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Chemical and Physicochemical characteristics of Roselle seeds oil

(*Hibiscus sabdariffa L.*)

الخواص الكيميائية و الفيزوكيميائية لزيت حبوب الكركدى

A dissertation Submitted To Sudan University of Science and Technology in partial fulfillment of the degree of B.Sc. (Honours) in Food Science and Technology

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الآية

قال تعالى:

(أَلَمْ تَرَ كَيْفَ ضَرَبَ اللَّهُ مَثَلًا كَلِمَةً طَيِّبَةً كَشَجَرَةٍ طَيِّبَةٍ أَصْلُهَا ثَابِتٌ وَفَرْعُهَا فِي السَّمَاءِ (24) تُؤْتِي أُكُلَهَا كُلَّ حِينٍ بِإِذْنِ رَبِّهَا وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ لَعَلَّهُمْ يَتَذَكَّرُونَ (25)

صدق الله العظيم

سورة إبراهيم الآيات (24-25)

DEDICATION

To our Families,

Teachers

.... and Friends

With respect.

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Prayers and thanks to **Allah**, who gave us good health and support to accomplish this study.

We are grateful thanks to our supervisor **Dr. Mahdi Abbas Saad Shakak** who was too patient with us during this study, also for his unlimited assistance and guideness.

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Abstract

This study was conducted at Sudan University of Science and Technology, college of Agricultural Studies, Department of food Science and Technology in order to recognize rosella seed oil and determine Physicochemical characteristic.

The seeds were cleaned and crushed for proximate analysis which include oil content , protein content , moisture content , total ash , crude fiber, and carbohydrate , then the results were recorded 21.1 , 27.1 , 7.2 , 6.7, 16.2 , 21.7 respectively.

The oil was extracted by solvents extraction, the physical properties of oil refractive index , moisture content , specific gravity and color were tested the result were recorded 1.4643 , 2.07 , 0.8985 yellow 70 red 2.9. respectively.

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, 103, respectively.

Rosella seed oil contain great amount of oil with good physical and chemical characteristics. It could be one of the promising source of vegetable oil production in Sudan.

المـــلخـص

تم اجراء هذه الدراسة بجامعة السودان للعلوم و التكنولوجيا كلية الدراسات الزراعية قسم علوم و تكنولوجيا الأغذية للتعرف على زيت حبوب الكركدى و تقدير خواصة الفيزكوكيميائية.

تم تنظيف الحبوب و كسرها لإجراء التحليل الكيميائي الذى يتضمن محتوى الزيت ، محتوى البروتين ، محتوى الرطوبة ، الرماد الكلي ، الالياف الكلية و الكربوهيدريتات. النتائج المتحصل عليها كانت كالاتي 21.1 ، 27.1 ، 7.2 ، 6.7 ، 16.2 ، 21.7 على التوالى.

تم استخلاص الزيت عن طريق المزيبات ،حيث كانت الخواص الفيزيائية كالاتى معامل انكسار ، رطوبة الزيت ، و الكثافة النوعية و تم اجرائها و النتائج المتحصلة كانت كالاتى 1.4643 ، 2.07 ،0.8985 ، اصفر 70 أحمر 2.9 على التوالى. ايضا الخواص الكيميائية قيمة البيروكسايد الاحماض الدهنية الحرة ، قيمة التصبن و القيمة اليودية كانت نتائجها كالاتى 1.2 ، 2.5 ، 192 ، 103 .

حبوب الكركدى تحتوى على كمية كبيرة من الزيت بخصائص كيميائية و فيزيائية جيدة و يمكن ان يكون مصدرا واعدا لإنتاج الزيوت النباتية في السودان.

TABLE OF CONTENTS

LIST OF TABLES

Chapter One

1.1 Introduction

Hibiscus sabdariffa L. (Roselle) a member of family Malvaceae; is cultivated in the tropical and subtropical regions for multipurpose uses. It is well known in Sudan with the name of Karkadeh". Roselle seeds have an economic value as a source of oil where it reaches 20% (Al–Wandawi, *et. al.* 1984). There is no doubt that the need of traditional edible oils will be increased due to the growth of population over the world and increases their demand. Therefore, current work on renewable resources in the oils and fats are required to increase the quantity and quality of edible oils. The Roselle plant grows in Upper Egypt and is capable to grow under special conditions to produce seeds. The fatty acid profile of karkade indicates its importance for human consumption as well as its suitability for vegetable oil processing (El–Sayed 1998). He reported that the Roselle seeds might be provided as a new source of edible oils.

