Al., (1992) with some modification . In 96-wells plate, the test samples were allowed to react with 2,2 Di (4-tert-octylphenyl)-1-picrl-hydrazyl stable free radical (DPPH) for half an hour at 37^{o}C. The concentration of DPPH was kept as (300µM).The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation the, decrease in absorbance was measured at 517nm using multi-plate reader spectrophotometer. Percentage radical scavenging activity by sample was determined in comparison with a DMSO

treated control group. All tests and analysis were run in triplicate (Shimada K, *et al.*, 1992).

3.3.4 Chemical properties

3.3.4.2 Sample preparation (Methylation)

2 mL of oil sample were taken in a test tube and added 7 mL from alcoholic NaOH prepared by dissolving 2g sodium hydroxide in 100 mL methanol and added 7 mL of alcoholic H_2SO_4 1% prepared by mixed 1 mL Conc H_2SO_4 H_2 SO_4+99 methanol and shacked by vortex for 3 minutes and leaved the contents to overnight. Added 2 mL of supersaturated NaCl and added 2mL of normal hexane and shacked for 3 minutes and collected the hexane layer. 5µL from hexane were collected and diluted with 5 mL diethyl ether added 1 gm from sodium sulphate as drying agent filter through syringe filter 0.45 µm then transferred the filtrate directly to the GC-MS vial and inject 1 µm directly to the GC-MS.

3.3.4.1The GC–MS condition

The qualitative and quantitative analysis of the sample was carried out by using **GC–MS** technique model (GC-MS, QP2010-Ultra, Simadzu company, Japan) with serial number 020525101565SA and capillary Colum (Rtx-5ms-30mm X 0.025µm). The sample was injected by using spilt mode. Helium as the carrier gas passed with flow rate 1.61ml/ min and the temperature program was started from 60 C^o with rate 10 C^o/ min to 300 C^o as final temperature degree with 5 minutes hold time. The injection port was 300 C^o, the iron source temperature 200 C^o and the interface temperature was 2500C^o. 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 29 minutes . Identification of

component for the sample was achieved by comparing their retention times and mass fragmentation patents with those available in the library, the National Institute of Standard and Technology (NIST, result were recorded).

3.3.4.3 Acid value

Acid value was determined according to handbook of food analysis. 5g of oil sample were accurately weighed in a 250 mL conical flask and 50 mL of diethyl ether added to 100 mL of freshly neutralized hot ethyl alcohol and about 1 mL of phenolphthalein indicator solution was added. The mixture was boiled for about five minutes and titrated against hot standard sodium hydroxide (0.1M) solution with shaking vigorously during the titration. The acid value was calculated by using the formula .

Acid value = 56.1VN /w

Where:

V : Volume in ml of standard sodium hydroxide .

N : Normality of the Sodium hydroxide solution.

W : Weight in g of the sample.

3.3.4.4 Saponification value

Saponification value was determined according to handbook of food analysis. 2.0g of oil sample were transferred into a 200 mL conical flask. 30 mL of ethanolic potassium hydroxide (0.5 N) was added, and cooling pipe was fixed to the flask .The flask gently heated and occasionally shacked while adjusting the heat so that back flow of ethanol will not reach the top of cooling pipe. After heated for 1 hour,

immediately cooled and added 1ml phenolphthalein indicator solution and titrated with HCl (0.5N) before the test liquid is solidified. Blank test performed for 3 times to obtain mean value of titration volume of 0.5N hydrochloric acid.

The saponification was calculated as followed.

Saponification value (mg / g) = (BLl－EPl) × TF × Cl × K1 / Wt

Where:

BLl：Blank level (mL).

EPl：Titration volume (mL) .

TF ：Reagent (HCl) factor (1.006).

Cl：concentration conversion coefficient (28.05 mg/mL).

(Potassium hydroxide in Eq:(56.11×0.5).

Kl ：Unit conversion coefficient

Wt ：Sample weight (g).

3.3. 4.5 Peroxide value

Peroxide value was determined according to Handbook of food analysis . 5g of oil were delivered into a conical flask with stopper. About 25 mL of solvent (15 mL acetic acid+10 mL chloroform) were added and shacked gently to dissolve the sample completely. One mL of saturated potassium iodide was added and immediately the flask sealed and shacked gently for one minute. The flask was left at room temperature in dark. 30 mL of pure water were added, and the flask sealed and stirred. The mixture was titrated with 0.01m/L sodium thiosulphate using starch as indicator.

The peroxide value was measured as followed:

Peroxide value (meq / kg) = (EP1－BL1) × TF × R /w t

Where:

EP1 ：Titration volume (mL)

BL1 ：Blank level (mL)

TF ：Factor of reagent (1.006)

R ：Constant (10)

Wt ：Sample weight (g)

3.3.4.6 Iodine value

Iodine value was determined according to Handbook of Food Analysis. To 300 ml conical flask with ground-in stopper 0.1g oil was added. 20 mL of carbon tetrachloride and 25 mL Hanus solution where added and the flask sealed. The flask content shacked for one minute. And kept sealed and left in a dark room (about 20°C) for 30 minutes with continuous shacking every 5 minutes. 10 mL of potassium iodide (15%) and 100 mLof water were added, and the flask sealed and shacked for 30 seconds. The flask content was titrated with 0.1mol/L sodium thiosulfate till the end point using starch as indicator.

The Iodine value was calculated as follow:

Iodine value (c g / g) = (BL1－EP1) × TF × C1 ×K1 / wt.

