بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies (SUST)

Extraction and Characterization of Hibiscus Sabdariffa L. Red Seeds Oil

استخلاص و تشخيص زيت بذور الكركدي الأحمر

A dissertation Submitted to the Sudan University of Science and Technology in Partial Fulfillment of the Requirements of the Degree of Master in Chemistry

By

Manal Abdalla Ahmed Mohammed

B. Sc Honors, SUST (2010)

Faculty of Chemistry

Supervisor

Dr. Kamal Mohamed Saeed

June, 2019

Dedication

I dedicate this work to my father and my mother (candle of my life)

My husband (Alsmani)

My brothers and sisters (Ezeldin, Esam Eldin, Monzir, Mawahib and Salma)

My beautiful girls

(Monjeda, Malaz and Asma)

My friend (Amna)

ACKNOWLEDGMENTS

All Thank and praise to Allah who give me strength to complete this work. I am grateful to my supervisor Dr. Kamal Mohammed Saeed for his suggestions, assistances and encouragement offered during this work.

Special thank to my husband Al Smani who help and give me the chance to complete this work. My deeply thanks to my brother Esameldeen and Ezeldin who supported me very much.

I am grateful to Maawia Mohmmed Essa from the Industrial Research and Consultancy Centre (IRCC) for his assistance in conducting some of the experiment atIRCC. I am also gratefulto Amna Mohammed Ahmed Musa,for herpatience, advices and wonderful cooperation.

Thank to all people who help and cooperate with me to complete this good work.

ABSTRACT

This study was aimed to extract the oil from Red Roselle seeds and studying the physicochemical properties of the oil. The seeds were purchased from the local market. The oil was extracted by hexane at 80^{o}C for 6 hours using Soxhlet extractor. The proximate analysis of the samples gives the following results: ash content (4.2%); moisture content (5.6%); fiber content (20%); oil (20%); carbohydrate content (22.2%) and the protein content (28.00%). The physical properties were studied (viscosity (57.6); refractive index (1.47); specific gravity (0.963) and the chemical properties; (acid value (4), saponification value (216); ester value (212); peroxide value (7). Also the oil was characterized by (IR) spectroscopy and (UV) spectroscopy and with GC/MS which identified 21 complexes, major components are: Hexadecanoic acid, methyl ester (19.69%); 9, 12 – Octadecadienoic acid (Z, Z) -, methyl ester (28.57%) and 9 – Octadecenoic acid (Z) - , methyl ester (28.01%). The study was concluded that the Roselle seeds are very useful and rich in fiber and protein content. Also the oil can be used in cosmetics as protective agent from cancer and sun –scalds.

مستخلص البحث

هدفت الدراسة الي استخلاص زيت بذور الكركدي ودراسة خصائصه الفيزيائية والكيميائية. جمعت عينات البذور من سوق محلي بمنطقة بحرى وتم استخلاص الزيت باستخدام مذيب الهكسان لمدة 6 ساعات عند درجة حرارة (80 درجة مئوية) وباستخدام الاستخلاص بجهاز (السوكسلت). وأظهرالتحليل العام لعينات البذور أن نسبة: الرماد (4.2%), محتوى الرطوبة (5.6%), محتوى الالياف (20%) الزيت (20%), الكربوهيدريت (22.2 %) وكانت اعلي نسبة هي البروتين (28.00%) . تم دراسة الخصائص الفيزيائية وكانت النتائج كالاتي: اللزوجة (57.6) ، معامل الانكسار(1.47) والكثافة النوعية (0.9063). وكذلك الخصائص الكيميائية (رقم الحموضة (4) ، رقم التصبن (216) ، رقم الاستر(212) ورقم البايروكسيد (7) . وكذلك تم تشخيص الزيت بواسطة جهاز الاشعة تحت الحمراء /GC) MS) وجهاز (UV) وجهاز الاشعة (IR).

حيث تم التعرف علي 21 مركب, المكونات الاساسية منها:

{Hexadecanoic acid, methyl ester (19.69%); 9, 12 – Octadecadienoic acid (Z, Z) -, .methyl ester (28.57%) and 9 – Octadecenoic acid (Z) - , methyl ester (28.01%)}

خلصت الدراسة الي ان بذور الكركدي ذات فوائد قيمة وغنية في محتواها من الالياف والبروتين كما ان الزيت يمكن ان يستخدم في مستحضرات التجميل كواقي للبشرة من اثار الشمس الضارة كالسرطان والحروق .

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Chapter One

Introduction and literature review

1.1- General Introduction

Roselle (Hibiscus Sabdariffa L) is an important base fiber crop is a member of the acacia family Roselle, now widely distributed throughout the tropics, it has been used for the production of best fiber and as an infusion, in which it may be known as cascades. Also Roselle can be used in many ways.

Roselle is known in Sudan by the colloquial name (Kerkade). In western Sudan, 'Kerkade' is grown successfully as rain crop, not for its seeds but for its calyces which are widely used to prepare invigorating refreshing drinks (Yagoub, 1998).

Roselle is a good source of iron, niacin, riboflavin and β-carotene. It has been suggested that increased intake of carotenoids especially β-carotene is inversely related to cancer risk (Colditz *et al*., 1985). 'Kerkrade' was found to be mainly due to the acid content. It contains a high percentage of ascorbic acid. Also 'Kerkrade' water extract is used both as a hot or more popular as cold refreshing drink (Ismail *et al*; 1985).

The plant is primarily cultivated for the production of bats fiber from the stem. The fiber may be used as a substitute for jute in making burlap. *Hibiscus*, specifically Roselle, has been used in folk medicine as a diuretic and mild laxative.

The seeds contain about 17 % oil. Seeds are also used as an aphrodisiac coffee substitute and excellent feed for chickens'. Roselle red - calyces which contain antioxidant are rich in riboflavin, ascorbic acid niacin, β-carotene, calcium and iron that are nutritionally important.

1.2. Objectives

The objectives of this study was to evaluate seed composition and seed oil characterization of Hibiscus seed obtained from local market by studying the followings:

1- Determination of proximate analysis of Roselle seeds such as; moisture content, oil content, protein content, ash content, fiber content and carbohydrate content.
2- Determination of some physical properties.
3- Determination of some chemical properties.
4- UV, GC/MS and IR characterization of seed oil.

1.3-Roselle names and classification

The Roselle is known as the *rosella* or *rosella fruit* in Australia. It is known as Belchanda among Nepalese, Tengamora among Assamese, Gal•da among Garos, Amile among Chakmas, Hanserong among Karbi, SilloSougri among Meitei and *mwita* among the Bodos. The Atongs call it "dachang" or "datchang". It is called as "gongura" by Telugu-speaking people from India. It is known as *Saril* or *flor de Jamaica* in Central America and "Sorrel" in many parts of the Caribbean. Among the Yoruba in South West Nigeria, it's known as "Isapa". In Chinese, Roselle is known as *luoshenhua*. In Thai, roselle is known as *gràjíap.Kraceī́yb*. In Burmese, roselle is known as *chin baung* .

The plant of 'Karkade' that possess binomial name (*Hibiscus sabdariffa L*) belong to the family Malvaceae and the species *sabdariffa* L. It classifies as following:

1.4- Scientific classification

Kingdom: Plantae

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Malvales

Family:Malvaceae

Genus: *Hibiscus*

Species: sabdariffa

1.5- Roselle origin

Roselle (*Hibiscus Sabdariffa* L) is properly a native of West Africa, Other suggested that it is form a Turkish origin.

1.6- Roselle botanical description

Roselle (*Hibiscus sabdariffa L.*) is an annual botanical plant belonging to the Malvasia family cultivated in Egypt. There are more than 300 species of hibiscus around the world one of them is roselle *(Hibiscus sabdariffa L.*) which is a member of the plant family malvaceae (Fasoyiro *et al*., 2005; Ismail *et al*., 2008). The calyces and leaves of the roselle are usually used for making jam, jelly, sauces and pickles, the petals of its flowers have been used in Egypt to prepare beverages, which have various important medical purposes Amin *et al*. 2008. Various studies have shown that Roselle seeds contain much protein and oil (8-10).

Roselle (*Hibiscus sabdariffa*) is an annual or perennial herb or woody-based subshrub, growing to 2 - 2.5 m (7 - 8 ft) tall and other mentioned that Roselle (*Hibisucussabdariffa* L.) is an annual bushy plant seldom more than 2.5 meters high. The branches often grow vertically and parallel to the main axis. The leaves with green red branches and alternate and are deeply three- to five-lobed, 8–15 cm (3–6 in) long, arranged alternately on the stems.

The flowers are solitary arising from axillaries' buds with short peduncles of 8–10 cm (3 - 4 in) in diameter. The calyx consists of ten pointed bracteoles (Purseglove, 1974).the sepals enlarge considerably after flowering. The petals are creamy rather than yellow and always carry a purplish spot near the base, andwhite to pale yellow with a dark red spot at the base of each petal, and have a stout fleshy calyx at the base of 1 - 2 cm (0.39–0.79 in) wide, enlarging to 3–3.5 cm (1.2–1.4 in), the staminal column carries a large number of stamens with kidney – shaped anthers. The ovary is superior and crowned with five globular stigmas in

the middle of the stamina column. The fruit has five chambers and contains between 20-40 kidney –shaped brownish-red seeds, fleshy and bright red as the fruit matures. They take about six months to mature. Hibiscus sabdariffa is self-pollinated (Pursegloe, 1969).