Roselle, *Hibiscus sabdariffa L.*, (Family Malvaceae) is one of the most important and popular medicinal plants. This crop is cultivated extensively present in India, Thailand, Sengal, Egypt and Sudan for its pleasant red coloured calyxes, which used for making jams, jelly's and bottled drinks. The yield of seeds reported to be (500–1000 kg/acre) (Abu-Tarboush 1996 and Al-Wandawi *et.al*. 1984). Its seeds contain a substantial amount of oil that resembles cotton seed oil (Ahmed and Hudson, 1979 and Ahmed, 1980). Furthermore, seed is an excellent source of proteins (25.2%) they also contain protease inhibitors, phytic acid and gossypol. However, this should not pose a problem in human nutrition if the seeds are properly processed (Abu-Tarbush and Ahmed 1996)

The fat quality is usually valued according to the content of essential fatty acid such as linolic, linolenic and arachidonic. Humans required some of these fatty acids in the diet to prevent fatty acid deficiency diseases including skin lesions, poor hair growth and low growth rate (Kinsella, 1987). These qualities are well presented in *Hibiscus sabdariffa* L. fatty acids.

1.2 Objectives of the study

1. To determine the chemical composition of roselle seeds.
2. To study the chemical and physicochemical properties of roselle seeds oil.

Chapter Two

Literature Review

2.1 Botanical classification

Kingdom: Planate (Plants)

Subkingdom: Tracheobionta (Vascular plants)

Superdivision: Spermatophyte (Seed plants)

Division: Magnoliophyta (Flowering plants)

Class: Magnoliopsida (Dicotyledons)

Subclass: Dilleniidae

Order: Marvels

Family: Mavaceae (Mallow family)

Genus: *Hibiscus L.* (Rosemallow)

Species: *Hibiscus sabdariffa L*

Genus *Hibiscus* which belongs to Malvaceae has more than 300 known species which are used as ornamental plants. It can grow up to 5-7 feet in height, with lobed leaves sometimes used for greens. The narrow leaves and stems are reddish green in color. The main edible part is the fleshy sepal, called a calyx, surrounding the seed boll in the flower. The size of the calyx varies with each variety, but ranges from ½ to 1 ½ inches in diameter (James, 1994). The origin of *Hibiscus sabdariffa* is not fully known, but it is believe to be native from India to Malaysia, where it is commonly cultivated, and must have been carried at an early date to Africa. It has been widely distributed in the Tropics and Subtropics of both

hemispheres, and in many areas of the West Indies and Central America has become naturalized. It was first introduced to West Indies and cultivated mainly as an ornamental plant.

Hibiscus sabdariffa L is relatively a new crop in Malaysia. It was introduced into Malaysia in early 1990s. Its commercial planting was first promoted by the Department of Agriculture in Terengganu in 1993 and has now spread to other states. Presently, the planted area is quite small approximately 150 ha (Osman *et.al*. 2011).

2.2 Roselle names

Roselle (*Hibiscus sabdariffa L*.) is known in different countries by various common names, including roselle, razella, sorrel, red sorrel, Jamaican sorrel, Indian sorrel, Guinea sorrel, sour-sour, and Queensland jelly plant (Mahadevan, Shivali, 2009 and Morton, 1987). In English-speaking countries it is known as roselle, Jamaican sorrel, red sorrel, Indian sorrel, rozelle hemp, natal sorrel and roselle. The Japanese name is rohzelu; also sabdriqa or lalambari in Urdu; and lal-ambari, patwa or laalambaar in Hindi (Kays, 2011). In French, roselle also is the word for the redwinged thrush. In Switzerland, the edible calyx is called Kerkrade. The roselle fiber is called India roselle hemp, roselle fiber, roselle hemp or Pusa hemp. Vernacular names for roselle include roselle, jelly okra, lemon bush, and Florida cranberry (Small, 1997).

2.3 Origin of roselle

Roselle may have been domesticated in western Sudan before 4000 BC; (Wilson, and Menzel, 1964). it was first recorded in Europe in AD 1576. It seems to have been carried from Africa to the New World by slaves for use as a food plant. Roselle was called Jamaican sorrel in 1707 in Jamaica, where the regular use of the calyces as food seems to have been first practiced (Lainbourne, 1913). The use of

the plant as “greens” was known in Java as early as 1658 (Lainbourne, 1913 and Wester, 1912). Taken to the New World, roselle was cultivated in Mexico, parts of Central America, the West Indies, and in southern Florida, Texas and California in the late 19th century. It is now grown for culinary purposes in much of the tropical world. The use of *Hibiscus sabdariffa* for fiber seems to have developed in regions other than Africa (Wilson, and Menzel, 1964).

Most breeding of Roselle has been for its fiber yield (Duke, 1993). Sudan is presently the major producer of Roselle; however, farmers regard it as a famine food. When drought is expected, farmers prefer to cultivate roselle rather than cereals because of its hardiness under adverse conditions (Mohamad, *et.al*, 2002).

