1.0 Introduction

Fats and oils are very important in the human diet due to the high content of essential fatty acids, which are necessary for the appropriate development of human tissues (Moya Moreno *et al.,* 1999). Many studies were conducted to study the utilization of wild plants as a source of unconventional oils and their maximum utilities as antioxidants chronologically (Mirghani *et al*., 1996; Mirghani, 1990; Mariod, 2000).

Inexpensive sources of protein that can be incorporated to value-added food products are in demand all over the world and most of the research is directed to various sources of proteins (Chandi and Sogi, 2007; Rangel *et al*., 2003 ; Sogi and Bawa, 2002) that may help in increasing the nutritional value of the products.

Plants have for a long time been a veritable source of drugs; man tends to ignore the importance of herbal medicine (Nair and Chanda, 2007). Scientists worldwide care about the scientific exploration of medicinal plants for the benefit of human beings. Many African plants are used in traditional medicine as antimicrobial agents but only few were documented (Bellomaria and Kacou, 1995; Lewis and Elvin-Lewis, 1997; Ahmad *et al*., 1998). In view of the fact that antimicrobials are sometimes associated with adverse side effects e.g. hypersensitivity, immunosuppressive and allergic reactions, it is therefore of interest to develop alternative antimicrobial drugs such as medicinal plants for the treatment of infectious diseases (Clark, 1996).

A number of potential phytoantimicrobial agents, such as phenolic compounds have been isolated from olives and virgin olive oil, and among these are polyphenols and glycosides, these phytoantimicrobial agents incorporating nutraceutical advantage while enhancing food safety and preservation (Keceli *et al*., 1998).

Polyphenols are naturally occurring secondary metabolites in all plant materials, and prominently found in herbs, vegetables, fruits, and seeds (Bravo, 1998). The most occurring polyphenols are flavonoids, which are benzo-γ-pyrone derivatives consisting of phenolic and pyrane rings, that can be divided into six classes including flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanidins (Heim *et al*., 2002; Vinson *et al*., 1995). The other common non-flavonoid polyphenols are phenolic acids. Owolabi *et al*., (2007) reported that medicinal plants play a key role in health care with about 80% of the world populations relying on the use of traditional medicine which is predominantly based on plants. Mariod *et al*., (2014) investigated wood extracts of *Combretum hartmannianum, Acacia seyal* and *Terminalia brownie* for their antimicrobial screening; they reported that the ethyl acetate extract gave the highest zone of inhibition against *Salmonella*, and all other extracts showed moderate zones of inhibition against all the bacteria tested.

Germination of seeds starts when the dry seed begins to take up water and is completed when the embryonic axis elongates; at this point reserves within the storage tissues of the seed are mobilized to support seedling growth. (Bewley*et al*., 2001). A number of studies reported that germination of cereal and legume seeds is an economical processing technology and have advantages and health benefits, and that the breakdown of seed-storage compounds and synthesis of structural proteins and other cell components take place during this process. Fats and carbohydrates that are often at surplus levels in Western diets are broken down, while dietary fiber that is mostly at a sub-optimal level

increases (Sierra, and Valverde, 1999; Urbanoetal., 1995).Yu-Haey Kuo *et al.*,(2004) reported that germination causes important changes in the biochemical, nutritional and sensory characteristics of legume seeds.

Kisra is a dietary staple food in Sudan. It is a morsel or piece of bread prepared from fermented sorghum flour and is consumed while still fresh. (Dirar, 1993). It provides most of the dietary proteins and energy in some parts of Sudan especially the rural areas. The baking time for each sheet of kisra is 15-20 seconds (Dirar, 1993).The average values for the composition of kisra are : 50% moisture,30-40% carbohydrate, 12% protein , 2% crude fiber and 1.5% ash (El-Tinay *et al*., 1985 and Dirar,1993). Kisra is prepared by mixing sorghum flour with water to give a thick paste which is allowed to ferment for 12-24 hours, following which the paste is thinned to the desirable consistency with water just prior to baking.

The overall objective of this study is :

To investigate the quality of oil and proteins and added-value end-products from *C.brocchiana* seeds, and study their nutritional value.

The specific objectives of this study are :

- To extract and develop phenolic rich fractions from leaves, stem, and oil of *C.brocchiana* plant
- To investigate the effect of germination on the chemical composition of the seeds
- To extract the oil by different methods
- To study the oil stability by peroxide value and FTIR spectroscopy
- To isolate and concentrate protein from defatted seeds
- To supplement Kisra, using protein concentrates

2.0 Literature Review

2.1. Euphorbiaceae Family

Euphorbiaceae, the spurge family, is a large family of flowering plants with 300 genera and around 7,500 species. Most spurges are herbs, but some, especially in the tropics, are shrubs or trees (Gibbs, 1974). This family occurs mainly in the tropics, with the majority of the species in the Indo-Malayan region and tropical America. A large variety occurs in tropical Africa, but they are not as abundant or varied as in these two other tropical regions. However, *Euphorbia* also has many species in non tropical areas such as the Mediterranean Basin, the Middle East. The fruit is usually a schizocarp, but sometimes a drupe. This family contains a large variety of phytotoxins (toxic substances produced by plants), mainly diterpeneesters, alkaloids, glycosides, and ricin-type toxins (Paul *et al*., 2014) . Many plants of Euphorbiaceae family are grown as ornamental plants and some species proved to be effective against genital herpes (Betancur-Galvis *et al*., 2002). Euphorbiacea family includes succulent or non succulent plants ranging from herbs and shrubs to trees and cactus types. Many of them contain a milky juice which is toxic, especially for cold-blooded animals. The fruits are usually three-celled capsules, each cell containing a single seed from which in some species, toxic, vesicating, and irritant seed oils may be obtained. The largest genera of the spurge family are those of Croton, with about 700 species, and of spurge or *Euphorbia*, with about 1600 species (Hecker ,1968).