Where:

EP1：Titration volume (mL)

BL1：Blank level (47.074mL)

TF ：Factor of titrant (1.006)

C1：Concentration conversion coefficient (1.269)

(Atomic mass of iodine: 126.9/100)

K1 ：Unit conversion coefficient (1)

Wt ：Sample weight (g)

Chapter Four

Results And Discussion

4.1 Result of extraction of Baoabab seeds

Table 4.1 Result of Extraction of Baoabab seeds

Sample	Weight of sample in gm	volume of extract in mL	Weight of extract in gm	Yield %
Adansonia seeds	450	98	89,75	19.94

The percentage of oil in seeds was found to be 19.94% while the other oil 20.8 that means the Yield of extraction was very good. The yield is than the value reported in soybeans oil with value 18%, cotton higher seed oil with value 14% .(Gunstone, ,2011) and African star apple with value 21.57% (K. Audu et al.,2013). The value is lower than the oil yield reported in palm oil, coconut oil and groundnut oil respectively .(Gunstone, 2011) (N. Cynthia et al.,2012). This may be because of the high moisture content, because for a good oil yield

4.2. Result of Physical analysis

4.2. 1. Density (ρ)

Density is proportion between weights of specific volume of material divisor on weight of volume for normativeness material.

Table 4.2 Result of Density.

Parameter	**Result g/cm^3**
Density(ρ)	0.9158
S.G	0.9167

- The obtained result of density and specific gravity was 0.9158, 0.9167 g/cm3 respectively While the other result for the same oil 0.7680 g/cm3 that support my research result (Hagir,2017). The density of the oil obtained is 0.9158 g/cm3 this indicates that Baobab oil is less dense than water. And the value obtained is similar to that reported for rubber seed oil with value 0.9158 g/cm3. (Asuquo, I. Anusiem. and E. Etim,2012).

-The oil had specific gravity of 0.9167; this is closer to 0.918 specific gravity reported for groundnut and less dense than 0.939 reported for neem seed (U.G. Akpan,*et al*., 2000).

4.2.2 Viscosity

The viscosity of a fluid is a measure of its resistance to gradual deformation by shear stress or tensile stress. It is a quantity expressing the magnitude of internal friction.

Table (4.3) Viscosity

Parameter	**Result cSt**
Viscosity400Ckinetic	40.58

- The obtained result of Viscosity 40.58 cSt while the other result for the same oil 38 that support my research result (Hagir,2017). The viscosity of baobab oil (40.58 cSt) was higher than that of groundnut and some other conventional oils such as soybean (31 cSt), cottonseed (36 cSt) and lower than sunflower (43 cSt) at 300C .(K.D. Kammann et al.,1985) and compared favourably with (61 cSt) of tomato seed (P.R.Cantarell *et al*.,1993)

.

4.2.3 Color

Table 4.4 Result of Color.

Parameter	**Result**
Color	1.5 Yellow

The obtained result of Color was 1.5 (Yellow) its stability as a liquid at room temperature while the other result for the same oil (3.5- 5.4) (Yellow and red) that support my research result (Hagir,2017).

4.2.4 Moisture content

Dry matter analysis is the simplest analysis can be performed in the lab. Moisture content is simply the loss in weight from evaporation of water.

DM= (weight of sample and pot+ weight of sample) / weight of sample after dryer)x100.

Moisture content = $\frac{\text{loss of weight (g) x 100\%}}{\text{Weight of Sample}}$

Table 4.5 Results of Moisture content

Parameter	**Result %**
Moisture Content	0.18

The moisture content is used to determine the stability and quality of the seeds. Studies has shown that plant with low moisture content have a longer shelf life (Y.Pomeranz,and C.Meloan , 2000) The moisture content in the Baobab seeds is moderately Low with a value 0.18%. As

result of the moderately lower moisture content, this may not affect to the stability and result to a longer shelf life of the oil .The obtained result of Moisture content was 0.18% while the other result for the same oil 7.4% that means the obtained result was better than the other oil (Hagir,2017).

4.2.5 Flash point

Flash point defines the temperature at which the decomposition products formed from frying oils can be ignited (AOCS Method Cc 9b-55). This temperature ranges from 275 C^o to 330 C^o for different oils and fats Baobab oil falls within this range. The minimum temperature at which the vapours of a fuel catch fire if in contact with a flame. The higher this value is the safer storage, transport and manipulation of the product will be.

Table 4.6 Result of Flash point

Parameter	**Result C^o**
Flash	280.90

4.3 Biological Results

4.3.1 Antimicrobial Activity

Table 4.6 Antimicrobial results

Plant name	**Solvent**	**Concent** mg\mL	**E.c**	**Ps.a**	**S.a**	**B.s**	**C.a**
Baobab	Dimethyl sulphoxide	100	00	00	00	00	00
		50	00	00	00	00	00
		25	00	00	00	00	00
		12.	00	00	00	00	00

Where:

B.s : Bacillus subtilis

S.a: Staphylococcus aureus

C.a: Candida albicans

E.c :Escherichia coli

Ps.a : 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 obtained result of Antimicrobial 00 that indicate the oil was not active for Antimicrobial.

4.3.2 Antioxidant

Table 4.7 Antioxidant Result

NO	**parameter**	**Result% RSA ± SD(DPPH)**
1 / sample	Baobab	46±0.0
2/ Standard	Propyl gallate	90±0.01

The obtained result of Antioxidant 46±0.00 while the standards of Propyl gallate 90±0.01 that indicate the oil have Antioxidant.

4.4 Chemical properties

4.4.1 The GC- Mass

Table 4.8 The GC-MS analysis of Baobab oil showed Seven components dominated by:

Peak#	Name	R.Time	Area	Area%
1	Hexadecanoic acid, methyl ester	15.785	3335759	30.16
2	Cyclopropaneoctanoic acid, 2-[2-[(2-ethylcy	17.220	165204	1.49
3	9,12-Octadecadienoic acid (Z,Z)-, methyl est	17.433	2622487	23.71
4	9-Octadecenoic acid, methyl ester, (E)-	17.516	4258505	38.50
5	Methyl stearate	17.781	490373	4.43
6	10-Octadecenoic acid, methyl ester	18.560	111290	1.01
7	Tetradecanoic acid, 12-methyl-, methyl ester	19.614	76244	0.69
	Total		11059862	100.00