Roselle is grown for its calyces, which are exported from the Sudan, China and Thailand, and it is also grown for its calyces in Mexico. In the Sudan it is collected by goat-herding nomadic tribes, but the product is frequently inferior because of poor processing conditions. Nevertheless, the Sudanese product is attractively bright red, very acidic, and it is extremely popular in Germany, which imports most of the crop. Export prices for the 1992–93 season for Sudanese, Chinese and Thai Roselle was of the order of $US1700.00/t (Duke, and duCellier, 1993).

Karkade (in Arabic) is grown in various parts of the Sudan, particularly Kordofan and Darfur. It is one of the cash crops cultivated by traditional farmers in Kordofan and Darfur States under rain-fed conditions, where large quantities are produced both for local consumption and for export. The total area under cultivation was estimated at 290,000 feddans (approximately 121,800 ha) in the 2000/2001 season, compared with 22,300 to 78,444 feddans (approx. 9370–32,950 ha) in the 1970s and 47998 to 59882 feddans (approx. 20,160–25,160 ha) in the 1980s. The increased area raised production from 454 tons in the 1960s to 26,000 tons in the 1999/2000 season (El-Awad, 2001).

Roselle is an important cash crop and a source of income for small farmers in Western Sudan, especially in North Kordofan State. The crop is grown mainly by traditional farming methods, exclusively under rain-fed conditions (El Naim, and Ahmed, 2010).

China and Thailand are also major producers, and control much of the world's supply. Thailand has invested heavily in Roselle production and their product is of superior quality, whereas China's product, with less stringent quality control practices, is less reliable and reputable. The world's best Roselle comes from the Sudan, but the quantity is low, and poor processing hampers quality. Mexico,

Egypt, Senegal, Tanzania, Mali and Jamaica are also important suppliers but production is mostly used domestically (Mohamad, *et. al*. 2002).

2.4 Roselle description and type

Roselle is an annual erect shrub that takes five months from planting to harvesting; it can also be regarded as a perennial .Species grown for their fiber are tall, with fewer branches, sometimes growing to more than 3–5 m in height (Wester, 1920) . Culinary varieties are many-branched, bushy, and generally 1–2 m tall. Stems may be green or red, depending on the seed source. Roselle has a strong taproot. The young plants have leaves that are unlobed , but as the plant grows the later-developing leaves are shallowly to deeply palmate, 3- or 5-parted (sometimes 7-parted). The large flowers have pale yellow petals (sometimes suffused with pink or red) and a dark red eye (Abdallah *et. al*. 2011).

The flowers are usually borne singly in the leaf axils. The sepals at the base of the large flowers and fruit vary from dark purple to bright red (sometimes white) at maturity, and are quite fleshy. The calyx increases from 1 to 2 cm in length before the flower is fertilized, then to about 5.5 cm (Occasionally longer) at mature (duCellier et. al. 1993). Some forms of roselle contain a pigment that gives a

brilliant red color to culinary products made from the plant; other forms are completely green. Edible types of Roselle are usually succulent, have well-developed lateral branches, and lack a hairy covering (Duke *et. al*. 1993).

Flowering is induced as the days become shorter and the light intensity decreases, beginning in September or later depending on the country. Flowers are red to yellow, with a dark center containing short peduncles, and have both male and female organs. The seed pods begin ripening near the bottom and proceed to the top. In Sudan, growers sometimes allow the seed to completely ripen and let the leaves drop prior to harvest (Small *et.al*, 1997).

There are two main types white, red Kerkrade. The sensory evaluation of cold and hot drinks made from both white and red Kerkrade revealed that there was no significant different as regard to the overall preference (Abdullah *et. al*. 2011).

2.5 Climate

Roselle requires a monthly rainfall ranging from 130–250 mm in the first three to four months of growth. Dry weather is well tolerated, and is desirable in the latter months of growth. Rain or high humidity at harvest and drying times can downgrade the quality of the calyces and reduce the yield (Bahaeldeen *et.al*. 2012).

2.6 Planting of roselle

Roselle is very sensitive to changes in the length of day. This photoperiodism requires the planting time to be set according to the length of the day rather than rainfall requirements. It is a deep-rooted crop, therefore deep plowing is recommended in preparing the seedbed. Seeds are planted at a rate of 6–8 kg/ha and approximately 2.5 cm deep. Seeds are usually planted at the beginning of the

rainy season, 60 cm – 1 m between rows and 45–60 cm apart. The reduced planting rate produces a larger calyx. Sowing is done by hand or using a modern grain drill. A good alternative tool would be a corn planter small enough to accommodate the hibiscus seeds. Thinning is also done by hand. There are over 100 cultivars or seed varieties of (Plotto *et*.a*l*. 2004).

Hibiscus sabdariffa The major commercial varieties are those grown in China, Thailand, Mexico and Africa principally Sudan, Senegal and Mali (Ahmed *et*.*al.* 2010).