2.2. *Chrozophora* Genus

Chrozophora genus is a plant of the family Euphorbiaceae and the sole genus comprised in the subtribe chrozophorinae. It comprises 7-8 species e. g. *Chrozophora brocchiana, C. senegalnsis, C. plicata, C. rotteri, C. tinctoria and C. oblongifolia* , which are mostly monoecious herbs under shrubs (Rafael *et al*., 2000). This genus is distributed in Pakistan, India, West Africa and Mediterranean regions. Previous phytochemical investigation of the genus Chrozophora resulted in the isolation of several types of chemical constituents including essential oils, terpens, sterols, phenylpropanoid glycosides, xanthones, chromone and flavonoids. It was reported that the plant contained essential oils and flavonoids. A literature search revealed only flavonoid a glycones and an acylated glucoside of apigenin. Hawas, 2007 reported isolation and structure elucidation of a novel brocchlin carboxylic acid from the aqueous ethanolic extract of *C.brocchiana*, together with eight known phenolic compounds, gallic acid, methylgallate, ethylgallate, ellagic acid, methoxyellagic acid, methylenedioxy ellagic acid, apigenin (Hawas, 2007).

2.2.1. Botanical Description

2.2.1.1. *Chrozophora brocchiana* (Vis.) Schweinf

A shrubby herb up to 60–150 cm tall; taproot stout and very long; stem ascending, knotty, much-branched from the base, white-velvety hairy with stellate hairs. Leaves alternate, simple; stipules small; petiole long; blade angular-ovate to triangular-ovate, 2.5–4 cm × 1.5–3 cm, base deeply chordate with 2 glands, apex rounded, margins undulate, upper surface sparsely hairy, lower surface velvety hairy, 3-veined at base. Inflorescence, a condensed axillary raceme, with male flowers at the top and female flowers at the base, bracts small. Flowers unisexual, regular, 5-merous; calyx velvety hairy, petals

deep red; male flowers with short pedicel, stamens up to 10 mm, filaments fused into a column; female flowers with pedicel elongating in fruit to 6 mm long, petals smaller than in male flowers, ovary superior, 3-celled, styles 3, fused at base, 2-fid at apex. Fruit a 3-lobed capsule c.1 cm long, densely covered with white or violet-tinged, shiny stalked scales, 3-seeded. Seeds ovoid, smooth, yellowish brown, covered by a thin, pale, shiny aril (Burkill, 1994). *C.brocchiana* occurs in the area of Cape Verde and Mauritania throughout the Sahel region east of Sudan, and is also found in Algeria, Ethiopia and Egypt, mostly in dried-up inundated flats or sandy river beds (Tignokpa *et al*.,1986).

2.2.1.2.*Chrozophora senegalensis* A. Juss

Chrozophora senegalensis closely resembles *C.brocchiana* but it has hairs, shorter petioles and non-elongating sepals in fruits. It is a herb with small green leaves, deep red flowers and violet tingled capsules, occurs on sandy soils. An under shrub, sometimes prostrate, in seasonally flooded flats and on river banks of the savanna region from Senegal to Northern Nigeria, and in Shari (Burkill , 1994).

2.2.1.3 .Chrozophora plicata (Vahl) A. Juss. ex Spreng

Prostrate or more or less erect, branched annual or perennial herb, up to 50 cm. Most parts densely covered in grayish stellate hairs. Leaves rhombic-ovate, up to 7 × 5 cm with a long petiole, plicate-undulate, especially when young, 3-5-veined from the base with 2 dark purple glands at the base; margin more or less entire or obscurely toothed. Flowers in leaf-opposed or pseudo-axillary inflorescences, covered in stellate hairs, unisexual. Male flowers, orange-yellow or pinkish; female flowers, crimson-red, Fruit up to 5 × 9 mm, 3-lobed, densely covered in stellate hairs, reddish or bluish-purple when ripe (Hyde *et al*,. 2014) . It occurs throughout tropical Africa to Northern South

Africa, Egypt, Syria, Palestine, and North-Western India to the Mediterranean (Burkill, 1994). It grows in warmer climate and temperate regions (Chopra, 1988 and Forster*et al*,.1999).