1.7- Distribution

1.7.1- Distribution in the World and Africa

The plant of *Hibiscus sabdariffa* L. (kaekade) is considered one of the oldest and ancient crops planted in Africa and in Sudan especially, the extents that become folklore plant that is linked to customs and myths for many African tribes. It is an annual plant that grows in the tropical and semi- tropical areas. The original area of 'Karkade' growth is between the horizontal lines 30 north and south of the equatorial line in sandy lands with good drainage. It is known that many factors can affect the chemical composition of food among which is the geographic area. In Cameroon, *Hibiscus sabdariffa* is most cultivated in the northern region where 90% of roselle are produced, 4% in the East region, 3 % in the center region and 3 % in other regions (Bidima and Melou, 2009).

1.7.2- Distribution and economic importance in Sudan

The growth of this plant is concentrated in western Sudan and especially in the great Kordofan state in the traditional rainfall zone.Kordofan produce 95% of 'Karkade' production in Sudan, 'Karkade' is grown as an essential and principle monetary crop in eastern Kordofan that contains the cities of Um Ruaba and Al Rahad, this area is known to produce and export the highest quality types of 'Karkade' in the world.

The crop is also planted around Al Obeid and eastern Kordofan and the sandy areas of southern Kordofan. 'Karkade' is also planted around Al Fashir in northern

Darfur and around Nyala and Eldeain and Boram in southern Darfur (Mohamed, 2007).

The principal production area in the Sudan is eastern Cordovan sands in an area encompassing ELRahad and Ummruaba.'Karkade' is grown on smaller scale around El Obeid, in western Kordufan near ELfashir and Nyala. Marcezell, (1985) reported that the estimated cultivated area is 59.882 feddans, the calyces average annual production is about 1,100 tons. Sudan became the world major supplier of Roselle dried calyces in 1960.In 1968 about 1,500 metric tons were exported (Mclean, 1973). In 1996 the exported quantity amounted to 5,994 tons (Alshoosh, 1997).

Because of its increasing economic importance, the areas planted by 'Karkade' increased until it reached about 58800 feddans, as the areas planted in the seventies (22300-78444 feddans), and the eighties (47998-59882 feddans). The increase in area caused an increase in total production; it reached its peak in the year 99/2000 with the production of 26000 tons, in comparison with 454 tons, produced in the early sixties. In spite of the increase in cultivated areas, the production per feddan was low that caused the total production to be lower than the export needs. The average production /feddan throughout the last four decades did not exceed 40 kg /feddan and in this period the production fluctuated between 10-68kg andfeddan (Alawad, 2002).

Although the economic importance of this crop is known as it generates annually sizeable amounts of foreign currencies, yet it is still viewed as a marginal crop by the country official and farmers.

1.8- Roselle varieties

It is important to mention that the types of Sudanese 'Karkade' are considered one of the best quality 'Karkade' around the world. Two types of 'Karkade' are now

produced i.e. red ‘Karkade’ and lemon green ‘Karkade’. The red type is widely spread and the demand for it increases every year. The highest quality among all the types is Al Rahad, this type is produced in Al Rahad,U m Ruaba and other places beside it. Other reported that two botanical varieties were recognized; variety sabdariffa, branched with red or pale yellow calyces, grown fiber. The main types of ‘Karkade’ calyces locally known in the Sudan as EL Rahad and EL Fashir.

There are more than 300 species of hibiscus around the world, one of them is Roselle (*Hibiscus sabdariffa*Linn.), which is a member of the plant family Malvaceae (Ahmed and Nour, 1981).

There are two main types of Roselle are recognized Hibiscus sabdariffa var altissma and Hibiscus sabdariffa var. sabdariffa.

Altissima is nearly branchless and can grow up to 3 - 5 m in height, its flowers are yellow, and calyces are red or green with high fiber but not used for food. The other distinct type of *Hibiscus sabdariffa*, grows in a bush with many branches. The flowers are axillaries or in terminal racemes, the petals are white with reddish center at the base of the stamina column. The calyx enlarges at maturity. The more economically important is *var. altissima*, which is cultivated for its jute - like fiber in India, East Indies, Nigeria and South America, whereas var. sabdariffa is another distinct type of Roselle and is also widely exploited for its calyces and fiber (Abu – Tarboush*et al*., 1997).

Roselle can be found in almost all warm countries such as India, Saudi Arabia, Malaysia, Indonesia, Thailand, Philippines, Vietnam, Sudan, Egypt and Mexico (Abu – Tarboush*et al*., 1997; Mat Isa A., Isa and Abd Aziz, 1985; Rao, P.U. 1996).

1.9- Uses of Roselle

1.9.1- Food uses and nutritional value

The food uses and nutritional value of Roselle were reported by Duke (1983). Many parts of 'Karkade' including seeds, leaves, fruits and roots are used in various foods.'Karkade' fruits are best prepared for use by washing, then, making an incision around the tough base of the calyces below the bracts to free and remove it with the seed capsule attached. The calyces are then ready for immediate use. Among them,the fleshy red calyces are the most popular. They are used fresh for making wine, juice, ja, jelly, syrup, ice cream, cakes and flavors. It is also dried and brewed into tea Alawad (2002). The young leaves and tender stems of 'Karkade' are eaten raw in salads or cooked with meat and other vegetables. The seeds contain about 17% oil. Seeds are also used as an aphrodisiac coffee substitute and excellent feed for chickens'. In quantity, the red –calyces which contain antioxidant are rich in riboflavin, ascorbic acid niacin, carotene, calcium and iron that are nutritionally important.

Roselle locally known as, 'Karkade', is grown as a rain –fed crop for its calyces. The seeds byproduct, was reported to be a promising new source of protein (El-Adawy and Khalil, 1994).

Furundu, a meat substitute, is traditionally prepared by cooking the 'Karkade' seed and then fermenting it for 9 days. Physicochemical and functional properties of raw and cooked seed and of furundu ferments were analyzed by Yagoub*et al*. (2004). Furundu preparation resulted in significant changes in 'Karkade' seed major nutrients.

Total polyphones and phytic acid were also reduced. The increase in total acidity and fat acidity coupled with a decrease in pH indicates microbial hydrolysis of the

major nutrients; proteins, carbohydrates, and fats.*In vitro* digestibility of the seed proteins reached the maximum value (82.7%) at the sixth day of fermentation, but thereafter it significantly decreased.

In western Sudan, 'Karkade' is grown successfully as rain crop, not for its seeds but for its calyces which are widely used to prepare invigorating refreshing drinks .in addition, the water extraction of the calyces are eaten raw as salad or cooked and the seeds are roasted. The tender leaves are as well essentially consumed as a pot-herb and the seeds can be conveniently converted to acceptable meaty –tasting fermented food (Yagoub, 1998).

In western Sudan the seed is subjected to a solid-state fermentation (SSF) process to produce a meat-substitute food known as furundu, the (SSF) has been reported to improve the nutritional and sensory value of a wide variety of legumes and oil seeds (Desphande*et al*., 2000).

1.9.2- Medicinal Uses

'Karkade' is used in many folk medicines. It is valued for its mild laxative effect and for its ability to increase urination, which is attributed to two diuretic ingredients, ascorbic acid and glycolic acid. It is used as cooling herb because it contains citric acid, providing relief during hot weather by increasing the flow of blood to the skins surface and dilating the pores to cool the skin. The leaves and flowers are used as a tonic tea for digestive and kidney functions. Alotion made from 'Karkade' leaves is used on sores and wounds (Duke, 1983; Perry, 1980) showed that the plant extract decreases the rate of absorption of alcohol in India, Africa and Mexico, all above - ground parts of the 'Karkade' plant are regarded as diuretic, cholerectic, febrifugal and hypotensive, decreasing the viscosity of the blood and stimulating intestinal peristalsis. Pharmancognosists in Senegal

recommend ‘Karkade’ extract for lowering blood pressure in 1962, sharaf confirmed the hypotensive activity of the calyces and found them antispasmodic, anthelmintic and antibacterial as well as in (1964) found the aqueous extract was effective against Ascarisgallinarum in poultry. Three years later, sharaf and co-workers (1967) showed that both the aqueous extract and the coloring matter of the calyces are lethal to Mycobacterium tuberculosis .in experiments with domestic fowel, ‘‘Karkade’’ extract decreased the rate of absorption of alcohols and so lessened its effect on the system (Morton, 1987) in East Africa, the calyx’s infusion, called, Sudan tea, is taken to relieve coughs. ‘‘Karkade’ juice, with salt, pepper, asafetida and molasses is taken as a remedy for biliousness.

In many homes in south western Nigeria, it is prepared traditionally either by steeping over night or by boiling with wood ash to neutralize the anti-nutrient before using it to prepare soup, stew and sauces. The processing of foods can improve nutrition, safety and occasionally lead to the fermentation of anti-nutritional and toxic compounds (Finto, 1995) .in African countries it is considered as medicinal plant and used for the treatment of cough and for wound dressing.

1.10- Chemical Composition of ‘Karkade

1.10.1- Moisture Content

Generally, the moisture content varies according to humidity, cultivar, area and environmental factor. Ibrahim *et al*. (1971) reported that the moisture content was about 9-14% of the dry calyces. Ismail (1980) reported that the moisture content of dry calyces was 12%. While Adam (2005) found moisture content of calyces of 11%. Alshoosh (1997) reported that the moisture content of ‘Karkade’ calyces for different seasons ranged from 5.4% to 13.4%. According to Ojokoh, *et al*. (2005) moisture content of ‘Karkade’ calyces 11.1%.