2.7 Harvest and storage of roselle

Hibiscus sabdariffa harvested from late November onwards. The harvest is timed according to the ripeness of the seed. The fleshy calyces are harvested after the flower has dropped but before the seed pod has dried and opened. The longer the capsule remains on the plant after the seeds begin to ripen, the more susceptible the calyx is to disease and sun cracking (Plotto *et*.a*l*. 2004).

The calyces ripen about three weeks after the start of flowering, which is 100–160 days after the plants are transplanted outdoors (Duke, and duCellier, (1993). The fruit ripens progressively from the bottom of the plant to the top. Harvesting is carried out by intensive hand labor, the calyces being picked singly at the appropriate stage. The fruit may be harvested when fully grown but still tender, when they can be easily snapped off by hand; later harvesting requires clippers (Morton, 1987). The fruit is easier to break off in the morning than at the end of the day. On average, each fruit yields about 7–10 g of sepals (Wester, 1912).

Drying is the traditional method for preserving foods. Roselle drying is done in one of two ways: by harvesting the fresh fruit and then sun-drying the calyces, or by leaving the fruit to partially dry on the plants and harvesting the dried fruit, keeping the crop well protected during the process.

Dehydration depends on the two fundamental processes of heat transfer (heat is transferred into the fruit) and mass transfer (subsequent removal of moisture from it) (Potter, and Hotchkiss, 1995).

In Sudan, the fully developed fleshy calyx is peeled from the fruit by hand and dried naturally in shade.

2.8 Pest control and weeds

Major diseases of hibiscus are stem rot and root rot. Prevention techniques include monitoring the water content in an irrigated field, and avoiding the planting of other crops that are also prone to these diseases. Insect damage is minor, but it does exist; pests include stem borer, flea beetles, abutilon moth, cotton bollworm and cutworm. Mealy bugs and leafhoppers are minor concerns, as is the cotton strainer. Plant enemies usually do not compete in a cultivated field (Plotto *et.al.* 2004).

Weeding can increase yield and calyx size. Roselle fields are generally weeded if necessary, and there are many weeds species observed in Sudan.

2.9 Uses of roselle

Many parts of roselle including seeds, leaves, fruits and roots are used in various foods. Roselle is a multi-use plant, whose outer leaves (calyx), also known as natal sorrel, (Ageless *et.al.* 1999) is frequently used in the production of jelly, jam, juice, wine, syrup, gelatin, pudding, cake, ice cream and flavoring. Its brilliant red color and unique flavor make it a valuable food product (Tsai, and Ou, 1996).

Roselle is an annual crop used in food, animal feed, nutraceuticals, cosmeceuticals and pharmaceuticals. The calyces, stems and leaves are acid in flavor. The juice from the calyces is claimed to be a health-enhancing drink due to its high content of vitamin C, anthocyanins and other antioxidants (Mohamed *et. al*. 2002).

In Sudan, the dry calyx is used to produce a flavorsome and healthy and dried calyces are used for tea, jelly, marmalade, ices, ice cream, sorbets, butter, pies, sauces, tarts, and other desserts (Duke, and Ayensu, 1985). The seeds have also been used as an aphrodisiac coffee substitute.

2.10 Medicinal value

Hibiscus sabdariffa is used in many folk medicines. It is claimed as a Thai traditional medicine for kidney stones and urinary bladder stones (Hirunpanich *et. al*. 2006). *Hibiscus sabdariffa* also is said to have diuretic effects, used effectively in folk medicines for treatment of inflammatory diseases (Dafallah and Al-Mustafa, 1996), and cancer (Chewonarin *et.al*. 1999). The positive effect of *Hibiscus sabdariffa* extract consumption to decrease blood pressure has been proved in study on both man and rats (Faraji *et.al.* 1999 and Onyenekwe *et.al.* 1999). More recently, the antihypertensive action of *Hibiscus sabdariffa* has been confirmed with experimental hypertension (Odigie *et.al.* 2003).

Oil extracted from seeds of *Hibiscus sabdariffa* has been shown to have an in vitro inhibitory effect on Bacillus anthraces and Staphylococcus albums (Gangrade *et.al.* 1979). An ethanol extract of the dried leaves of the plant also has been shown enable to reduce aflatoxin formation (El-Shayeb and Mabrook, 1984).