2.2.1.4. *Chrozophora tinctoria A. Juss*

It is an herb or under shrub, monoecious, indumentums consisting of very dense, sessile and peduncle stellate or lepidote hairs, next to simple hairs. Stipules narrowly triangular, scars very indistinct. Leaves spirally arranged and simple. Flowers actinomorphic, staminate flowers usually 2 per node, pistillate flowers usually single and fruits, slightly lobed capsules, triangular in transverse section, dehiscing usually septicidally and partly loculicidally into 3 bivalved parts, outside densely stellate, inside glabrous, thin-walled; column slender, with frayed remnants of the septa, apically triangular; septa single veined. Seeds 3 per fruit, obovate, angular; covered by a thin, incomplete sarcotesta; the latter carunculate apically, embryo flat; endosperm copious (Forster *et al*., 1999) . It is an annual plant, native to a number of countries in Africa, temperate and tropical Asia and Europe, and commonly known as ‘dyer’s-croton (GRIN Database, 2006). Süleyman,(2000) reported that *C. tinctoria*, is an annual plant and is the only species of chrozophora found in Turkey, that used as a source of dyeing material in Carpet, Kilims and in other crafts in Western Anatolia and it should benefit the economy of Turkey.

2.2.1.5.*Chrozophora rottleri (Geiseler) juss*

It is an annual herb, prostrate or ascending; monoecious, the flowers borne in sessile auxiliary racemes with staminate flowers in upper and pistillate flowers in the lower part of raceme, main stem up to 50 cm long, stellate-pubescent. Leaves alternate, 2-5 x 1-4 cm. It is an erect herb with silvery hairs; lower part of stem is naked, upper part hairy and has slender tap-root. The

three-lobe leaves are alternative, thick and rugose. The plant occurs naturally in tropical African, Asia and India Prota 11 (1) (2010).

2.2.1.6 *Chrozophora oblongifolia (Delile) A. Juss. Ex spreng*

This plant is distributed in Egypt, Saudi Arabia, Yemen, Oman, Sudan (Red Sea coast), Eritrea, Djibouti, Somalia, and Socotra. It is an erect shrub, sub shrub or woody herb up to 1 m high,. Fruit rounded-trilobate, 5-6 x 7-9 mm, somewhat muricate, lepidote, bluish-purple. Seeds triangular-ovoid, 4-5 x 3-4 mm, coarsely tuberculate, yellowish-gray

A much-branched shrub, with stems stout and woody, but sometimes herbaceous and dying after flowering in the first year; stems rather harshly white or tawny stellate-pubescent. Leaves distinctly petioled, ovate-rhomboid or oblong to lanceolate Capsule 3-coccous, blue-purple, about 1/2 in. wide, loosely clothed with floccose flat scales with fringed denticulate margins. (Cordell, 2000).

2.2.2. Chemical Composition, Bioactive Compounds and Antioxidant Activity

Chrozophora brocchiana

In previous studies the seed of *C.brocchiana* had 37-40% oil and 26.2 % protein, and the fatty acids composition of the oil showed linoleic acid as the major component, followed by oleic, stearic and palmitic acids (Mirghani, 1990 and Hussein *et al*., 1994), The triaclyglycerol composition of *C. brorcchiana* showed LLL 15.8%, OLL 14.9%, PLL 7.5%, OOL 7.7%, PLO 16.9%, OOO 2.8%, POO 11.7%, POP 3.9%, SOO 4% SOP 6% and SOS 2.3%. The ratio of mono-acidic: di-acidic: tri-acidic regardless the type of fatty acids and their position in the TAG was 1:2.65:1.17. Prota 11(1) (2010) and Hussein *et al*., 1994) , and the chemical content of the aerial parts revealed an unusually

high silica content 72.2%. Hawas, (2006) reported that the aqueous methanol extract of the aerial parts of *C.brocchiana* contains brocchiana carboxylic acid, an analogue of brevifolin carboxylic acid, as well as gallic acid, methyl gallate, ethyl gallate, ellagic acid, mono- and di-methoxy ellagic acid, apigenin and luteolin 7-O-glucoside .

Chrozophora senegalensis

The leaves of this species contain carbohydrates, saponins, tannins, steroids (Hassan *et al*., 2004) glycoside and alkaloids (Audu*et al*,. 2008). Antonio Vet *et al*., (2006) reported that the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay led to the isolation of three new flavonoid glycosides namely quercetin 3-O-(6″-caffeoyl)-β-D-glucopyranoside-3′-O-β-D-glucopyranoside, quercetin 3-methylether-7-O-α-L-rhamnopyranosyl-(1→6)-(2′-p-coumaroyl)-β-D-glucopyranoside, acacetin 7-O-(6″-p-coumaroyl)-β- D -glucopyranoside and along with five known flavonoids, one phenolic derivative, and three megastigmane glycosides.