1.10.2-Ash content

The ash content of foodstuff represents the inorganic residue remaining after the organic matter has been burned. Mclean (1973) reported that ash represents about 9% of the dry calyces and that the analysis of ash showed the presence of sodium, potassium, magnesium, aluminum, iron, sulfate, phosphate, chloride and carbonate. Ibrahim *et al.* (1971) reported arrange between 8-11 % 'Karkade' calyx's ash. While Adam, (2005) showed that the ash content was about 10% for 'Karkade'. The ash content of 'Karkade' calyces for different seasons ranged from 7.13% to 14.66% Alshoosh (1997). In addition,Ojokoh (2006) showed that the ash content of calyces was 12.8%.

1.10.3- Carbohydrates content

The content of carbohydrate ranged from 54.9% to 65.8% for different seasons (Alshoosh), 1997) Ibrahim *et al.* (1971) detected glucose and arabinose in the water extract of 'Karkade' from Sudan. According to Ojokoh*et al.* (2005) carbohydrate content of *calyces* 55.9%, USDA (2004) showed in every 100 grams of edible portion in 'Karkade' there 11.31 grams of Carbohydrate.

1.10.4- Fat content

Lipids are a group of heterogeneous compounds, which are classified together because of their solubility in organic solvents. This solubility differentiates them from other constituents such as protein carbohydrates and nucleic acid in seeds. They include free fatty acids, mono, di, tri-glycerides, phospholipids, sterolesters and glycerol.

Mclean (1973) reported that the seeds have been found to keep over a year without variation in the oil or fatty acid content. The oil also exhibited good stability in both free fatty acid content and color over 120 days' storage test. It is claimed to

have good cooking properties compared to cotton seed oil. The fat was determined as 0.16% for red calyces. Adam (2005) and Alshoosh (1997) showed that the fat content of 'Karkade' for different seasons ranged from 0.77% to 1.15%. Ojokoh (2005) reported that, the content of fat for calyces 3.9%.

1.10.5- Protein content

Protein is polymers of amino acids. Though there are hundreds of thousands of different proteins that exit in nature; they are all made up of different combinations of amino acids. Proteins are large molecules that may consist of hundreds, orthousands, of amino acids. In the study made by Ibrahim *et al*. (1971) 13 aminoacid were given by the protein hydrolyzates of the whole and spent calyces, six of which were essential amino acids. While the protein by hydrolyzed from the water extract gave only nine amino acids. Also showed that th

e protein content of the calyces of Sudanese cultivars ranged from 7.05-9.45% the protein content of calyces for different seasons ranged from 4.3% to 13.6% Alshoosh (1997). Ojokoh, (2006) reported that, the content of protein for calyces was 4.8%.

1.10.6- Organic Acids

Organic acids are classified chemically according to the number of carboxylic acid groups or according to other functional groups present.

Harborn (1973) reported that organic acids are water soluble colorless liquids or relatively low melting solids. The majority are non-volatile. Organic acids are important constituents of food products, not only as chelating agents for iron and copper but also for the control of PH and inhibition of enzymes (Sistrunk and Cash, 1973). 'Karkade' is characterized as a fruit higher rich in organic acids; oxalic, malic and succinic (Wong, *et al.*, 2002). Flavor characteristics of 'Karkade'

were found to be mainly in the acids content. The presence of citric, malic, latic, tannic, tartaric and oxalic acids have all been reported by Mclean (1973). Some of the organic acids shown to be present in aqueous extract of calices of 'Karkade' are the citric acid, ascorbic acid and hibiscus acid. Ibrahim *et al.* (1971) detected malic, citric, oxalic and ascorbic acid in the water extract of 'Karkade' calices. The two major acids in 'Karkade' calices as determined by Kafaga and Koch (1980) were citric and hibiscus. Alshoosh (1997) reported that hibiscus acid content of the calices of different lines of 'Karkade' ranged from 20.77% to 28.53% and also he found that citric acid content ranged from 17.43% to 21.84%. Hassan (1988) found that total acidity as citric acid for different lines ranged from 18.73% to 20.72%. Ismail (1980) found that the acid content of 'Karkade' calices was 20.90%.

1.10.7- Ascorbic Acid (Vitamin C)

Vitamin C (chemical names: ascorbic acid and ascorbate) is six carbon lactone which is synthesis from glucose by many animals.

Ascorbate is found in many fruits and vegetables. Citrus fruits and hibiscus are particularly rich sources. Diets with high vitamin C content have been associated with lower cancer risk, especially for cancers of the oral cavity, stomach, colon and lung. The populations at risk of vitamin C deficiency are those for whom the fruit and vegetable supply is minimal. Epidemics of scurvy are associated with famine and war, when people are forced to become refugees and food supply is small and irregular. Persons in whom the total body vitamin C content is saturated can subsist without vitamin C for approximately two months before the appearance of clinical signs, and as little as 6.5-10.0 mg/day vitamin C will prevent the appearance of scurvy (Finch *et al*.,1998).

This vitamin acts as a scavenger to harmful elements in the body .it is one of the powerful antioxidants, vitamin C neutralizes free radicals (harmful elements naturally occurring in the body and environment factors) it helps the cells and tissue against damage that could lead to disease, including cancer and heart diseases. Vitamin C also helps the body to fight infections (Block, 1991).

The presence of vitamin C in 'Karkade' has been confirmed by many analysts among them Mclean (1973) who showed that vitamin c concentration varies from 21 to 89.4 mg /100 g, in both fresh and dried calyces. Ibrahim *et al.* (1971) reported ascorbic acid in 'Karkade' water extract with concentration of 7.12 mg/100g. Alshoosh (1997) reported that the vitamin C in 'Karkade' calyces ranges from 92.06 to 97.26 mg/100g. And Goraphy (2001) recorded the range of vitamin C65.00 to 125.00 mg/100g.

1.10.8- pH value

PH can be viewed as an abbreviation for power of hydrogen or more completely, power of the concentration of the hydrogen ion .The mathematical definition op pH is a bit less intuitive but in general more useful.

PH of Roselle calyces varies between 2.57-2.80 was obtained from samples analyzed in the Sudan (Ibrahim *et al*., 1971). According to Alshoosh (1997) the pH of 'Karkade' calyces for seasons 93/94, 94/95 and 95/96 ranged from 2.95- 3.42, 2.4-3.42, 2.4-3.05 and 2.7-3.2 respectively.

1.10.9- Color

The pigments and color precursors of fruits and vegetables occur for the most part in the cellular plastid inclusion such as the chloroplasts and the other chromo lasts, and to a lesser extend dissolved in fat droplets or water within the cell protoplast

and vacuole these pigments are classified into four major groups, which include the chlorophylls, caroteiod, anthocyanins and anthoxanthins pigments.

Belonging to the latter two groups also is referred to as flavonoids, and includes the tannins (Norman, 1973). Natural plant produced anthocyanin pigments, the substances that are largely responsible for intense red to blue color in many foods and related flavonoid phytochemicals are considered to be responsible for a range of unique and broad – spectrum health benefits.

Anthocyanins are the most important and wide spread group of coloring materials in plants. These intensely colored water- soluble pigment are known to be responsible for nearly all the pink, scarlet, red, mauve, violet and blue colors in the petals and leaves of higher plants (Barouillard, 1982). Anthocyanins are glycosylated polyhydroxy and polymethoxy derivatives of 2-phenyl benzopyrylium (flavylium) salts.

1.10.10- Antinutritional value

As with other foodstuffs certain nutritional inhibitors and toxic substance are associated with ‘Karkade’. Anti- nutritional factors can be classified broadly as those naturally present in the plant) e.g. Anthocyanins is all based chemically on a single aromatic structure. In general, anthocyanidins do not accumulate in the plant and the pigments occur in flowers and fruits mainly in the glycosylated form (Harborn, 1967). Alshoosh (1997) showed that the color intensity in ‘Karkade’ range from 0.51 to 0.77.

1.10.10.1- Total polyphenols

Polyphenol refers to acomplex Family of phenolic compounds, which are widely distributed in plants, and the level of them varies classified into phenolic acids (simple phenols), flavonoides and tannins.

Simple phenols and flavonoides are relatively low molecular weight compounds, representing the vast majority plant phenolics that are mostly soluble. The free phenolic acids, however, are particular concern because of enzymatic oxidation to quinines and subsequent binding to Lysine and methionine inside chain of protein.

Polyphenols have several functions in plant; they have anti-pathogenic, anti-herbivore and allelopathic properties (Brice and Morrison, 1982; Ray and Hastings, 1992). Phenolic compounds are partially responsible for the sensory and antinutritional quality of plant foods (Bravo, 1998).

1.10.10.2- Tannins

Tannins, the polyphenolic compounds of higher plants have the ability to bind polymeric substrates like proteins, carbohydrates and reported to be toxic to gastrointestinal microorganisms (Bhat*et al*., 1998). The inhibitory effect of tannins has been shown to be due to the reducation of enzymes activity, days functioning (Goel*et al*., 2005). Based on their molecular structure differences and adverse hydrolytic relativities, tannins are classified into two groups:(i) hydrolysabletannins, and (ii) condensed tannins (proanthcyanidins), that are resistance to enzymatic degradation (Sosulski, 1979; Bravo, 1998). Hydrolysable tannins bind readily with proteins to from indigestible complexes, and they are thus considered effective antinutritional compounds for herbirous animals. Both groups of tannin are polymers of gallic or ellagic acid esterifies with acore molecule, commonly glucose or alcohol and the condensed tannins are flavonoid polymers (Salunkhe*et al.*, 1989). Reports are available that micro organisms can degrade hydrolysable tannins but there are evidences that these have the ability to tolerate condensed tannins (Krause *et al*., 2005).