2.11 Nutritional properties of roselle

Nutritional properties of roselle calyces were previously reported. (Duke 1983 and Mat *et.*al. 1985) they found that 100 g of fresh Roselle calyces contain 84.5% of

moisture content. The calyces contain 49 calories, 1.9 g protein, 0.1 g fat, 12.3 g total carbohydrate, 2.3 g fiber and 1.2 g ash (Duke and Atchley 1984). Different values were observed by another study conducted in Sudan, in which calyces contained 15% moisture content, 5% protein, 15% total carbohydrate, 12% fiber and 7% ash (Gabb, 1997). Nutritional data from U.S. Department of Agriculture also reported different value than previous findings, namely 1% protein, l.2% carbohydrate and 0.6% fat. The results differ probably because of the different varieties, genetic, environment, ecology and harvesting conditions of the plant (Atta 2003). Fresh roselle calyces contain 1.72 mg Ca, 57 mg Fe, 300 μg β-carotene equivalent, and 14 mg ascorbic acid 100 g (Duke 1983).

Nutritional properties of roselle seeds previous studies had significantly shown that Roselle seeds contained high amounts of protein, dietary fiber, and magnesium and calcium. The seeds from Egypt contain 7.6% moisture, 34.0% protein, 22.3% fat, 15 fiber, 23.8% nitrogen-free extract, 7.0% ash and 0.3% Ca (Samy 1980). Another study from India found that the seeds contain 6-8 moisture, 18-22% crude protein, 19-22% fat. 5.4% ash, 39-42% total dietary fiber l 19-128 mg Ca. 596-672 mg P. 4.0 mg Zn, 3.1 mg Cu, 393- 396 mg Mg. 0.08-0.18 Cr, 0.36-0.51 mg riboflavin and 0.9 mg nicotinic acid (Rao 1996).

Chapter Three

Materials and Methods

3.1 Materials

Roselle seeds (*Hibiscus sabdariffa*) were collected from Al-Fasher Market in North Darfur State in harvesting season (2015). The seeds kept in a plastic container and transported to laboratory for analysis.

3.2 Methods

3.2.1 Roselle a proximate chemical analysis

The moisture, ash and crude fiber contents were analyzed according to standard methods described in AOAC (1997).

Nitrogen was assayed using Kjeldahl method and the nitrogen content was converted to protein by a multiplication factor of 6.25 (AOAC**,** 1997**)** Total Carbohydrates were determined by difference using a standard method of (AOAC 1997). All the proximate analyses were carried out in triplicate and the results expressed as percentage of the sample analyzed.

3.2.1.1 Moisture content

The dry seed (5g) was weighed into a clean dry aluminum dish with a known weight. The sample was dried in vacuum oven at a temperature of 105^0 C for 6 hours, cooled in desiccators and weighed. And weighing was repeated twice until there was no difference in the two successive weights.

The moisture content was calculated following the method of AOAC, (1997).

$$\text{Moistur Content \%} = \frac{(\text{W2} - \text{W1}) - (\text{W3} - \text{W1})}{(\text{W2} - \text{W1})} \times 100$$

Where:

W1= weight of empty crucible.

W2= weight of crucible with the sample.

W3= weight after drying.

3.2.1.2 Crude oil content

The dry seed (50g) was extracted with Petroleum either solvent using Soxhlet apparatus for 6 hours. The crude oil extracted was concentrated in a rotary evaporator and dried by heating in a vacuum oven at 50C for one hour. The Percentage crude oil content was then determined gravimetrically (AOAC**,** 1997).

$$\text{Crude oil content \%} = \frac{\text{weight of extracted oil}}{\text{weight of dry sample}} \times 100$$

3.2.1.3 Total ash

The dry seed sample (5g) was placed in a dry clean porcelain dish and heated progressively for 6 hours at 550C until, grey -reddish ash was obtained according to (AOAC**,** 1997). The sample was cooled in a dessicator, weighed and total ash calculated using the following formula:

$$\text{Total ash \%} = \frac{(\text{weight of dish} + \text{ash}) - (\text{weight of dish})}{\text{Weight of sample}} \times 100$$

3.2.1.4 Crude fiber

About 2g of Roselle seed powder was transferred into a 200 ml Labeled beaker after which 1.25 % Sulphuric acid (50 ml) and distilled water (150 ml) were added. The sample mixture was then boiled for 30 minutes under reflux flask and later treated with 1.33% potassium hydroxide (50 ml) and 150ml water the solution was re-boiled again for 30 minutes and registered using vacuum crucible filtrate on system. The sample in the crucible was rinsed with water followed by acetone. The samples was put into a pre-weighed crucible and transferred to the oven to dry for 4 hours, cooled in desiccators and weighed .The weighed sample was used in the furnace set at 660 C for 5 hours until it became grey ash which was cooled in the dedicator and weighed (AOAC**,** 1997). The weight of ash was then calculated as follows:

$$\mathrm{CF\%} = \frac{(\mathrm{W1} - \mathrm{W2})}{\mathrm{Ws}} \times 100$$

Where:

CF= crude fiber.

W1= weight of crucible with sample before ashing.

W2= weight of crucible with sample after ashing.

Ws= weight of sample.