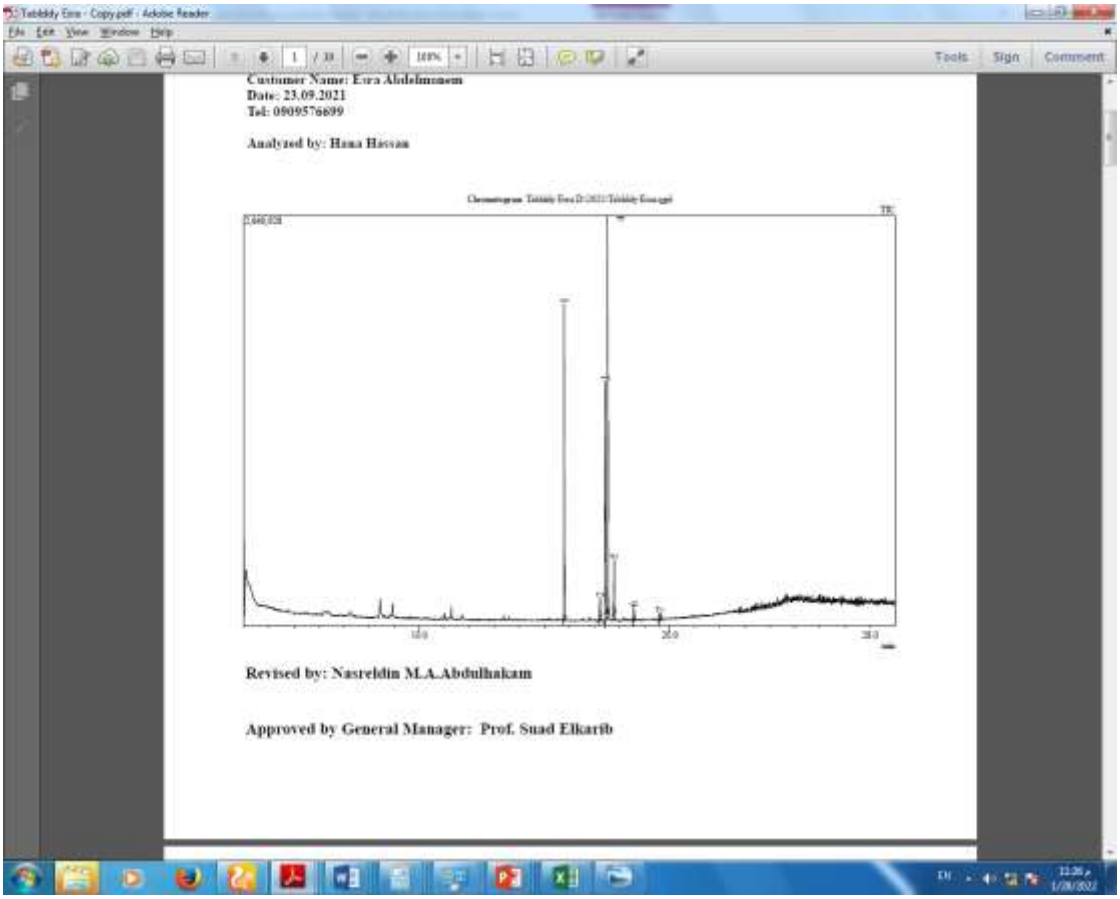

Fig 4.1 the GC.Ms Spectrum of Baobab Seeds oil

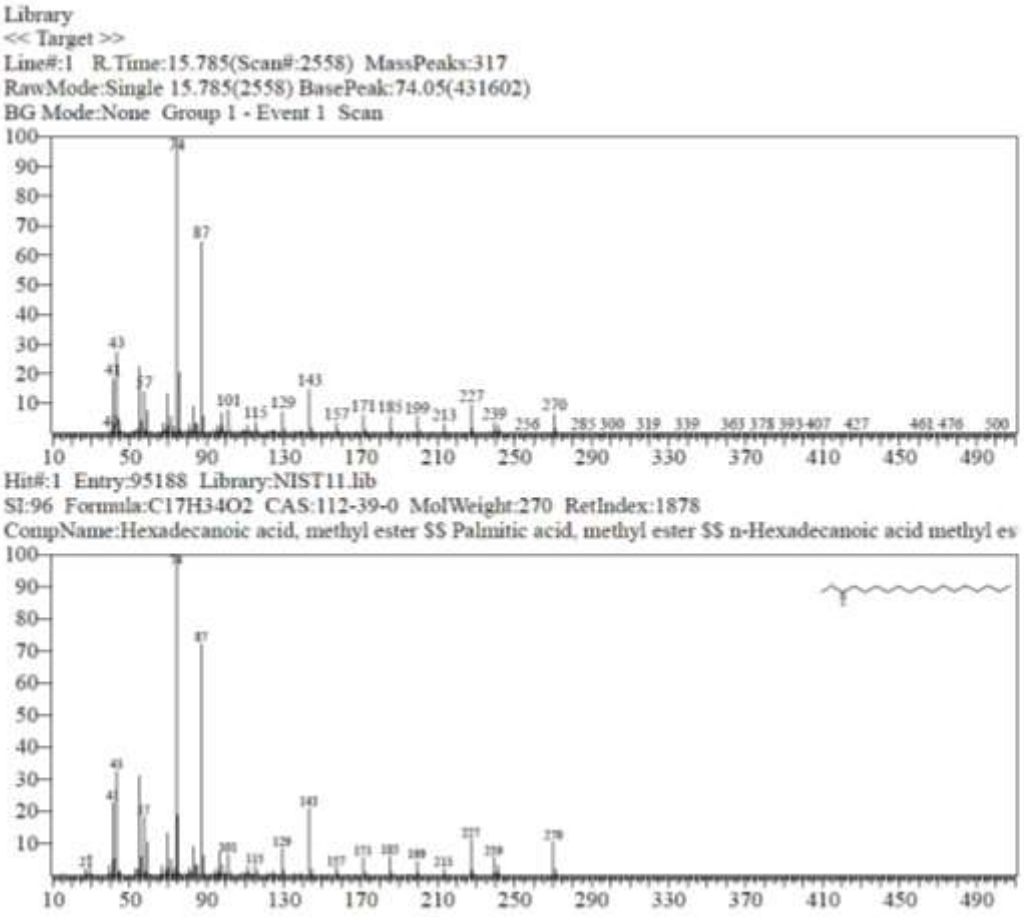

Figure 4.2 The GC-MS spectrum for Hexadecanoic acid, methyl ester