Chrozophora tinctoria

This plant is used for producing a dark dye substances having a high solubility in water it produced dark blue dye (Başlar and Mert,1999) and flavonoids (Hashim *et al*., 1990). The natural dyes obtained from these plants include three main colors; red, yellow and blue. It is possible to obtain other colors from mixtures of these colors (Süleyman, 2000). Dye obtained from *C.tinctoria,* due to its high solubility in water, produced dark red color, but it did not show reaction with wool fiber, as such, dyeing of wool fibers was of low grade. Wool fibers are thus giving as light colored appearance. The lowest color intensity was 3.213 and the highest was 6.408 (Ugulu *et al*.,2009). The HPLC analysis of the methanol extract of the aerial parts of *C.tinctoria* yielded five flavonoid glycosides, these were quercetin 3-O-rutinoside (1,rutin),

acacetin 7-O-rutinoside (2), apigenin 7-o-B-D-[(6-p-coumaroyl)]-glucopyranoside (3), apigenin 7-O-B-D-glucopyranoside (4) and apigenin 7-O-B-D-[6-(3,4-dihydroxybenzoyl)]-glucopyranoside (named, chrozophorin 5), the last one was reported as a new natural product **(** Delazar *et al*,. (2006).

Chrozophora plicata

The fatty acid composition of *C.plicata* oil showed that the content of linolenic and oleic acids were varied from 60-75% and this oil resembles cottonseed oil in its proportions of linoleic, oleic and saturated acids. The plant has hydrocarbons, cholesterol, stigmasterol, β-sitosterol, β-amyrin, squalene, octacosanol, hexacosanol and tetracosanol (Radwan *et al*., 2000). The methanolic extract of the whole plant showed inhibitory activity against the yeast α-glucosidase and was considered as a target molecule for future anti-diabetic drugs (Tabussum *et al*., 2013). The *C.plicata* leaves extracts had strong exhibited fungitoxicity against p-aphanidermatum (Radwan *et al*., 2000), and the fruit produced dyes, stains, inks, tattoos and mordants (Tabussum *et al*., 2013).

Chrozophora rottleri

The oil from the seed of *C.rottleri* was reported to be rich in linoleate, while the leaves and root contain xanthone glycosides and chromone glycoside. The tannin was found in the whole plant (Dipankar *et al*.,2011). Another study revealed the presence of alkaloids, carbohydrate, glycosides, tannins, steroids, flavonoids and saponins in the chloroform extract of *C. rottleri* (Madane *et al*., 2013). Maharaj and Prabhakaran (2013), reported that *C. rottleri* had adverse allelopathic effects on the germination and growth of rice seedlings.

Chrozophora oblongifolia

The methanol hot aqueous extract of *C.oblongifolia* contains some bioactive compounds e.g. flavonoids, tannins, terpenoids. The extract exhibited antimicrobial activity with MIC values ≤ 125 μg/mL against Gram-positive bacteria and antioxidant activity, antiseptic for wounds and for hemorrhoids (Mothana *et al*., 2011). Fourteen novel dolabellane diterpenoids have been isolated from the aerial part of *C.oblique* (the old name) all of them are naturally acylated at the C-16 hydroxyl group with 3-hydroxy-3-methylglutaric acid (Mohamed *et al*., 1995).

2.2.3.Food and Feed Uses

The *C.brocchiana* plant is not grazed by stock in Senegal and is said to cause vomiting and diarrhea for animals, nor is it grazed in Sudan, but camels and Grant's gazelle are recorded eating it in Kenya. In Niger though, it is sought after by goats and at certain times of the year also by cattle. It is known to be acrid and poisonous in India. The fresh shoots of *C.plicata* force-fed to Nubian goats and desert sheep caused poisoning (diarrhea, dyspnoea, dullness and loss of appetite) and death of all animals (Adam *et al*.,1999).

The plant is known as Argassi in Sudan, where in eastern states the boiled seeds of *C.brocchiana* are used for food. In central Sudan, a sweet, non-drying oil is pressed from the seed extracted by traditional mills. Argessi oil has a potential of a new type of vegetable oil Prota 11(1) 2010. *C. tinctoria* is used in coloring foods, textiles, cosmetics and pharmaceutical preparations (Ugulu *et al*.,2009).

2.2.2.4. Medicinal Uses

Chrozophora genus has several interesting medicinal uses, the plant ash of *C.brocchiana*, is applied to sore and the crushed leaves were rubbed on the affected sites to treat stitch in the side. The aerial parts are taken in decoction to

strengthen lactating mothers and their children, and to treat fever and dysentery ,while powdered dried leaves in water are taken to treat diarrhea. Root sap in water is used as ear drops to treat otitis (Schmelzer,2007). Analysis of the chemical content shows no particular reason for a beneficial action as a wound-dressing; however, there is an unusually high silica content Prota 11 (1) (2010). While *C.senegalensis* plant has been reported as an astringent for treatment diarrhea mainly caused by *Salmonella* specie, and in Senegal a root decoction is given to suckling babies to treat diarrhea (Yushau , 2011 and Prota 11 (1) 2014. It is boiled with cereal foods and the pregnant women used a decoction of it as a body wash, also used as a remedy for syphilis; and treatment of intestinal pain, typhoid and boils (Tignokpa *et al*., 1986; Etkin, 1997; Usman *et al* ., 2007). The fruit juice is used as eye drops to treat more severe cases, a maceration of leaves and roots is used to treat loss of hair and diabetes, and aqueous extract of the aerial parts caused an in-vivo hypoglycemic response in rats (Burkill. 1994; Benoit-Vical *et al*., 2008).