Tannin content of ‘Karkade’ calyces ranged from 0.39 -0.5%, 0.38-0.57% and0.38-0.56% for season 93/94, 94/95 and95/96 respectively were reported by Alshoosh (1997).According to Ojokoh*et al.* (2005) tannin content of fermented calyces

using controlled fermentation ranged from 1.32-1.53 % and 5.3% for unfermented calyces. In addition,Ojokoh (2006) showed that the tannin content of fermented calyces was 1.21%, 1.32% for calyces fermented with wood ash and 5.3% for unfermented calyces. Moreover, the content of tannic acid of red calyces about 2 mg/100g, 2.33 mg/100 for red calyces soaked in wood and 1.4 mg/100g for green calyces soaked in wood ash (Adanalwo and Ajibade, 2006).

1.11- Composition of seed

The fat and protein contents of the seeds were 22.43% and 32.46%, respectively Apparent viscosity (at 20°C), 13.42 cps (Mahgouband Hayat, 2009).

Ghislain*et al.* (2014) postulated that roselle seed Crude protein (22 to 26), lipid (18 to 22), fiber (18 to 23) and ash content (4.3 to 6.4%). Roselle seeds contain about 14.9% protein, 21.2% crude fiber, 14.6% fats and oils, 35.6% carbohydrate. Roselle seed oil contains phytosterols and tocopherols (Nyam*et al.* 2009).

Roselle seeds cultivated in many countries such as Egypt, India, Mali, Malaysia, Nigeria, and Sudan have been found to contain high amount of protein, dietary fiber, lipids, and minerals (Samy, 1980; Rao, 1996; Fatoumata*et al.*, 2011; Hainida*et al.*, 2008; Balogun and Olatidoye, 2012; Abu El Gasim*et al.*, 2008).

The antioxidant and probiotic potential of Mbuja have been investigated by Mohamadou *et al*., (2007). However, little study has been done on the chemical composition and protein quality and digestibility of Roselle seeds cultivated in Cameroon. The whole seeds are said to have high protein value; however, these grains are not already used as source of protein in the food habits in Cameroon nevertheless these can be utilized as thickener agent in the sauce like soyabeans or groundnut usually used. The raw Roselle seeds are known to have bitter taste because of the anti-nutritional components like tannins. These anti-nutritional components can be in activated by processing methods such as moist heat

treatment, dry heat treatment and soaking in water (Price *et al*., 1979; Yacoub and Abdalla, 2007).

Roselle seed oil was extracted and characterized, and its physicochemical parameters are summarized: acidity, 2.24%; peroxide index, 8.63 meq/kg; extinction coefficients at 232 (k (232)) and 270 nm (k (270)), 3.19 and 1.46, respectively; oxidative stability, 15.53 h; refractive index, 1.477; density, 0.92 kg/L; and viscosity, 15.9 cP. Roselle seed oil belongs to the linoleic/oleic category, its most abundant fatty acids being C18:2 (40.1%), C18:1 (28%), C16:0 (20%), C18:0 (5.3%), and C19:1 (1.7%) (Mohamed *et al.*, 2007).

The physical properties of the oil extracts showed the state to be liquid at room temperature and indicated that the oil had refractive index, 1.4652.

Chemical prosperities of the seed oil are peroxide value, 3.15 (meq O2/kg oil); free fatty acid, 0.82%; iodine value, 97.78%; saponification value, 198.45 and viscosity, 15.15 (mPa.s at 25°C) (Bouanga-Kalou*et al.* 2011).

Roselle (*Hibiscus sabdariffa* L.) seed is a valuable food resource due to it is rich in protein content and micro nutrients. It is also an excellent source of fiber (Omabuwajo*et al.*2000). Roselle seeds contain 18.3 % of total dietary fibers (Hainida et al. 2008). Roselle seed might be useful as low cost source of dietary fiber substitute in dietary supplement or food ingredient in food industry.

According to Tosh and Yada (2010), the edible seeds from pulses are rich food source of dietary fibers that enhance various health benefits.

Roselle seeds can be considered good ratio of soluble to insoluble fiber fraction (Hainida*et al*., 2008).

Total carbohydrate was determined by difference. All determinations were done in triplicate. Total carbohydrate is obtained by difference. Total carbohydrate = 100 % − (%moisture+ %ash+ %protein+ %fat+ % crude fiber).

Nyam (2014) was found that proximate composition of Roselle seed powder contain 6.6% Moisture (%), 1.1 Ash (%), 13.0 Crude protein (%), 17.4 Crude fat (%), 24.7 Crude fiber (%) and 37.3 Total carbohydrate (%)

The moisture, ash and crude fiber contents were analyzed according to standard methods described in Association Organization American Chemist (AOAC) (2000). Total Carbohydrates were determined by difference. All the approximate analysis was carried out in triplicate and the results expressed as percentage of the sample analyzed.

El-Deab and Ghamr (2017) reported that Roselle seed composition is that protein content 23.25% crude fiber, 16.25%, ash content 5.73 fats and oils, 19.36%, carbohydrate 21.7.

And GC analysis showed five fatty acids were identified: palmitic, stearic, linolic, gamma-linoleic, and alpha-linoleic acid. The forms differed both in respect of the presence and the content of particular fatty acids (Kosakowska *et al*., 2005).

1.12- Oil Extraction

Variations in oil yields may be due to the differences in the extraction methods used and location of the plant (Eltayeib and AbdElaziz, 2014).

The yield of the extracted oil for both red and white was found to be 21.1%.

1.13- Oil content

The comparative study on the composition of fatty acids and sterols in the seed oil of four forms of Roselle (Hibiscus sabdariffa L.) cultivated in Egypt was carried

out. The total oil content in the seeds of the investigated forms ranged from 15.31 to 18.99 percent.

1.14- Composition of oil

This high value for proximate analysis make the Roselle seeds a rich source of nutrients. Major fatty acid found was Oleic acid (38.46%) followed by linoleic (33.25%), and palmitic (20.52%) (El-Deab and Ghamry, 2017).

The main unsaturated fatty acids in the oil are Oleic (47.0555%, 47.8868%), Linoleic (30.5836%, 30.7931%) and Elaideic acid (14.359%, 15.1603%) and the saturated acids are Palmitic acid (3.9494%, 3.9198%) and Myristic acid (1.9609%, 1.9845%) (Eltayeib and Abd Elaziz, 2014). These values were arranged for red and white calyces respectively. Comparing the study results with the standards and guideline of edible oils set by the FAO/WHO and the Sudanese Standard and the Metrology Organization (SSMO) the study recommended that the Roselle seed oil can be an economic source of healthy edible fat and for other food industry applications and suggest further study on the effect of storage time on the physicochemical characteristic of the oil (Eltayeib and AbdElaziz, 2014). Roselle seed oil were Myristic (1.9609%, 1.9845%) and palmitic (3.9494%, 3.9198%) acids and the main unsaturated fatty acids are oleic (47.0555%, 47.8868%), linoleic (30.5836%, 30.7931%) and elaidic (14.3159%, 15.1603%) for red and white Calyces (Eltayeib and AbdElaziz, 2014).

Eltayeib and AbdElaziz (2014) reported that Fatty acid in roselle seed oil for white calyces (1.9845) and Red calyces (1.9609), Myristic acid in roselle seed oil for white calyces (3.9198) Red calyces (3.9494), Palmitic acid for White calyces (30.7931) Red calyces (30.5836), Linoleic acid in Roselle seed oil for White calyces (47.8868) Red calyces (47.0555), Oleic acid for White calyces (15.1603)

Red calyces (14.3159), Elaidic acid in Roselle's seed oil for White calyces (0.2744) Red calyces (0.2518), Stearic acid in Roselle seed oil for White calyces (0.4404) Red calyces (0.2583), Arachidic acid in Roselle seed oil for White calyces (0.6735) Red calyces (0.7062) and C cis - 8,11,14 - Eicosatric acid for White calyces (0.8672) Red calyces (0.9185).

Major fatty acids were linoleic, oleic and palmitic (Al-Okbi*et al.*, 2017). Recently, Ali and Al-Anany (2017) reported the presence of lauric (1.25%) and linolenic (0.4%) in addition to the above mentioned major fatty acids. The variability in oil percentage and fatty acid**s** could be attributed to the method of extraction and or the geographic origin of the plant. The present results of fatty acids fall within the range of the aforementioned studies.

1.15- Oil characterization

The oil had a refractive index (1.467, 1.466), saponification value (189.7, 189.1), iodine value (119,119), peroxidevalue (4.6, 4.7), acid value (3.57, 3.55), viscosity (22.5, 22.5), specific gravity (0.90, 0.90). Eltayeib and AbdElaziz (2014) postulated that Physicochemical properties of Roselle seed oil for both White calyces Roselle and Red calyces Roselle (21.1%). Specific gravity for both White calyces Roselle and Red calyces Roselle (0.90), Refractive index for White calyces Roselle (1.446) and Red calyces Roselle (1.447), Viscosity for both White calyces Roselle and Red calyces Roselle (22.5), Saponification value for White calyces Roselle (189.1) and Red calyces Roselle (189.7), Acid value for White calyces Roselle (3.5533) and Red calyces Roselle (3.577), Iodine value for both White calyces Roselle and Red calyces Roselle (119), Peroxide value for White calyces Roselle (4.7) and Red calyces Roselle (4.6).

The physical properties of oil refractive index, specific gravity and color were tested the result were recorded 1.4643, 0.8985 and 0.067 at, respectively (El-Deab and Ghamry, 2017).

Also the chemical properties which are peroxide value, free fatty acids, saponification value, and iodine value were tested the results were recorded 1.2, 2.5, 192 and 103, respectively. Total unsaturated and saturated fatty acids shows that the crude oil had 73.40% and 26.57% respectively (El-Deab and Ghamry, 2017).