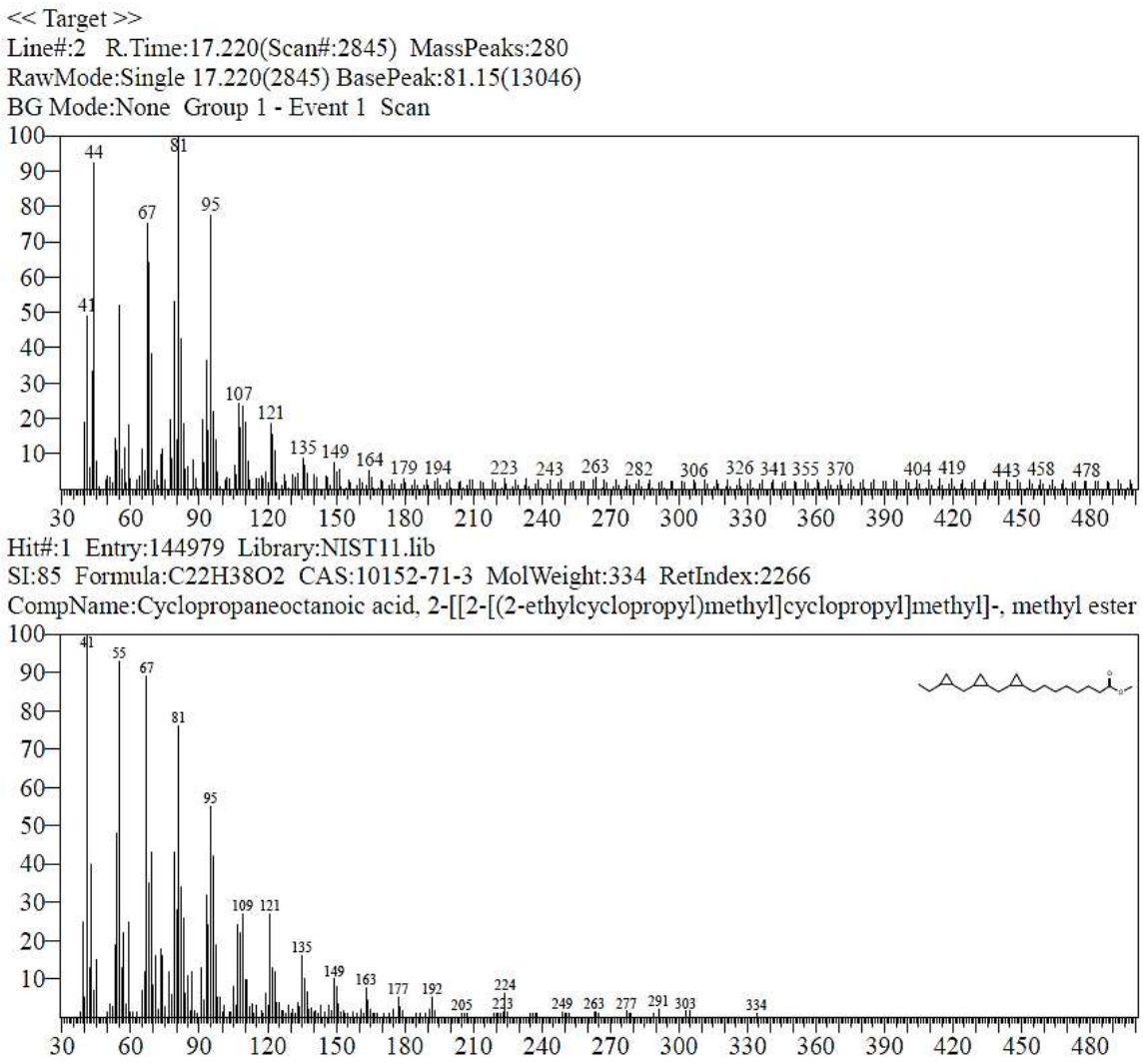

Figure 4.3 The GC-MS spectrum for Cyclo propane octanoic acid, 2-2-[(2-ethylcyl

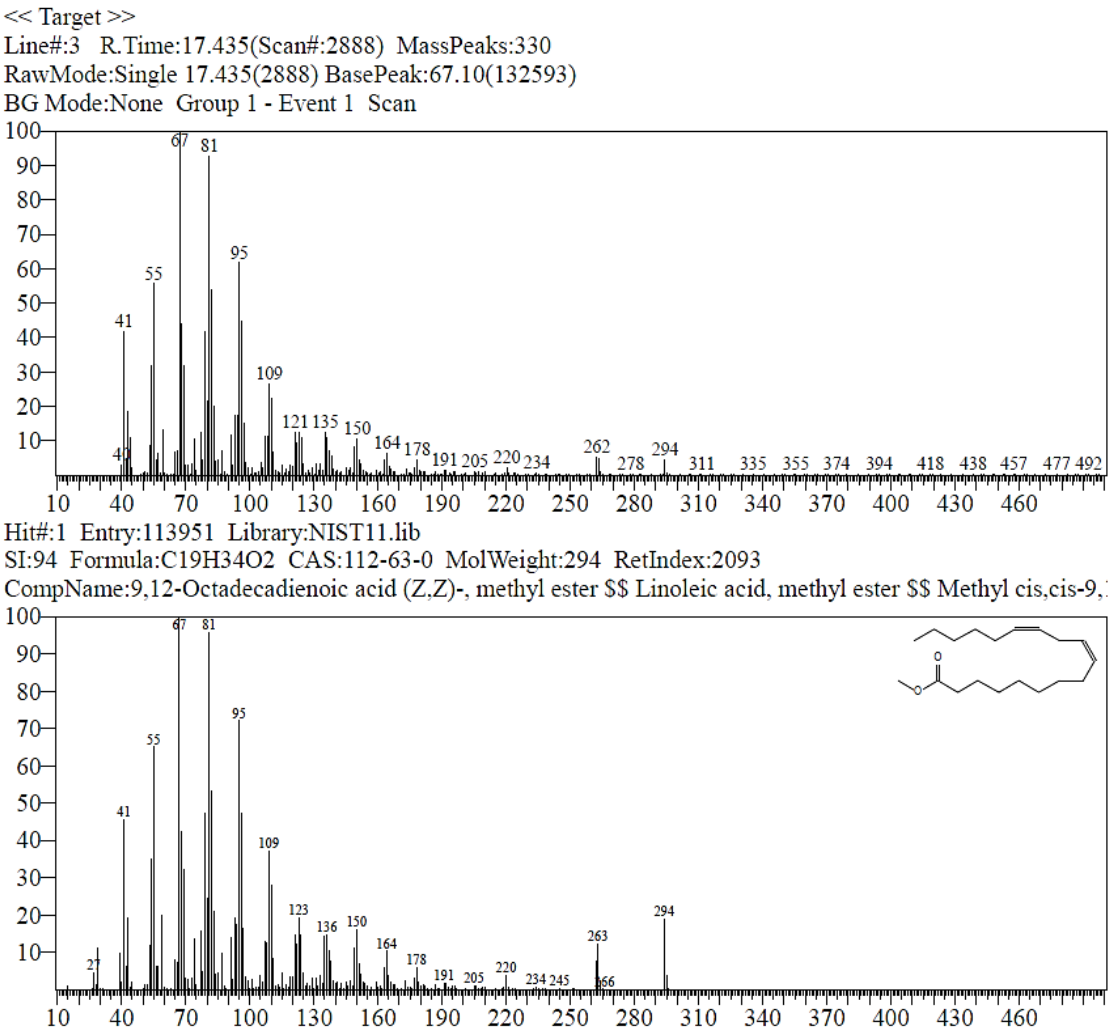

Figure 4.4 The GC-MS spectrum for 