Tignokpa *et al*., (1986) reported that leaves and stems extracts of *C. senegalensis* showed a high antiplasmodial activity against two chloroquine-resistant *Plasmodium falciparum* strains, without toxicity in vitro and no toxicity in vivo in mice. The leaf extracts alone showed antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*; with highly active on *Salmonella typhi* (Usman *et al.,* 2007). In Sudan, *C. oblongifolia* stem and leaves extracts are used to treat gonorrhea, and the chloroform and methanol extracts showed considerable antidiabetic activities. (Prota 11 (1) 2010 and Ugulu *et al.*, 2009) reported that *C.tinctoria*, has a high solubility in water, and produced dark red color, but it did not show reaction with wool fiber. The plant is used traditionally to treat warts, also has been used as an emetic, cathartic, and for the treatment of fever elsewhere (Delazar*et al*., 2006 and Delazar *et al*., 2005).

C.plicata has an emetic, drastic and corrosive property. Its seeds are used as cathartic (Gamble, 1967). The leaf extracts exhibited strong fungi toxicity against *P. aphanidermatum*, the plant poisoning causes salivation, dyspnea, bloat, dullness, diarrhea, paresis of the hind limbs, recumbence and lateral deviation of the head and neck (Galal and Adam,1988).

C.rottleri is traditionally used for the treatment of various diseases. In Sudan people use stems or whole plant as powder and apply it to wounds to improve healing. The plant is also used in Saudi Arabia and India to treat Jaundice and for purifying blood. An infusion of seeds and leaves is taken as a laxative in Ethiopia ; in Senegal the plant is not browsed by most stock, except occasionally by sheep and goats, as it causes vomiting and diarrhea, whereas in Kenya, camels graze it. The fruits yield a purplish blue dye, which is used to dye mats in East Africa (Prota 11 (1) 2010. The fruit juice is given in cases of cough and cold in Nepal (Manandhar and Manandhar , 2000). The leaves of *C.rottleri* are used as a depurative agent and they are very much beneficial in treatment of skin diseases (Khare, 2007) .The seeds are used as cathartic like Ghodtapde and credited with purgative properties. Priyanka P *et al*., (2010) reported that, the aqueous extract of the leaves of this plant has a significant anti-helmintic property against Pheritima posthuma (Indian Earth worm). The aqueous extract of *C.rottleri* possessed phytotoxic activity on rice, wheat and mustard. In an experimental study by Suparna and Tapaswi,(1999) they reported that, the leaf extracts of *C.rottleri* exhibited higher inhibition of shoot, root and radial elongation than the stem and root.

2.2.5. Toxicity

Several toxic dolabellane diterpene glucosides, dolabellane diterpenoids and phenylpropanoid glucosides have been isolated from *C.obliqua*. Although rats fed 10% leaves in their diet had a low growth rate, bouts of soft feces, lesions of

internal organs and alterations in blood and urea, no death occurred among the rats (Galal and Adam, 1988 and Adam *et al.*,1999).

The fresh shoots of *C.plicata* force-fed to Nubian goats and desert sheep caused all animals to die, and the main signs of poisoning were salivation, dyspnoea, bloat, loss of appetite, dullness, diarrhea, paralysis of the hind limbs and lateral deviation of the head and neck (Prota 11 (1) 2010. *C.brocchiana* oil and it's by products (cakes) did not show toxicity using the brine shrimp (Artemia salina) lethality test (Hussein *et al*., 1994). In addition, the seeds of these plants contain a great number of other compounds, which have a potential value as food and for production of non-food products.

3.0 Materials and Methods

3.1. Materials

3.1.1. Samples

The fresh leaves, stems and seeds of *C.brocchiana* plant were collected From Ghibaish, Western Kordofan state, and sorghum flour obtained from Medani market, Gezira state, Sudan.

3.1.2. Solvents and Reagents

All solvents used were of analytical grade. Methanol, n-hexane, Ethyl acetate, sodium hydroxide, hydrochloric acid, methyl ester, potassium hydroxide, sodium sulphate, n-heptane, diethyl ether, ethanol, potassium iodide, sodium thiosulfate, H_2SO_4, ethyl alcohol (96%) phenolphthalein indicator, ninhydrine, sodium chloride, diethyl ether, copper sulphate-sodium sulphate, HCl 0.1N ,ammonia-boric acid sulphuric acid, boric acid (2%),sodium hydroxide solution (33%), H_2SO_4 (0.255N). NaOH (0.313N), M potassium hydroxide ethanolic, n-heptane/tert. butyl methyl ether, acetic acid-chloroform,

3.2. Methods

3.2.1. Preparation of samples

The plant materials were air-dried in the laboratory and then ground into powder form using a mortar and sieving and then stored in air tight bottles. The oil was extracted by hexane using Soxhlet apparatus from the ground seeds, and by mechanical cold press using traditional mill.

3.2.2. Germination process

Germination of the seeds was achieved follwing Akpapunam *et al*., (1997). Thus,500g of *C.brocchiana* seeds were washed with 5% (w/v) NaCl solution in Petri dishes containing wet filter papers. The dishes containing the seeds were kept at room temperature (30°C) and allowed to germinate for 72 h

in light. The seeds were watered at regular intervals (three times a day). Germinated seeds were removed and dried in air for 6 h. The dried samples were milled into flour of 300µm particle size and stored for analysis. Germination treatment was processed in duplicate .