El-Deab and Ghamr (2017) reported that Roselle seed oil (21.1%). Specific gravity (g/cm^3) (0.8985), color at 420 nm (0.076), Refractive index (1.4643), Saponification (192.64), Iodine value (wijs) (103.00), Peroxide value (meo L kg) (1.96), free fatty acids (as oleic) and oxidative activity (h) (21.30).
The results presented in this article suggest that Roselle (*Hibiscus sabdariffa*) could be used as a source of protein, vegetable oil, crude fiber, high relative percentages of unsaturated fatty acid, natural antioxidants including phytosterols and tocopherols (El-Deab and Ghamr, 2017)

The major unsaturated fatty acids found in Roselle seed oil are oleic and linoleic acid. The presence of high linoleic acid shows that Roselle seed oil could be a good source of essential fatty acids.

The main oil constituents as described by El-Deab and Ghamr (2017) are Myristic (C14:0), Palmitic, (C16:0), Palmitoleic acid (C16:1), Stearic (C18:0), Oleic (C18:1), Linoleic (C18:2) Linolenic (C18:3), saturated fatty acids 25.27, UN saturated fatty acids 74.73 and Unsat. Sat. 2.96:1.

1.16- Oil uses

Roselle seeds are waste that is left behind during processing of Roselle for juices or other related products. Disposing of waste is highly undesirable both economically and environmentally (El-Deab and Ghamry, 2017).

Roselle (Hibiscus sabdariffa L.) is more than an eye-catching crop and has been used in number of dishes, beverages and conventional remedy of diseases for centuries. It is popular for its edible fleshy calyces and leaves that are used for making salads, tea, juices, jams, jellies, ice-cream, and many other products. In many countries of the world fresh calyces of Roselle are harvested to produce pro-health drink due to its high vitamin C and anthocyanins contents. But in Bangladesh the Roselle leaves and calyces are used as vegetables and its fiber is used as jute substitute. Roselle is also famous for its high nutritional and medicinal values. Nutritional analysis of the calyces of Roselle showed that they are high in calcium, iron, niacin and riboflavin. It is also a source of antioxidants, anthocyanins which acts as free radical scavengers and inhibit lipid per-oxidation. Consumption of Roselle products such as fresh juice, tea, jam, jelly or in the form of capsule rich in anthocyanin protect human body from the harmful reaction of free radical by antioxidant activity. Roselle is a multipurpose crop and has great potential to increase the income of farmers, producers, processors of Bangladesh by fetching higher market price both from export and local market (Islam et al., 2016).

1.16.1- Medicinal uses and health benefits

Roselle is a multipurpose plant and all above ground parts of Roselle is used as traditional medicine for the treatment of several diseases in Africa, Senegal, India, Thailand and Mexico (Ngamjarus*et al*. 2010). Many medicinal applications of the

Plant parts of Roselle have been reported in different countries of the world (Ageless, 1999; Lin *et al.*, 2011; Fullerton *et al*., 2011).

Roselle tea reduces the blood pressure in hypertensive and pre-hypertensive persons (Muhammad and Shakib 1995; Faraji and Tarkhani 1999).

Generally, Roselle is considered as traditional medicine for the remedy of diuretic, mild laxative, cancer, cardiac and nerve diseases. Every fraction of Roselle plants including leaves, fruits, roots, seeds are utilized in various foods. Among them, red fleshy calyces are employed for making fresh beverage tastes like Ribena, juice, jam, jelly, syrup, gelatin, pudding, wine, cakes, ice-cream and flavors and also dried and brewed into tea (Rao, 1996; Tsai *et al*., 2002).

Roselle seeds are used to produce biodiesel and also used as animal feed as the seeds contain 17.8 to 21% non-edible oil (Ahmed, 1980) and 20% protein (Ahmed and Nour, 1981).

1.16.1- Nutritional value

Roselle contains high amount of vitamin C and anthocyanins which makes it unique for nutritional characteristics. Nutritionists have reported that Roselle calyces are high in Ca, K, Mg, Na, niacin, riboflavin and iron. Nutritional composition of 100 g fresh Roselle calyces, leaves and seeds (Islam *et al.,* 2016).

Moisture (8.2 g), Protein (19.6 g), Fat (16.0 g), Fiber (11.0 g), Energy (411 kcal), Ash (7.00g). Calcium (356 mg), Phosphorus (462 mg), Iron (4.2 mg), Thiamine (0.1 mg), Riboflavin (0.15 mg), Niacin (1.4 mg), Ascorbic Acid (Carbohydrates10.00 g) (Leung *et al.* 1968; Duke and Atchley, 1984; Morton, 1987, Morton and Dowling, 1987)

Chapter Two

Materials and Methods

2.1- Materials

Roselle (*Hibiscus sabdariffa*) Seed were obtained from local market.

2.2- Methods

2.2.1- Sample Pre Treatment

The seeds were cleaned and crushed using mortar and pestle. They kept for further applications.

2.2.2- Approximate analysis

2.2.2.1- Moisture content

Moisture was determined according to method described by Association Organization American Chemist (AOAC) (2006) method. Three grams of well-mixed sample were weighed accurately in clean preheated dish of known weight by using sensitive balance. The uncovered sample and dish were kept in an oven provided with a fan at 105 ^{0}C and let to stay overnight. The dish was covered and transferred to desicator and weighed after reaching room temperature. The dish was again heated in the oven for another two hours and reweighed. This was repeated until constant weight was obtained. The loss of weight was calculated as present of sample weight and expressed as moisture content.

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

Where:

W_1= weight of sample before drying.

W_2= weight of sample after drying.

2.2.2.2- Crude oil:

Total fat was determined by Association Organization American Chemist (AOAC) (2006) method. Two grams of crushed seed sample were placed in extraction thimble and plugged by a piece of cotton, and then the thimble was placed in soxhlet extractor. A dry and accurately weighted flask was fitted to the extractor, then solvent (Normal Hexane) was poured into the flask, and then extractor, flask and condenser was fitted together. Water was allowed to flow through the condenser. Heat was applied from an electrical heater. The system allowed to continuous evaporation and Siphoning. Extraction period was 6 hours. After extraction period the solvent was distilled off and the flask with oil was dried in oven for 30 min at 100^0C, cooled in desiccators and weight.

The oil content was calculated according to the following equation:

$$\text{Fat content } \% = \frac{Wt_2 - W_{t1}) \times 100}{\text{SW}}$$

Where:

Wt_1= weight of empty flask.

Wt_2= weight of flask with extracted oil (after solvent evaporation).

SW= weight of sample.

2.2.2.3- Crude protein:

Nitrogen content determinations were made on the sample by micro kjeldahl apparatus following AOAC (2006) method. 0.2 gm of sample was weighed accurately into a micor kjeldahl flask, 0.4 gm of catalyst mixture (90% potassium sulphate and 10% cupric sulphate) and 3.5 ml of concentrated sulphuric acid were added, the flask was placed in the digestion equipment for 3 hours. Then the sample transferred to distillation flask; 20 ml of 40% NaOH were added to

distillation apparatus. The system brought to distillation. The ammonia evolved was received in 10 ml of 2% Boric acid solution. The trapped ammonia is titrated against 0.02 N HCL using universal indicator (methy1 red + bromocresol green).

$$\text{Nitrogen (N) \%} = \frac{\text{volume of HCL} \times 0.02\text{N} \times 14 \times 100}{\text{Sample weight} \times 1000}$$

Protein % = N% × 6.25.

2.2.2.4- Crude fiber:

Crude fiber was measured using Pearson method. Three grams of defatted sample were placed in 1 litter conical flask. Twenty ml of H_2SO4 (0.255 N) was added to the conical flask and placed on digestion apparatus with readjusted hot plate and boiled exactly for 30+2 min, it was rotated periodically to keep solids from adhering to sides, and water level was maintained in the flask by adding water. The conical flask removed and the content filtered through Buchner funnel using filter paper (What man No. 52). The conical flask rinsed with hot water several times and washed through buncher. The residue transferred to the conical flask and 200 ml of sodium hydroxide (0.313N) was added and allowed to boil for 30min. Then conical flask removed and filtered as above with filter paper (Whatman No.541). The residue first washed with enough 1% HCL to make the paper and contents acid (use indicator paper at funnel tip), and then with hot water was added to remove acid. Then wash with alcohol and diethy1 ether until substantially all the water removed. The air dried residue transferred to ashing crucible and dried to constant weight in drying oven, cooled in desicator and weighed, then ignited at 500 ^{0}C in muffle furnace. Then the ashed sample cooled in desicator and reweighed. Then the fiber content calculated as follow:

Crude fiber % = $\frac{\text{Loss of weight on ignition}}{\text{Weight of sample}} \times 100$

2.2.2.5- Ash content

Total ash was determined according to AOAC (2006) method. Three grams of well mixed sample were weight in porcelain crucible of known weight. The crucible ignited at 550 ^{0}C in a muffle furnace until light gray ash was obtained. The content of the crucible was cooled in desicator and weight soon after it reached room temperature. Percentage of ash calculated from the increase in the weight of the crucible. Ash content was calculated using the following equation:

$$\text{Ash content \%} = \frac{W_1 - W_2}{S} \times 100$$

Where:

W_1 = weight of the crucible with sample.

W_2 = weight of the empty crucible.

S = weight of sample.

2.2.3- Physicochemical properties of the oil

2.2.3.1- Specific gravity

The dry pycnometer filled with prepared sample in such a manner to prevent trap of air bubbles after removing the cap of the side arm. The stopper was inserted in the pycnometer immersed immediately in water bath 30.0 + 0.2 and holded for 30 minutes. Any oil came off 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 ensuring the temperature did not fall below 30 ^{0}C.