9,12-Octadecadienoic acid (Z,Z)-, methyl ester

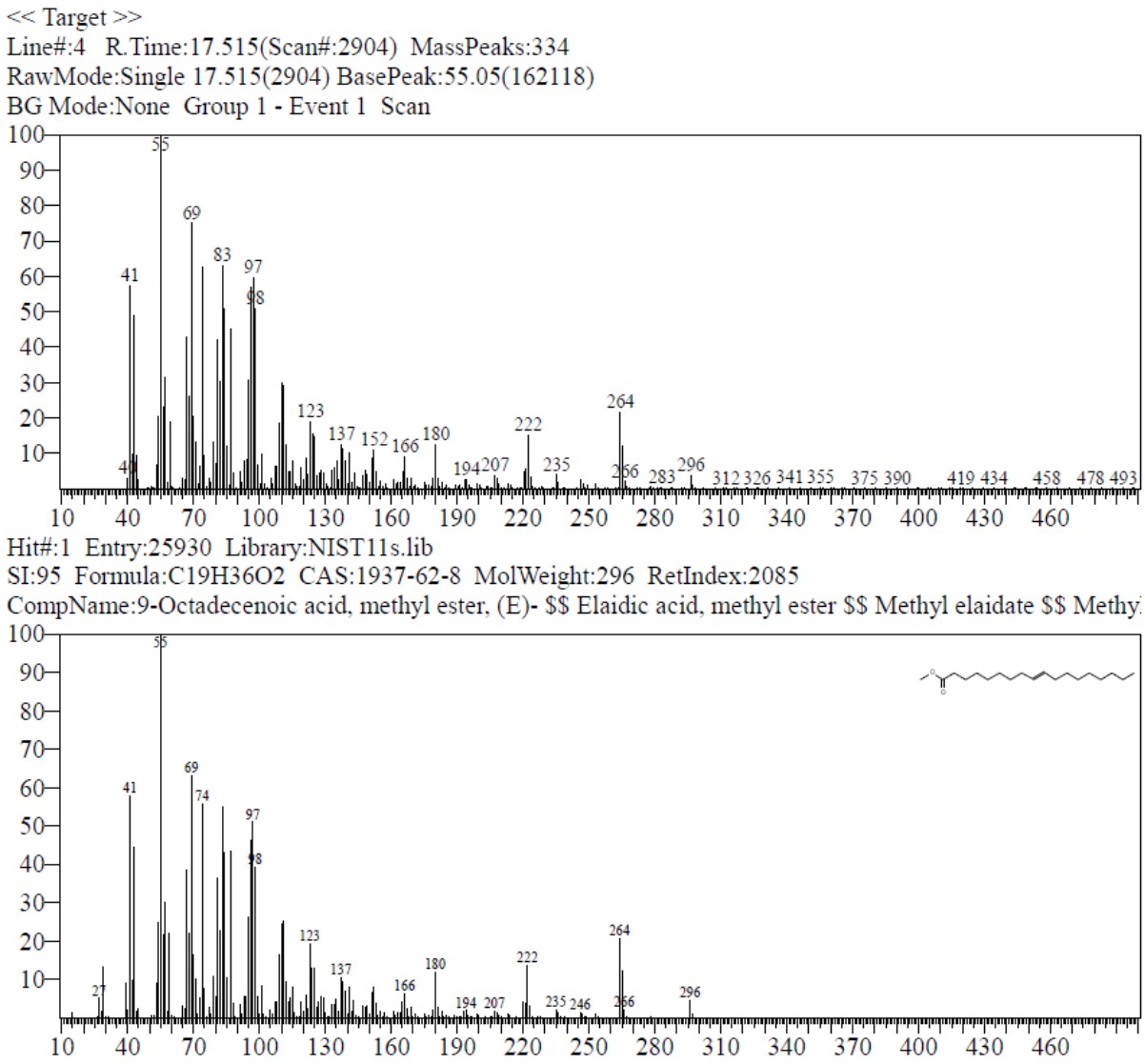

Figure 4.5 The GC-MS spectrum for 9-Octadecenoic acid, methyl ester, (E)- 9-Octadecenoic acid, methyl ester, (E)-