3.2.3.The Seed Chemical Analysis

3.2.3.1.The moisture content

The moisture content of *C.brocchiana* seed was determined according to the standard methods of the Association of Official Analytical Chemists (AOAC, 2005).The moisture in weighed sample is removed by heating in an oven (under atmospheric pressure) at 105˚C. Then, the difference in weight before and after drying was calculated as a percentage from the initial weight. In brief, A sample of 5g±1mg was weighed into a pre-dried and tarred dish. Then, the sample was placed into an oven (NO. 03822, FN 400,Turkey) at 105˚C until a constant weight was obtained. After drying, the covered sample was transferred to a desiccator and cooled to room temperature before reweighing. Duplicate results were obtained for each sample and the mean and ± SD values were reported according to the following formula:-

$$\text{Moisture content [\%]} = \frac{[m2 - m3] \times 100}{[m2 - m1]}$$

Where;

m1 = Wt of dish +cover, g

m2 = Wt of dish +cover+ sample before drying, g

m3 = Wt of dish +cover+ sample after drying, g

[eq.1]

3.2.3.2. Crude Protein Content

The protein content was determined in all samples by macro- Kjeldahl method using a copper sulphate-sodium sulphate catalyst in digestion according to the official method of the AOAC (2005).

The method consists of sample oxidation and conversion of nitrogen to ammonia, which reacts with the excess amount of sulphuric acid , forming ammonium sulphate. After that the alkaline solution and the ammonia-boric acid complex were titrated against standard solution of HCl (0.1N). Accordingly, the protein content was calculated by multiplying the total N_2 % by 6.25 as a conversion factor for protein. In brief 2g of each sample were accurately weighed and transferred together with 1g of Kjeldahl catalysts and 25 ml of concentrated sulphuric acid into a Kjedahal digestion flask. After that, the flask was placed into a Kjeldahl digestion unit for about 2 h, until a colorless digest was obtained and the flask was left to cool to room temperature. The distillation of ammonia was carried out in 25ml boric acid (2%) by using 100ml distilled water and 70ml sodium hydroxide solution (33%). Finally, the drops of mixed indicator (Bromocreasol green and methyl red) were added until a brown reddish color was observed.

Calculation:

$$\text{Protein content [\% DM]} = \frac{[\text{ml HCl sample} - \text{ml HCl blank}] \times 0.1 \times 0.014 \times 6.25 \times 100}{\text{Sample weight (g)}} \quad \text{[eq.2]}$$

3.2.3.3 Fat Content

AOAC (2005).The method determined the substances, which are soluble in n-hexane (40-60°C) and extractable under the specific conditions of Soxhlet extraction method. Then, the dried hexane extract (fat content) is weighed and reported as a percentage of the initial dry matter.

A sample of 5g±1mg was weighed into an extraction thimble and covered with cotton that was previously dipped in n-hexane **.**Then the sample and a pre-dried and weighed extraction flask containing about 100 ml n-hexane was attached to the extraction unit (Electrothermal, town, England) at 50–60°C in a Soxhlet apparatus for 6 h. Then the flask was disconnected from the unit and the solvent was redistilled. Later, the flask with the remaining crude hexane extract was put in an oven for 3h, cooled to room temperature in a desiccator, reweighed and the dried extract was registered as fat content [%DM] according to the following formula:-

$$\text{Fat content [\% DM]} = \frac{\text{dry extract weight (g)} \times 100}{\text{Sample weight (g)}}$$

[eq.3]

3.2.3.4.Crude Fiber Content

Crude fiber content was determined according to the official method of the AOAC (2005). The crude fiber was determined gravimetrically after the sample was digested in chemical solutions. The weight of the residue after ignition is then corrected for ash content and was considered as crude fiber.

About 2g ± 1mg of a defatted sample was placed in a conical flask containing 200 ml of H_2SO_4 (0.255N). The flask was then fitted to a condenser and allowed to boil for 30 minutes. At the end of the digestion period, the flask was removed and the digest was filtered under vacuum through a porcelain filter crucible. Further, the precipitate was repeatedly rinsed with distilled

boiled water followed by boiling in 200 ml NaOH (0.313N) solution for 30 min under reflux condenser. Then, the precipitate was filtered and rinsed with hot distilled water, 20 ml ethyl alcohol (96%) and 20 ml diethyl ether. Finally the crucible was dried at 105°C to a constant weight, cooled (in a desiccator), weighed and ashed in a muffle furnace (No.20.301870, carbolite, England) at 550-600°C until a constant weight was obtained. After cooling to room temperature, the difference in weight was considered as crude fiber.

Calculation:

$$\text{Crude fiber [\% DM]} = \frac{[\text{ dry residue + crucible (g) }]-[\text{ ignited residue + crucible (g) }] \times 100}{\text{Sample (g)}}$$

[eq.4]

3.2.3.5 Ash Content

Ash was determined by incineration (550°C) of known weights of the samples in a muffle furnace (Gallenkamp, size 3) (Method No 930.05) [AOAC, 2005].