3.2.1.5 Crude protein

Protein can be determined through the following stages:-

3.2.1.5.1 Digestion stage:

The dry Roselle seed powder (2g) was placed in kijeldahl tube and a 4g mixture (catalyst; sodium sulphate and copper sulphate) was added the mixture was digested with concentrated sulphuric acid (25ml) for 2 hours in fume hood until the solution became clear to light green.

3.2.1.5.2 Distillation stage:

Distilled water (120ml) was added to the solution and allowed to cool. Sodium hydroxide (45%) was also added without agitation. The flask was then connected to the distillation bulb with the tip of the condenser immersed in a standard acid solution (boric acid 2%)

Containing 5 drops of the indicator. The flask was then heated to release ammonia into the indicator solution.

3.2.1.5.3 Titration stage

The excess standard acid in the distillate was titrated with 0.1N standard HCL The conversion factor of 6.25 was used (AOAC**,** 1997) and % of Nitrogen calculated as Below:

$$\mathrm{CP\%} = \frac{(\mathrm{T} - \mathrm{B})\ \mathrm{x}\ \mathrm{N}\ \mathrm{x}\ 14\ \mathrm{x}\ 100\ \mathrm{x}\ 6.25}{\mathrm{Ws} \times 1000}$$

Where:

CP= crude protein.

T= Titration reading.

B= Blank titration reading.

N= HCl normality.

Ws= sample weight.

3.2.1.6 Total carbohydrates

Total carbohydrates were determined by difference using the method in **(**AOAC**,** 1997).

$$\text{Total Carbohydrate\%} = 100 - (\text{MC} + \text{AC} + \text{FC} + \text{CF} + \text{CP})$$

Where:

MC= moisture content.

AC= Ash content.

FC= fat content.

CF= crude fiber.

CP= crude proteins.

3.2.3 Oil extraction

3.2.3.1 Preparation of the Seed for oil Extraction

The seeds were decupled, cleaned and crushed and later dried in the oven for three hours at 50 °C to ensure that moisture content was reduced to the bears minimum.

3.2.3.2 Oil extraction

The prepared seed were oven dried at 70°C until a constant weight was obtained, then grinded into equal sizes. The extractor used was Soxlet apparatus with petroleum ether as solvent. After extraction, the mixture of the solvent and extract was allowed to cool and then filtered to remove solid particles. The filtrate was concentrated under vacuum in a rotary Evaporator (Akpan *et.al*. 2005). The results obtained were noted. The extracted oil was analyzed for the physical and chemical properties. All reagents used were of analytical grade.

3.2.4 Physic-Chemical characteristics of the extracted Roselle seeds

3.2.4.1 Moisture content determination

Five grams (5 g) of the cleaned sample was weighed and dried in an oven at 80 °C. After every 2 hours, the sample was removed from the oven and placed in the desiccators for 30 minutes to cool. It was then removed and weighed (Akpan *et.al***.** 2005). The percentage moisture in the seed was then calculated from:

$$\text{Moisture content } \% = \frac{(\text{W1} - \text{W2})}{\text{W1}} \times 100$$

Where:

W1 = Original weight of sample before drying (g).

W2 = Weight of sample after drying (g)

3.2.4.2 Specific gravity determination

The specific gravity bottle was cleaned with acetone, ether and dried in an oven at 60 o C. The weight of the empty bottle was taken, after which the bottle was filled with the oil sample and properly covered. The weight was then recorded using a weighing balance, after which the sample was removed from the bottle. The bottle

was properly washed and filled with distilled water, after which the weight was taken and finally, the specific gravity was computed using the relationship below (Akpan *et.al*, 2005).

$$\text{Specific gravity } \% = \frac{(\text{Wo} - \text{W})}{\text{W1} - \text{W}}$$

Where:

W = Weight of empty bottle (g),

Wo = Weight of the bottle and oil content (g).

W1 = Weight of bottle and water content (g).

3.2.4.3 Saponification value determination

0.5M KOH was prepared in 95 % ethanol, 5g of oil sample was weighed and 50 ml of KOH was added, 50 ml of the blank solution was also measured into a conical flask. The two samples were then connected to a reflux apparatus and allowed to boil for an hour until the reflux is completed, 1 ml of phenolphthalein was added to the mixture and the resulting mixture was titrated while hot against 0.5 M HCL acid solution. The volume of the acid used to attain the end point was recorded, the blank determination was carried out using the same procedure described above until the color changes from blue to transparent white, then the volume of acid used was noted, the Saponification value was determined using the relationship below (Akpan *et. al*. 2005).

$$\text{Saponification value } (\text{S.V}) = \frac{56.1 \times \text{T } (\text{V0} - \text{V1})}{\text{M}}$$

Where:

T= Morality of the standard KOH solution used (M),

Vo = Volume of acid used for the first titration with oil sample (cm3),

V1 = Volume of acid used for the second titration blank solution (cm3),

M = Mass of the oil sample used (g)