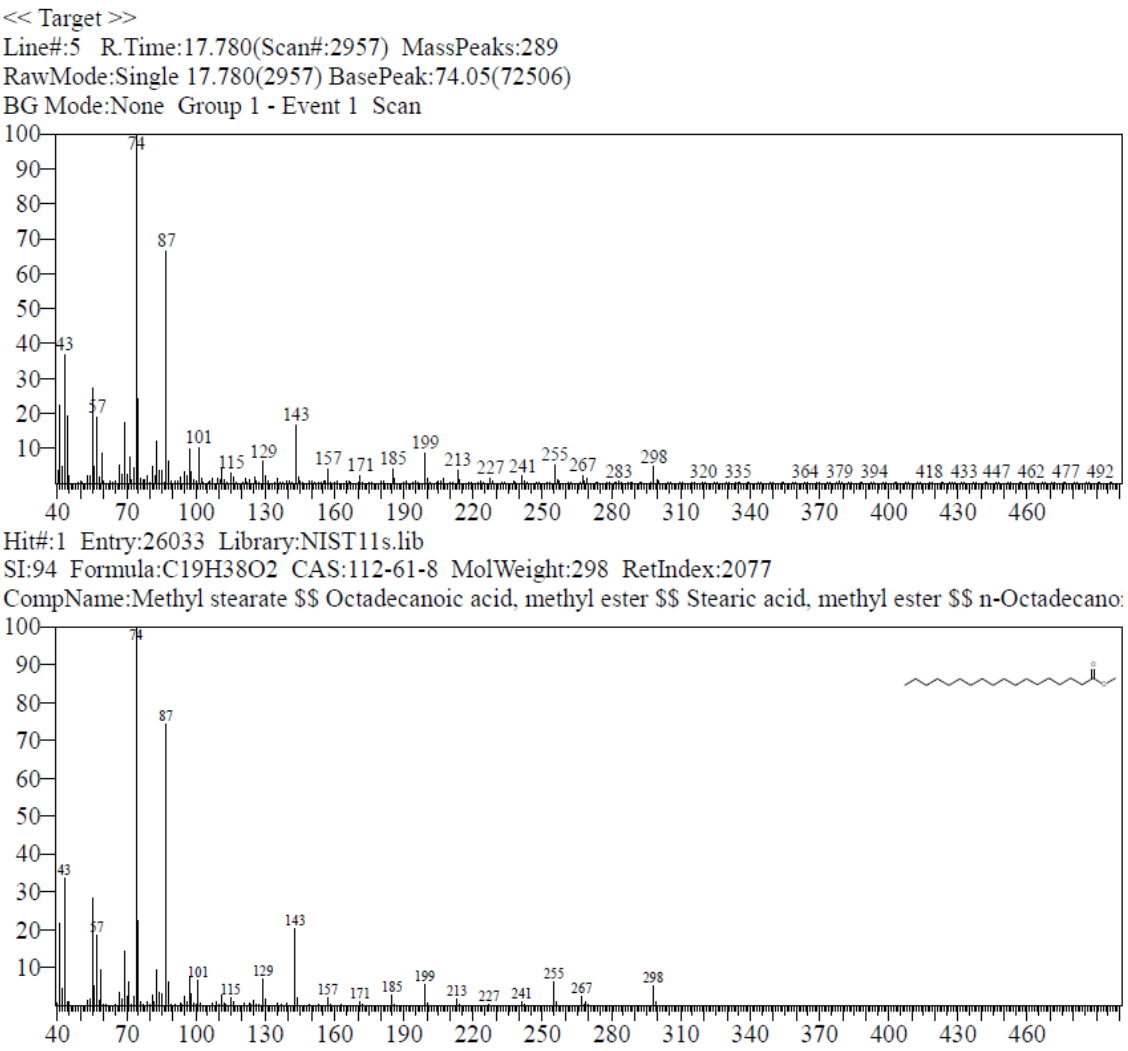

Figure 4.6 The GC-MS spectrum for Methyl stearate

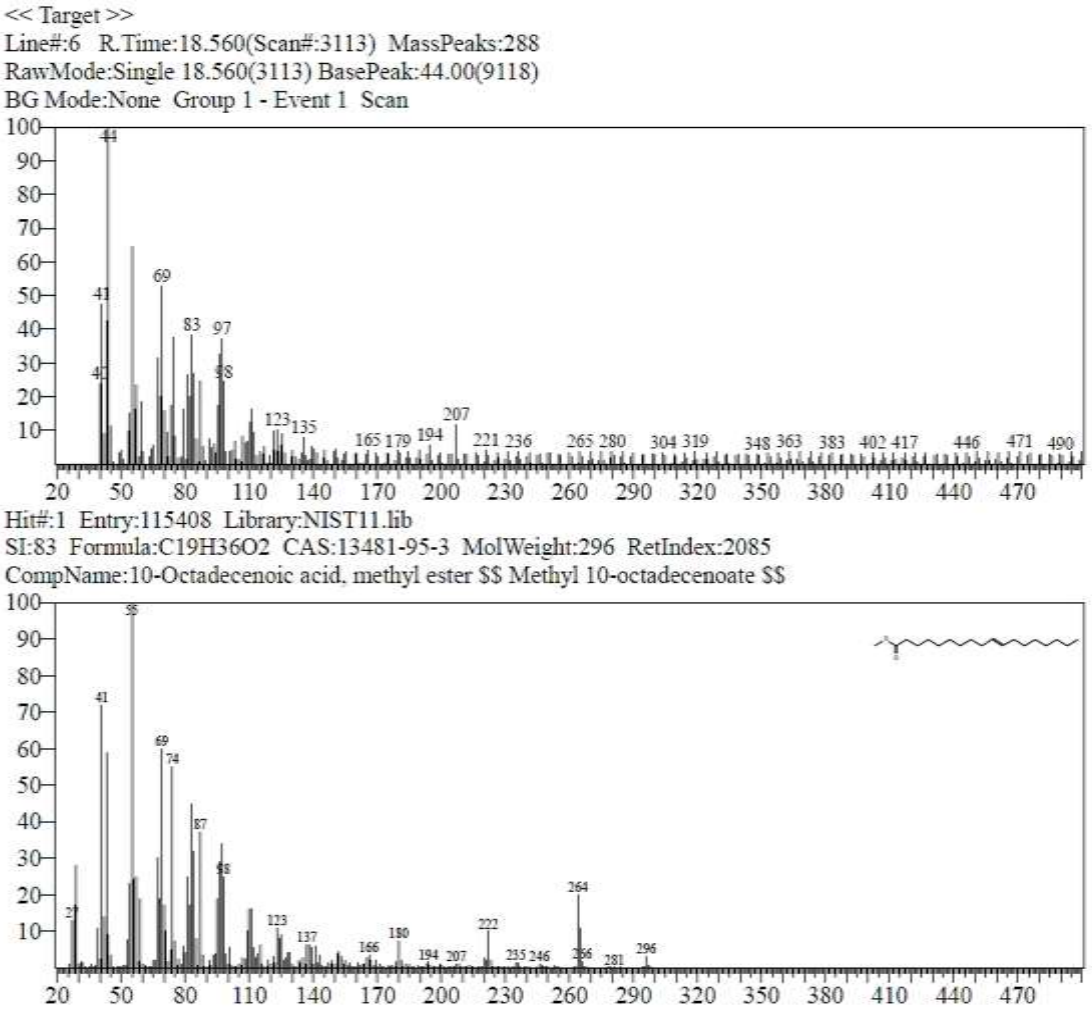

Figure 4.7 The GC-MS spectrum for 10-Octadecenoic acid, methyl ester

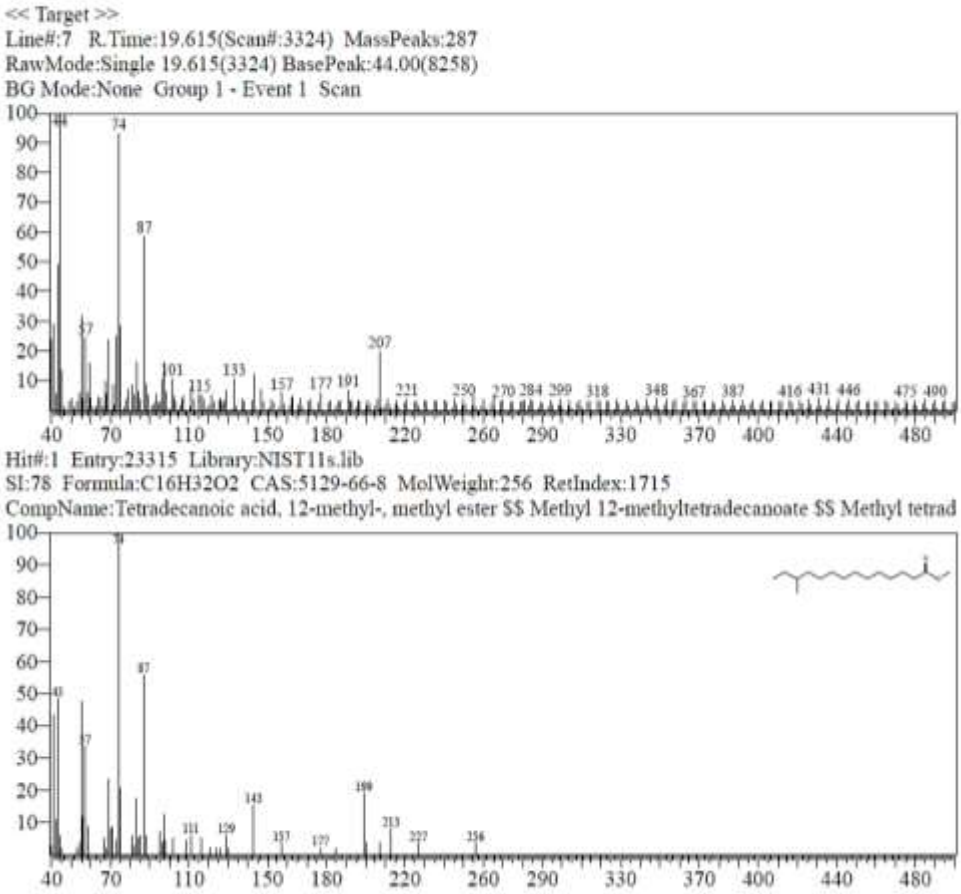

Figure 4.8 The GC-MS spectrum for Tetra decanoic acid, 12-methyl-, methyl ester.

The GC-MS spectrum of the studied oil revealed the presence of Seven components. The oil contains the following components as major constituents:

9,12-Octadecadienoic acid (Z,Z)-, methyl ester (23.71%)

9-Octadecenoic acid, methyl ester, (E) (38.50%)

Methyl stearate (4.43%)

Hexadecanoic acid, methyl ester(30.16%)

While the GC-MS analysis of the other Baobab oil revealed a presence of 20 components as major constituents (Abdalgader,2016):

Oleic acid (Area:30.26%).

-9-Octadecenoic acid methyl ester (22.40%).

-9,12-Octadecadienoic acid methyl ester (18.73%).

4.4.2 Acid Value (AV)

Acid value is common parameter in specification of fats and oils. It is defined as weight of KOH in mg needed to neutralized the organic acid percent in 1 g of fat and it is measure of free fatty acids (FFA) present in fat or oil.

$$AV=\frac{\text{Titration-Blank X N.KOH X Mwt of KOH}}{\text{Wt of Sample}}$$

N.KOH :- Normalty of base=0.1Mwt of KOH=56.1

FFA*2=AV→ FFA=AV/2.

Table 4.9 Result of Acid value.