The inorganic materials, which vary in concentration and composition, are customarily determined as a residue after being ignited at a specified heat degree.

A sample of 5g±1mg was weighed into a pre-heated, cooled, weighed and tarred porcelain crucible and placed into a muffle furnace at 550-600°C until a white gray ash was obtained. The crucible was then transferred to a desiccator and allowed to cool to room temperature and weighed. After that, the ash content was calculated as a percentage based on the dry matter of the sample.

Calculation:

$$\text{Ash content [\% DM]} = \frac{\text{residue weight (g)} \times 100}{\text{Sample weight (g)}}$$

[eq.5]

3.2.3.6. Soluble Carbohydrates and Starch

The soluble carbohydrates content of the sample was calculated by subtracting the total sum of (moisture, fat, protein, fiber and ash) from 100%.

%Carbohydrate = 100 - (%Moisture + %Fat + %Ash + %Crude fibre + %Crude protein).

3.2.3.7. Mineral Analysis

The mineral content of *C.brocchiana* seed cake were measured in duplicate according to the standard methods of the AOAC (AOAC 2005) using atomic absorption spectrometer (3110;Perkin Elemer. Waltham, MA, USA) .

3.2.3.2.Physical Characteristics of Seeds

The weight of 100 whole seeds chosen randomly was determined using a sensitive balance (Mettler- Toledo, Columbus, OH, USA). The seed measure was determined using a vernier caliper (Qingdao Co., Ltd., Shandong, China), the mean and standard deviation were calculated. The weighed hundred seeds were decorticated manually, care being taken not to lose any hulls or kernel. Hulls and kernels were weighed and the proportion weight of the hulls and kernel was computed. All analyses were carried out in duplicate.

3.2.3.3.Physicochemical Properties of the oil

3.2.3.3.1. Oil Colour using Spectrophotometric Method

The color of oil from ungerminated and germinated seeds was determined spectrophotometrically following method CT 39 (IQA) NP-1819 (1984), and the apparatus used was a digital spectrophotometer (hitachi, LTD, Tokyo, Japan).

3.2.3.3.2. Refractive Index (RI)

This was carried out according to the AOCS official method Cc 7-25 (2006); the refractive index was measured at 25°C, and the apparatus used was digital refractometer PR-303, Atago, town, Japan.

3.2.3.3.3.Unsaponifiable Matter

Unsaponifiable matter was determined using diethyl ether method according to the American Oil Chemists Society (AOCS, 2006). Five grams of the dry-filtered samples were weighed and then transferred into a 250 ml round-bottom flask containing 50 ml of 2 M potassium hydroxide ethanolic solution. The flask was fitted with a condenser and heated to slight boiling, with continuous stirring until the solution became clear (indicating saponification completion). After heating for a further 30 min, the contents of the flask were transferred to a 500 ml separatory funnel, washed several times with 50 ml of distilled water; afterwards, 80 ml of ethyl ether were added and shaken for approximately one minute and the mixture was allowed to settle. The lower aqueous phase was separated off (3 times) and collected in a second reparatory funnel. The ethyl ether extracts were combined in a separatory funnel and washed with distilled water until the wash water gave a neutral reaction. The wash water was discarded from the extracts, dried with anhydrous sodium sulphate and filtered into a pre-weighed bottle (W_0). The ethyl ether was then distilled, then brought to complete drying in an oven at 100°C for approximately 1 h, and then weighed after cooling in a desiccator (W_1). The contents of unsaponifiables were calculated as follows:

$$\text{Unsaponifiables (\%)} = \frac{W_1 - W_0 \times 100}{\text{Oil (g)}}$$

Where, W0: a pre- weighed bottle,W1:weighed bottle after cooling

3.2.3.3.4. Free fatty acids (FFA%)

Free fatty acids (FFA%) were determined using AOCS (2006) method Ca 5a-40. In brief: the liquid sample was dissolved in a specified amount of hot neutralized alcohol, and 2 ml of phenolphthalein indicator were added, and then titrated with sodium hydroxide. The contents of free fatty acids were calculated as follows:

$$\text{Free fatty acids as oleic (\%)} = \frac{\text{ml of alkali} \times N \times 28.2}{\text{Weight of sample}}$$

Where N: Normality of the sodium hydroxide solution

3.2.3.3.5.Peroxide value (PV)

The peroxide value of a fat is a measure of its content of reactive oxygen, in term of millimoles of peroxide, or milliquivalents of oxygen per kg of fat. Peroxide value was determined following the Method Cd 8-53 (AOCS 2006). In brief, 5g sample were dissolved in 30 ml of a 3:2 acetic acid-chloroform mixture and 0.5 ml of saturated potassium iodide solution was added, the liberated iodine was titrated with 0.1N sodium thiosulfate. All analyses were done in duplicate and the obtained mean value was used.

$$\text{Peroxide value (meq of peroxide per 1000g of samples} = \frac{(S-B\ (N)X(1000)}{\text{Wt of sample}}$$

Where,

S: sodium thiosulphate volume, B: blank and N:normality

3.2.3.3.6.Fatty Acids Composition (FAC)