3.2.4.4 Peroxide value determination

A known weight (5g) of sample was weighed into cleaned dried boiling tube 1 gram of potassium iodine (KI) powder was added to the oil and 20 cm3 of the solvent mixture (i.e, glacial acetic acid and chloroform in the ratio 2:1). Then the boiling tube was placed in boiling water bath so that the liquid mixture boils within 30 seconds and allowed to boil vigorously for not more than 30 seconds, the content after boiling was quickly poured into a flask containing 20 cm3 of 5 % potassium iodine (KI) solution and the tube was washed out twice with 25 cm3 of water. Then the mixture was titrated with 0.002 M sodium sulphate using fresh 1 % starch solution, a blank titration was carried out at the sample time, the peroxide value was calculated using the relationship below (Akpan *et.al*. 2005).

$$\text{Peroxide value} = \frac{\text{T} \times \text{M} \times 1000}{\text{W}}$$

Where:

T = titer value of $Na_2S_2O_3$ = (Sample titer – Blank titer,)

M = Morality of $Na_2S_2O_3$

W = Weight of sample / (g)

3.2.4.5 Refractive index determination

The refractive index was determined using Abbey refractometers. The glass prism of the refractometer was thoroughly cleaned with alcohol to ensure that it is free from dust, a drop of oil sample was placed on the lower prism and smeared, then closed with the other covering prism and the light source of the refractometers was switched on, while viewing through the telescope.

The coarse adjustment knob was rotated until the black shadow appears central in the cross wire indicator and while still viewing through the telescope, the fine knob adjustment was made until the rainbow-colored fringe which appeared on the black dividing line disappeared, the coarse knob was rotated to give fine adjustment and make the black shadow appear exactly central in the cross wire indicator. The reading under the telescope and that of the fine adjustment knob were noted and divided by 10,000, this value was then added to the value obtained through the telescope to give the value of the refractive index of the oil at room temperature (Akpan *et.al*. 2005).

3.2.4.6 Free fatty acids

Free fatty acids content was carried out according to the (British Standard Institute Method 1958) . About (5g) of the oil was weighed accurately into 250 ml conical flask. 50 ml mixture of 95% alcohol and ether solvent (1:1) were added. The solution was neutralized after addition of one ml of phenolphthalein indicator. The contents of the flask were then heated with caution until the oil was completely dissolved .The contents of the flask were then titrated with 0.01N KOH with constant shaking until a pink colour persisted for 15 seconds .The number of ml of 0.1 N KOH recorded as % .

Calculation:

$$\text{Free fatty acid as oleic acid } = \frac{\text{V x N} \times 56.1}{\text{W}}$$

Where:

V: Volume of titration (ml).

N: Normality of KOH.

W: weight of sample.

3.2.4.7 Iodine value

The iodine value (I V) of the oils which quantifies their unsaturation level was determined according to (AOAC 2000). Approximately (0.2g) of oil was accurately weighed and placed in a dry and clean flask specially offered for the test .A 10 ml of chloroform was used for dissolving the oil . A 25 ml of pyridine sulphate dibromide solutions was added and finally 20 ml of KI (0.1 N) were added to the contents of the flask was then stoppered and the mixture was allowed to stand for 10 minutes in a dark place . The stopper and the side of the flask were rinsed with enough amount of distilled water , the contents of the flask was then stoppered and the mixture was allowed to stand for 10 minutes in a dark place .The stopper and the side of the flask were then shaken and titrated against 0.1N sodium thiosulphate solution using starch liquid as indicator . A blank determination was carried out simultaneously .

Calculation:

Iodine value (IV) = (B-A) x 0.01269 x 100 / S

Where:

B: Volume (ml) of sodium thiosulphate in blank solution.

A: Volume (ml)of sodium thiosulphate in test active solution.

S: Weight (gm) of the oil sample.

Iodine factor = 0.01269

3.2.4.8 Color

The color intensity of oils was recorded using LovibondTintometer as units of red, yellow and blue according to the (AOCS 2005). Samples of oils were filtered through filter paper immediately before testing. An appropriate cell (2" cell) was filled with oil and placed in the tintometer near-by window for light .The instrument was switched on and looked through the eye piece . The yellow colour was adjusted to 35, then slides were adjusted until a color match was obtained from combination of red and blue. The values obtained by matching were recorded as red, yellow and blue.