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

Where:

A: weight in gm of bottle with oil at 30 ^{0}C.

B: weight in gm of bottle at 30 ^{0}C.

C: weight in gm of bottle with water 30 ^{0}C.

2.2.3.2- Refractive index

The refractive index of the oil was determined by (AOAC, 1990). The refract meter was first adjusted at 1.3330 at 20^{0}C with pure distilled water as a blank reading. A drop of the fixed oil was placed in the instrument and telescope was adjusted so that the cross hairs were distinct and in focus. The adjustment of the knob was rotated until the lower part of the field was dark and the upper part was light and a clear definite boundary appeared. The coarse adjustment knob was moved first and then the fine adjustment knob until the boundary line coincided with the intersection of the cross hair in the telescope. The instrument was read when temperature is stable.

2.2.3.3- Viscosity

Viscosity was determined according to Diamante and Lan. The absolute viscosities of the different vegetable oils were determined using a Lamy Viscometer RM100 (Lamy, France), a rotating viscometer with coaxial cylinder. Approximately 25 mL of oil was placed in the Tube DIN 1 outer cylinder, and then the bob MK Din-9 was inserted. The radius of the tube (R_a) is 16.25 mm and the radius of the bob is (R_i) 15.5 mm. The length of the bob is 54 mm. The correct mode was set for the appropriate measuring system (MS 19) and the measurement time was fixed at 60 seconds. The torque of each sample at the

different temperatures was recorded at a range of shear rate (ϒ) from 64.5 to 4835 s^{-1}. All viscometric measurements of the samples were carried out in triplicate. Every replicate was run twice the mean torque value of the two runs was recorded for each replicate at a given shear rate. The shear stress was obtained from

$$T = \frac{1+\delta^2}{2\,\delta^2} \times \frac{M}{2\,\pi\,L R i^2}$$

Where:

T = shear stress (Pa), δ = ratio of to, R = radius of the tube (m), R_i= radius of the bob (m), L = length of the bob (m), and M = torque reading (N·m).

2.2.4. Chemical properties of the oils

2.2.4.1. Acid value

Acid value was determined according to Handbook of Food Analysis. The oil mixed thoroughly before weighing. About 5g of cooled oil sample accurately weighed in a 250 ml conical flask and 50 ml added to 100 ml of freshly neutralized hot ethyl alcohol and about one ml of phenolphthalein indicator solution. The mixture boiled for about five minutes and titrated while hot against standard alkali solution shaking vigorously during the titration. The weight of the oil taken for the estimation and the strength of the alkali used for titration shall be such that the volume of alkali required for the titration does not exceed 10 ml.

$$\text{Acid value} = \frac{56.1 \times V \times N}{W}$$

Where:

V = Volume in ml of standard potassium hydroxide or sodium hydroxide used

N = Normality of the potassium hydroxide solution or Sodium hydroxide solution; and

W = Weight in g of the sample

The acidity is frequently expressed as free fatty acid for which calculation shall be

Free fatty acids as oleic acid $= \frac{28.2 \times V}{W}$

Per cent by weight W

Acid value = Percent fatty acid (as Linoleic) × 2

2.2.4.2- Saponification value

Saponification value was determined according to ISO 3657: 2002. About 2.0g of sample were transferred into a 200mL conical flask. Twenty ml of 0.5 mole/L potassium hydroxide Ethanol, and fix a cooling pipe to the flask. The flask gently heated and occasionally shaked while adjusting the heat so that Back flow ethanol will not reach the top of cooling pipe. After heated for 30 minutes, immediately cooled, and titrated with 0.5mol / L HCl before the test liquid is solidified. Blank test performed for 3 times to obtain mean value of titration volume of 0.5mol/L hydrochloric acid.

The saponification was calculated as followed:

Saponification value (mg / g) = $\frac{W2 - W_1}{S} \times 28.05$

Where:

W_2 = Volume of titrated 0.05 N (KOH) with sample

W_1 = Volume of titrated 0.05 N (KOH) with blank

S = Sample size (g)

2.2.4.3- Peroxide value

Peroxide value was determined according to ISO 3960: 2007. Five g of the sample were delivered into a conical flask with stopper. Thirty mL of solvent were added and gently shake to dissolve the sample completely. The air inside flask gently replace with nitrogen to remove remaining oxygen. Five ml of saturated potassium iodide were added and immediately seal the flask and gently shake it for one minute. The flask left at room temperature 20°C in a dark room. Thirty mL of pure water were added, and the flask sealed and stirred. Titration with 0.01mol/L sodium thiosulfate was performed to measure peroxide value. Likewise, perform blank test to obtain blank level.

The peroxide value was measured as followed:

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

EP1 ：Titration volume (mL)

BL1 ：Blank level (0.00mL)

TF ：Factor of reagent (1.006)

R ：Constant (10)

SIZE ：Sample size (g)

2.2.5- GC- MS Analysis of the oil

Two ml of the sample was mixed throughly with 7 ml alcoholic sodium hydroxide (NaOH) that preparec by dissolving 2 g in 100 ml of methanol. Seven ml of a coholic sulfuric acid (1 ml H_2SO_4 to 100 ml methanol) was then added. The mixture was then shaked for 5 minute. The content of the test tube was left to stand over night. One ml of Super saturated sodium chloride was then added and the content being shaken. Two ml normal hexan was added and the contntent were

shaked throughly for three mintues. Then the n-hexane layer (the upper layer of the test tube) was taken using disposable syringe. Five microliter from the n-hexane extract was diluted with 5 ml of diethyl ether. Then the mixture was filtered throgh syringe filter 0.45 micro meter and dried with 1 g of un hyrous sodium sulphate as drying agent and I micro liter of the diluted sample was injected in the GC- MS instrument.

2.2.5.2- Method of analysis

The qualitative analysis of the sample was carried out by using GC-MS technique model (GC/MS-QP 2010 – Ultra) from Japan, Simadzu company, with serial number 020525101565 SA and capillary column (Rtx – 5ms-30m x 0.25 mm x 0.25 µ m). the sample was injected by using spilit mode, helium as a acarrier gas passed with flow rate 1.61 ml/min, the temperature programe was started from 60°C with at flow rate 10c/min, to 300 °C as final temperature degree with three hold time, The injection port temperature was 300 °C, the ion source temprerature was 200°C and interface temperature 250°C. The sample was analysed by using scan mode in the range m/z 40-500 charges to ratio and the total run time was 27 mimute. The identification of components for the sample was achieved by comparing their retention index and mass fermentastionpatents with those available in the libarary, the National Institute of Standards and Technology (NIST). Resuts were recoded.

2.2.6- UV Analysis

One oil drop was taken and placed in beaker of 100 ml then 5 ml of n-hexane were added and shacked thoroughly to reach solution consistency. The cell was filled with n-hexane and inserted in the device and the resultsrecoded. The cell was

emptied and was read in the instrument at wave length 200 - 800 nm). The absorptions results recoded.

2.2.7- IR Analysis

One drop of the oil was put in disk of (KBr), then another disk of (KBr) was used above the one to give filmy and planer surface, then it was put in holder and was read in the IR instrument.

Chapter Three

Results and Discussion

3.1- Approximate analysis of Roselle seeds

The approximate analysis of Roselle seeds was illustrated in Table (3.1). The results displayed that moisture content (5.6%), ash content (4.2%), fiber content (20 %), oil content (20 %), protein content (28 %) and carbohydrates (22.2 %)

Table (3.1): Approximate analysis of Roselle seeds

Parameters	Values
Moisture content (%)	5.6
Ash content (%)	4.2
Fiber content (%)	20
Oil (%)	20
Protein (%)	28.00
Carbohydrates (%)	22.2

Roselle seedsprotein higherthan that found by Soheir and Heba (2017) (14.9%) protein, while crude fibre was near to Soheir and Heba (2017)21.2% crude fiber. Oil content were lesser than Soheir and Heba (2017) findings 14.6% and carbohydrate were found to be lesser than Soheir and Heba (2017)35.6%.

3.2- Physical properties of Roselle seed oil

The physical properties of Roselle seeds oil were illustrated in Table (3.2). The results showed that density was (0.9063), refractive index (1.4700) and viscosity (57.6).

Table (3.2). Physical properties of Roselle seed oil

Parameters	Results
Specific gravity	0.9063
Refractive index	1.400
Viscosity (cP)	57.6

3.3- Chemical properties of Roselle seeds oil

The Chemical properties of Roselle seeds oil were illustrated in table (3.3). The results showed that acid value was (4.0), Saponification value was (216), Peroxide value was (7) and ester value was (212).

Table (3.3). Chemical properties of Roselle seed oil

Parameters	Results
Acid value (mgKOH/g oil)	4
Saponification value	216
Peroxide value (meq O2/Kg oil)	7
Ester value	212

3.4 - Characterization of Roselle seed oil

UV curved was obtained by using Roselle oil in the range of (205, 233 and 272 nm). This indicates the oil can be used as protective agent against sun radiations table (3.4). This indicates that the medium UV rays absorbed by Roselle oil can protect skins from cancer and sun scalds when the oil was used.