Parameter	**Result KOHg/ g oil (as oleic acid)**
Acid value (AV)	0.49

The obtained result of Acid value was 0.49% while the other result for the other baobab oil was 2.5% that means the oil was weak acid (Hagir, 2017). The acid value obtained are 0.49 this value is lower when compared with that of African star apple oil with value 4.50. The values are also lower when compared to that of groundnut oil with value 2.61 (S. Adebayo *et al*., 2012), (Gunstone, ,2011) The value obtained is an indication that the oil cannot easily go rancid.

4.4.3 Saponification value (SV)

Saponification value is expressed by potassium hydroxide in mg required to saponify one gram of fat. →SV= 28.05*(b-a) /Wt of sample

Table 4.10 Saponification Value (SV)

Parameter	**Result mg KOHg-1**
Saponification Value (SV)	190.70

The obtained result of Acid value was 190.70 % while the other result for the other baobab oil was 165.495 % (Hagir,2017).

The saponification value which gives an idea of the approximate chain length of the oil, and was found to be 190.70 mg KOHg-1. This value is higher than the value reported in cashew nut oil with value 146 KOHg-1, cotton seed oil with value 189 KOHg-1 and groundnut oil with value148.67 KOHg-1 (N. Idowu, and A. Abdulhamid ,2013) ,(B. Orhevba, and A. Efomah ,2012) (Gunstone, ,2011) also higher than Jojoba seed oil with value 88 KOHg-1 . Jojoba seed oil is use in liquid soap making, antifoaming and shampoo ,(B. Orhevba, and A. Efomah ,2012) . This indicates that the oil could be used in soap making, and shampoo, since its saponification value falls within the range of these oils. It has been reported by that, he saponification value and molecular weight has an inverse relationship (C. R., Ekeanyanwu *et al*., 2012),) this means that, the shorter the saponification value higher molecular weight and the longer its' chain length. The saponification value obtained which are moderately high, shows that it has low molecular weight and short chain length.

4.4.4 Peroxide Value

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.

$PV = V*N*1000 / wt$

V: $ml\ of\ tiosulfate\ sodiumtitrate.$

N: n$ormalityoftiosulfatesodium.$

Table 4. 11 Results of Peroxide value.

Parameter	**Result** mg Eq.O_2/ g oil
Peroxide Value	2.98

The obtained result of peroxide value value was 2.98 mEq.O2 kg-1 while the other result for the other baobab oil was 5 % that means oil have not rancidity (Hagir,2017) . The peroxide value (PV) gives an idea of the level of rancidity in oil. The PV was found to be 2.98 mEq.O_2 kg-1. This value is lower when compared to the values obtained in groundnut oil with value 22.25 mEq.O2 kg-1 and African nut- meg with value 4.13±0.40 .(Gunstone, ,2011) (C. R., Ekeanyanwu *et al*. ,2012) . Studies has shown that there is a relationship between moisture content and peroxide value; decrease in moisture content result in a decrease in peroxide value. (R. Amina *et al*., 2013). From the PV obtained the low peroxide value maybe as a result of low moisture content in the seeds, this is an indication of the oil to not get rancid easily, during process or storage.

4.4. 5 Iodine value (IV)

Iodine value or iodine adsorption value or iodine number in chemistry is the mass of iodine in grams of chemical substance, it used to determine the amount of unsaturation in fatty acids →IV=10* Titration

Table 4.12 Result of Iodine value.

Parameter	**Result Ig/g oil**
Iodine value (IV)	97.25

The obtained result of Acid value was 97.25 I g/g oil while the other result for the other baobab oil was 96.00 I g/g (Hagir,2017).

Iodine value which gives the degree of un-saturation of the oil was found to be 80.02g/g. As a result of the value, the oil is classified as non-drying oil. Iodine

value above 100 is classified as drying while those below are classified as non-drying (K. Julius *et al*., 2013). The iodine value obtained (97.25 I g/g) is higher than the value reported for African star apple with value 35 I g/ g, also cashew nut oil with value 41.87±2.30 I g/g (S. Adebayo *et al* .,2012) (N. Idowu, and A. Abdulhamid ,2013) higher than groundnut oil with value 89.46g/100g, cotton oil with value 94.7g/100g .(Gunstone, ,2011).(B. Orhevba, and Efomah,2012) , due to its relative low iodine value, which results in classifying it as non-drying, it indicates that the oil has a low content of unsaturated fatty acids and can be employed in soap making and lubricant oil, which will help to reduce the dependence on edible oil in making such products .

Chapter Five

Conclusion And Recommendations

Chapter Five

5. Conclusion and Recommendations

5.1 Conclusion

Oil was extracted from Boabab seeds powder by hexane as a solvent. The solvent was separated by using Rotary evaporator. The physical analyzes carried out are (density, specific gravity, viscosity, color, flash point and moisture content). The chemical properties (value of acidity, saponification value, iodine number, and peroxide number) and also Biological analysis was carried for antioxidant antibacterial .The results showed that the oil does not contain antibacterial but it does contain an antioxidant. The baobab seed oil was also subjected to gas-liquid chromatography attached to a mass spectrometer. The results were interpreted using the data of the NIST library for mass spectrometry, and the compositions were confirmed by studying the disintegration pattern. The results of gas chromatography analysis showed that the oil contains seven components.

5. 2 Recommendations

It is recommended to conduct more research on the issue, to get the maximum amount of Baobab oils, the following points must be taken into consideration:

1-Optimum selection of Baobab seeds it must be fresh dried seeds and Smoothing seeds well

2-Using a solvent that has a high purity.
3- Develop appropriate technology to cracking of Baobab seeds to extract all oil seeds content
4-Inserting new seeds in industrial plants.

5- Using Baobab oils as useful cosmetic product for skin and heir

6- Conducting Phytochemical screening for major secondary constituents such as (triterpenes, flavonoids, alkaloids and tannins)

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Appendices

Fruits of *Adansonia digitata*

Leaves of *Adansonia digitata*

Baobab Seeds before and after dryer and after crushed

Soxhlet Extractor

Seed oil