Chapter Four

Results and Discussion

4.1 Approximate chemical analysis of roselle seeds:

Results in table (1) show that the moisture content , protein , fat , ash , fiber and carbohydrates (7.2% , 27.1% , 21.1% , 6.7% , 16.4% , 21.7%) respectively. While protein found to be greater than that mentioned by (Alwandawi *et.al.* 1984) 25.2% and lower than reported by (Hainida *et.al.*2008) and (Nady, 2014) 33.4% and 31.1% while the crude fat mentioned by (Alwandawi *et.al.* 1984) 21.1%, which is equal to the found result and similar with that reported by (Nzikou *et.al.* 2011) and (Nady, 2014) 21.8 , 21.6. While moisture content was found to be lower than that mentioned by (Hainida *et.al.* 2008) and (Nady, 2014) 9.9% , 9.2% and greater than that recorded by (Nzikou *et.al.* 2011) 6.4% , while fiber found to be agreement with that reported by (Alwandawi *et.al.* 1984) 16.3 , and similar with mentioned by (Nzikou *et.al.* 2011) 16.4 , ash was found to be greater than that noticed by (Alwandawi *et.al.* 1984) 5.1 , and similar to (Nzikou *et.al.* 2011) and (Nady, 2014) 6.2 , 6.8 and lower than that reported by (Hainida *et.al.* 2008) 7.4 , carbohydrates was found to be lower than that recorded by (Alwandawi *et.al.* 1984) and 26.6 , and agreement with that reported by (Nzikou *et.al.* 2011) 21.2.

The results differ probably because of the different varieties, genetic, environment, ecology and harvesting conditions of the plant

4.2 Physical characteristic of roselle seeds oil:

Results in table (2) show that the moisture content, color, refractive index and specific gravity (2.07, yellow 70 red 2.9, 1.4643, 0.8985) respectively. While refractive index found to be similar to (Bligh and Dyer 1959) and (Nady, 2014) where are 1.4697 and 1.4675 respectively, specific gravity was found to be similar to (Nady, 2014) 0.8995.

The results differ probably because of the different varieties, genetic, environment, ecology and harvesting conditions of the plant

4.3 Chemical characteristic of roselle seeds oil:

Results in table (3) show that the free fatty acid, iodine value, peroxide value and saponification value (2.5, 103, 1.2, 192) respectively. While free fatty acid found to be greater than that reported by (bligh and dyer 1959) 0.88 , and similar to (Nzikou, *et.al.* 2011) 2.24 , iodine value was found to be lower than that recorded by (Nady, 2014) 116 and greater than that mentioned by (bligh and dyer 1959) and (Nzikou, *et.al.* 2011) 98 and 81 respectively , while peroxide value was found to be lower than that recorded by (Nady, 2014) and (bligh and dyer 1959) 6.5 , 2.03 respectively , saponification value was found to be similar than that reported by (Nzikou, *et.al.* 2011) , and lower than that mentioned by (Nady, 2014) and (bligh and dyer 1959) , 197 all , while iodine value was found to be greater than that reported by (Nzikou, *et al.*, 2011) and (bligh and dyer 1959) 81.4 , 98 respectively, and lower than that recorded by (Nady, 2014) 116.

The results differ probably because of the different varieties, genetic, environment, ecology and harvesting conditions of the plant

Table (1) approximate chemical composition of roselle seeds:

Composition	Mean ± SD (%)
	n = 3
Moisture content	7.2 ± 0.125
Crude Protein	27.1± 0.412
Crude oil	21.1 ± 0.2
Crude Fiber	16.2 ± 0.17
Ash	6.7 ± 0.2
Carbohydrates	21.7 ± 1.107

Table (2): physical characteristic of roselle seeds oil:

Physical characteristic	Mean ± SD n = 3
Color Yellow Red Blue	 70.0 ± 1.12 2.9 ± 0.04 0.0
Moisture Content	2.07 ± 0.4101
Refractive Index	1.4643 ± 0.0015
Specific Gravity	0.8985 ± 0.004

Table (3): Chemical characteristic of roselle seeds oil:

Chemical characteristic	Mean ± SD n = 3
Free fatty acids (as olic acid)	2.5 ± 0.020817
Iodine Value	103 ± 1.5
Peroxide value	1.2 ± 0.0714
Saponification Value	192 ± 2

Chapter Five

Conclusion and Recommendation

5.1 Conclusions

The roselle seeds have a considerable amount of oil content and a high amount of protein. It has a low value of peroxide value due to presence of antioxidants in oil , also it has acceptable levels in saponification and iodine value. It proved to be one of the promising potential sources.

5.2 Recommendations

We recommend to:

- More studies are needed for the oil fatty acids composition and its shelf life.
- More attention and care should be taken for growth and maintenance of Roselle tree.
- Sudan is considered potentially rich source for different types of oil seed crops , more wise polices are need to secure the production of *Hibiscus sabdariffa* and lowering its cost.
- To use seed kernels of *Hibiscus sabdariffa* as a supplement food source in the tropical and subtropical regions.

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Appendices:

Figure (1) Roselle seeds.

Figure (2) Roselle seeds oil.