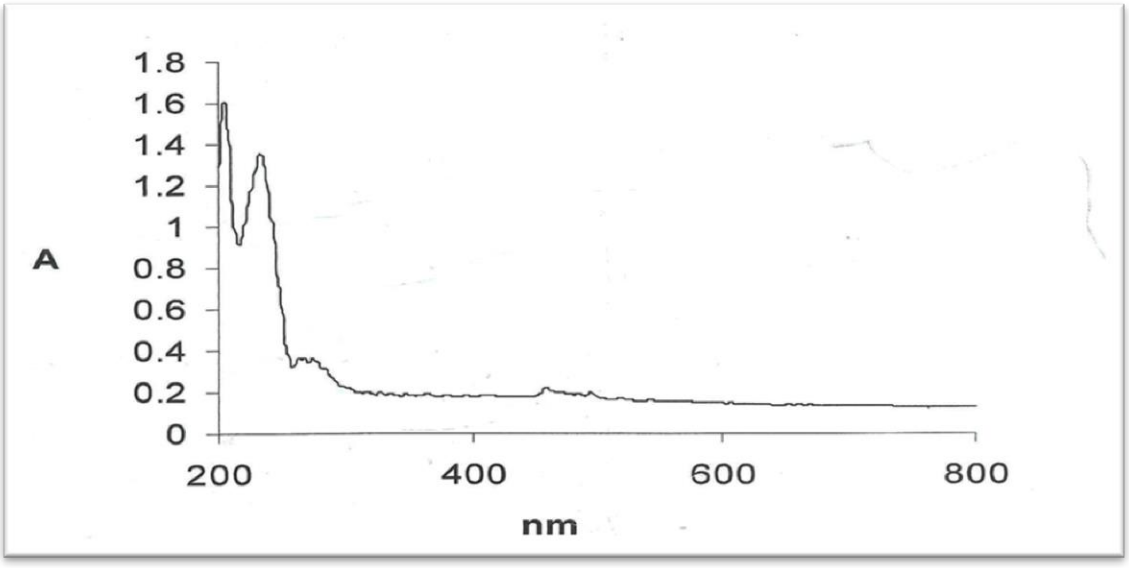

Figure (3.1) UV-absorption of Roselle seed oil

Table (3.4): UV absorption of Roselle seeds oil:

nm	Absorption
205	1.604
233	1.354
274	0.367

3.5- IR characterization of Roselle seeds oil

The IR characterization of Roselle seeds oil was illustrated in Figure (3.2). The results showed the presence of (2924.91 and 2857.08) asymmetric and symmetric C-H stretching for SP^3 hybridized system. C = O ester at 1745.14, C-H of (CH_3) (CH_2) bending and Rocking of (CH_2) n at 722.42. 3004.96 C-H stretching for unsaturated system. (1457.28, 1370.29, 1236.27 and 1162.67) C-H pending and C-O stretching

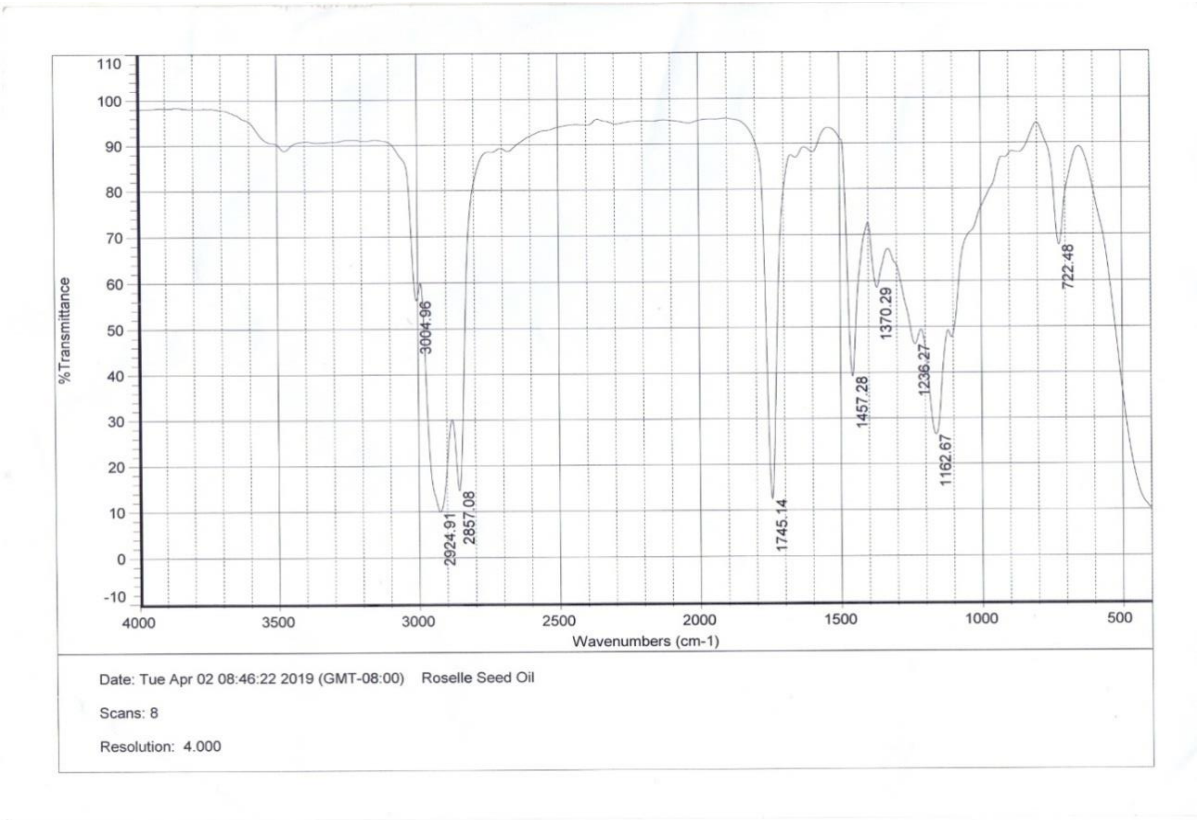

Figure (3.2). FTIR spectrum of Roselle seeds oil

3.6- GC- MS

GC - MS analysis of Roselle was showed major components oil (More than 10%) such as Hexadecanoic acid, methyl ester, 9, 12 – Octadecadienoic acid (Z, Z) -, methyl ester and 9 – Octadecenoic acid (Z) - , methyl ester (Table 3.5) and appendix (i).

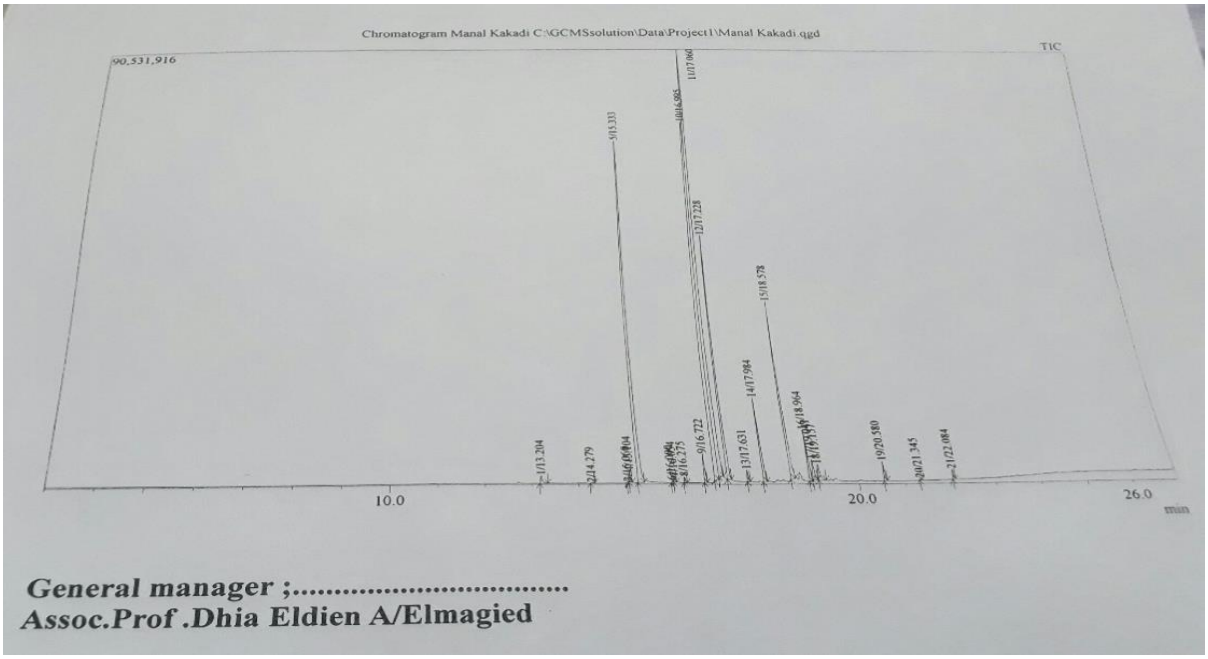

Figure (3.3): GC- MS Characterization of Roselle seeds oil

Table (3.5): Major components of Roselle oil (More than 10%)

Peak	Area %	Name
5	19.69	Hexadecanoic acid, methyl ester (olic acid)
10	28.57	9, 12 – Octadecadienoic acid (Z, Z) - , methyl ester (linoleic acid)
11	28.01	9 – Octadecenoic acid (Z) - , methyl ester (linolenic acid

Table (3.5): Major components of Roselle oil (More than 10%)

While the minor components of Roselle oil (1 - 10%) were Methyl stearate, 10 - Nonadecenoic acid, methyl ester, Cyclopropaneoctanoic acid, 2- [[2-[(ethyl, Eicosanoic acid, methyl ester and Tetracosanoic acid, methyl ester (Table 3.6) and appendix (i).

Table (3.6): Minor components of Roselle oil (1- 10%)

Peak	Area %	Name
12	8.52	Methyl stearate
14	2.61	10 – Nonadecenoic acid, methyl ester
15	6.05	Cyclopropaneoctanoic acid, 2-[[2-[(ethyl ester)]]]
16	1.54	Eicosanoic acid, methyl ester

Minor components of Roselle oil (Less than 1%) are Methyl tetradecanoate / Pentadecanoic acidmethyle ester/ 7-Hexadecanoic acid, methyl ester,(z)-/ 9-Hexadecanoic acid, methyl ester,(z)-/ 9-12 Hexadecanoic acid, methyl ester/ Cis - 10 - 6 - Hexadecanoic acid, methyl ester/ Hepadecanoic acid, methyl ester/ 8, 11 – Octadecadienoic acid, methyl ester/ Methyl 2- Octylcyclopropene- 1 – ocytanoate/ PGH1, methyl ester/ 7- Tetradecenal. (Z)- Docosanoic acid, methyl ester/ Tricosanoic acid, methyl ester (table 3.7) and appendix (i).

Table (3.7). Trace components of Roselle oil (less than 1%)

Peak	Area %	Name
1	0.35	Methyl tetradecanoate
2	0.02	Pentadecanoic acid, methyle ester
3	0.03	7-Hexadecanoic acid, methyl ester,(z)
4	0.31	9-Hexadecanoic acid, methyl ester,(z)
6	0.05	9-12 Hexadecanoic acid, methyl ester
7	0.20	Cis - 10 - 6 - Hexadecanoic acid, methyl ester
8	0.19	Hepadecanoic acid, methyl ester
9	0.88	8, 11 – Octadecadienoic acid, methyl ester
13	0.33	Methyl 2- Octylcyclopropene- 1 – ocytanoate
17	0.89	PGH1, methyl ester
18	0.80	7- Tetradecenal. (Z)
19	0.58	Docosanoic acid, methyl ester
20	0.04	Tricosanoic acid, methyl ester
21	0.32	Tetracosanoic acid, methyl ester

Conclusion

The Roselle seed oil was extracted with solvent extraction with hexane at $80^{o}C$ for 6 hour and found that:

Approximate analysis showed that Roselle seeds contain high amount of fiber and protein.

From UV analysis the oil can be use in cosmetics as protective agent from cancer and sun scalds.

The GC-MS study 21components fatty acid composition as olic acid (19.69 %), linoleic acid (28.75 %) and linolenic acid (28.01 %).

The presence of high linoleic acid shows that Roselle seed oil could be a good source of essential fatty acids and the stability of the oil is high and may be use as anti oxidant by blending with other vegetable oil.

Recommendations

The following are suggested for further work in this field:-

To encourage the research of other possible part of Roselle that contain appreciate percentage of oil such as leaf.

To study the possibility to utilize this oil in pharmaceutical applications.

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Appendices:

Appendix (i)

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	13.204	3590039	0.35	Methyl tetradecanoate
2	14.279	184695	0.02	Pentadecanoic acid, methyl ester
3	15.060	265874	0.03	7-Hexadecenoic acid, methyl ester, (Z)-
4	15.104	3187123	0.31	9-Hexadecenoic acid, methyl ester, (Z)-
5	15.333	199997877	19.69	Hexadecanoic acid, methyl ester
6	16.000	519824	0.05	9,12-Hexadecadienoic acid, methyl ester
7	16.054	2065020	0.20	cis-10-Heptadecenoic acid, methyl ester
8	16.275	1939529	0.19	Heptadecanoic acid, methyl ester
9	16.722	8959399	0.88	8,11-Octadecadienoic acid, methyl ester
10	16.995	290152820	28.57	9,12-Octadecadienoic acid (Z,Z)-, methyl e
11	17.060	284453747	28.01	9-Octadecenoic acid (Z)-, methyl ester
12	17.228	86558710	8.52	Methyl stearate
13	17.631	3338474	0.33	Methyl 2-octylcyclopropene-1-octanoate
14	17.984	26490770	2.61	10-Nonadecenoic acid, methyl ester
15	18.578	61429268	6.05	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethyl
16	18.964	15637797	1.54	Eicosanoic acid, methyl ester
17	19.041	9029752	0.89	PGH1, methyl ester
18	19.157	8171852	0.80	7-Tetradecenal, (Z)-
19	20.580	5887221	0.58	Docosanoic acid, methyl ester
20	21.345	447127	0.04	Tricosanoic acid, methyl ester
21	22.084	3286304	0.32	Tetracosanoic acid, methyl ester
		1015593222	100.00	

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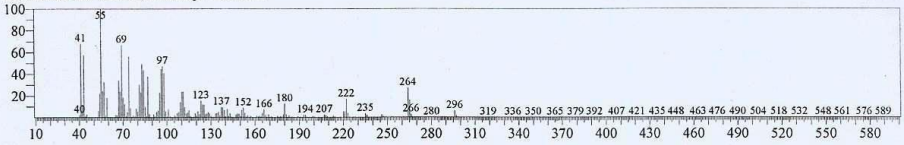

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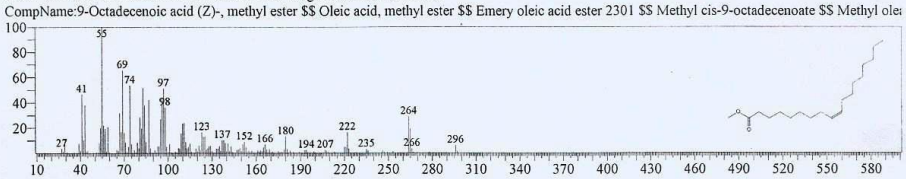

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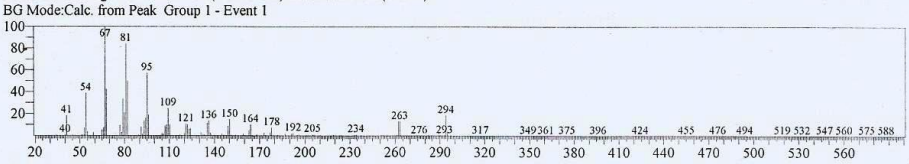

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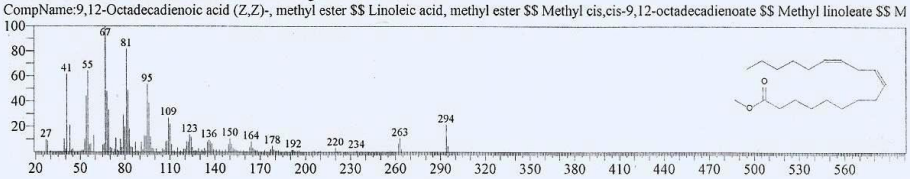

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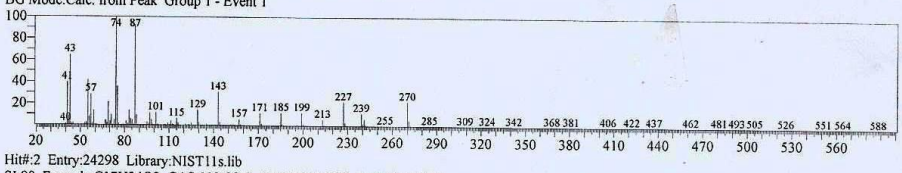

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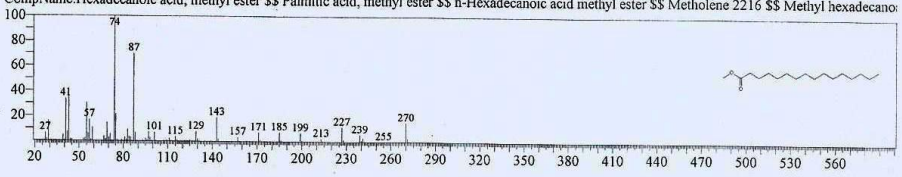
SI:90 Formula:C17H34O2 CAS:112-39-0 MolWeight:270 RetIndex:1878

CompName:Hexadecanoic acid, methyl ester $$ Palmitic acid, methyl ester $$ n-Hexadecanoic acid methyl ester $$ Metholene 2216 $$ Methyl hexadecanoa



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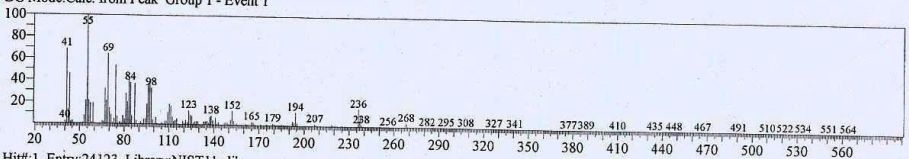

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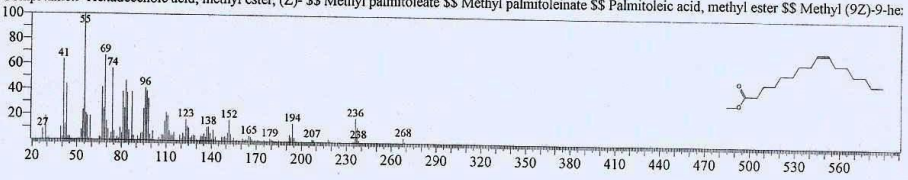

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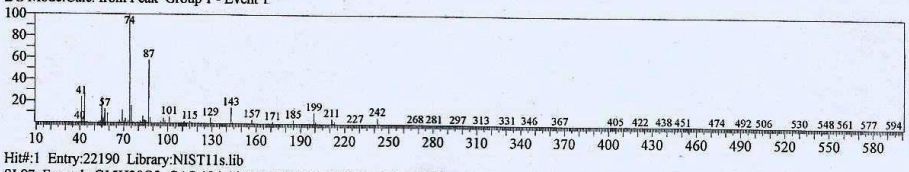

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