Oils from germinated and ungerminated *C.brocchiana* seeds were derivatized to methyl esters following the method of Christie (1989). Methyl ester sample (1µL) were syringed into a Gas Chromatograph (GC-2010A, Shimadzu, Tokyo, Japan) provided with a flame ionization detector and a

BPX70 capillary column of 30m length, and 0.32 mm id. (SGE,Melbourne, Australia). The starting temperature was 140°C for 2 min, which was then increased at 8°C min^{-1} to 220°C where it was held for another 5 min. The oven, the injector and the detector ports were set at 140, 240 and 260°C, respectively. The carrier gas was helium with column flow rate of 1.10 ml min on a 50:1 split ratio. The fatty acid peaks were identified by comparing the retention times with those of a mixture of standard FAMEs (Sigma Chemicals, Deisenhofen, Germany). All determinations were carried out in duplicate and mean value ± SD were reported.

3.2.3.3.7.Tocopherols

For determination of tocopherols of oil from ungerminated and germinated *C.brocchiana* seeds a solution of 250 mg oil in 25 mL *n*-heptane was directly injected in an HPLC , using a Merck-Hitachi low-pressure gradient system, fitted with a L-6000 pump, a Merck-Hitachi F-1000 Fluorescence Spectrophotometer Detector (wavelengths for excitation 295 nm, for emission 330 nm) and a D-2500 integration system. Twenty samples were injected by a Merck 655-A40 Auto-sampler onto a Diol phase HPLC column 25 cm x 4.6 mm ID (Merck, Darmstadt, Germany) at a flow rate of 1.3 mL/min. The mobile phase used was n-heptane/tert. butyl methyl ether (99+1, v/v) (Balz M. *et al*., 1992). All analyses were carried out in duplicate.

3.2.3.4. Oxidative Stability

3.2.3.4.1. Peroxide Value

The oxidative stability of oils extracted from ungerminated and germinated *C.brocchiana* seeds, was studied by using 60 g of each oil transferred in duplicate to 100-mL glass beakers. The samples were stored in forced-drafted air oven at 70°C for 72 h (Lee *et al*., 2004). The oxidative stability of oil was determined by the increase in peroxide value, which was determined in duplicate according to AOCS official methods (AOCS 2006).

3.2.3.4.2. Fourier Transform Infrared Spectroscopy (FTIR)

The IR spectra were gathered utilizing a Vector 22 FTIR spectrometer (8400S; Shimadzu) with OPUS software (version 3.1). A film of oil (five drops) was placed on the attenuated total reflectance (ATR) device, which was equipped with a ZnSe crystal (Van de Voort *et al*.,1994). The spectra were obtained using 128 scans and rated against the spectrum of the clean crystal. The range from 4000 to 400 cm1 with a resolution of 4 cm1 was used to obtain spectral information. After each measurement, the ATR plate was carefully cleaned by wiping it with analytical-grade acetone and dried with a soft tissue before it was filled with the next sample. Two spectra replicates were obtained for each sample.

Statistical Analysis

All experiments were carried out in duplicate unless otherwise stated; results are expressed as means ± SD. Statistical analysis was carried out using a one-way ANOVA with a significance level of $p \leq 0.05$. The software used for the statistical analysis was the SPSS for Windows statistical package (v.10.0.6; SPSS, Chicago, IL, USA).

3.2.3.5. Amino Acids Composition

The amino acid content (except for tryptophan) in ungerminated seeds of *C.brocchiana* plant was determined using an Amino Acid Analyzer (L-8900 Hitachi-hitech, Tokyo, Japan) under the experimental conditions recommended for protein hydrolyzates. Samples containing 5.0 mg of protein were acid hydrolyzed with 1.0 mL of 6 N HCl in vacuum-sealed hydrolysis vials at 110°C for 22 h. Ninhydrine was added to the HCl as an internal standard. The tubes were cooled after hydrolysis, opened and placed in a desiccator containing NaOH pellets under vacuum until dry (5–6 days). The residue was then dissolved in a suitable volume of NaS buffer, pH 2.2 filtered through a

Millipore membrane (0.22-lm pore size, Millipore, Billerica, MA, USA) and analyzed for amino acids by ion-exchange chromatography in a Beckman (model 7300, Pickering Laboratories, Inc. Mountain View, CA, USA) instrument, equipped with an automatic integrator. Amino acid nitrogen was determined by multiplying the concentration of individual amino acids by corresponding factors calculated from the percentage N of each amino acid (Mosse, 1990). The ammonia content was included in the calculation of protein nitrogen retrieval, as it comes from the degradation of some amino acids during acid hydrolysis (Yeoh *et al*., 1996; AOCS 1993). The ammonia nitrogen content was calculated by multiplying the ammonia content by 0.824 (N = 82.4% NH3).

Amino acids calculation

The amount of amino acid obtained was calculated (g/100g) by the formula:

X= Area of Asp in the sample X Area of internal std (AABA) X amount of std X dilution factor

Area of Asp std X Area of sample internal std X sample weight

Where:

X=represents the amount of amino acid (g/100g)

3.2.3.5.1.Energy Value (kcal)

The sample calorific value was estimated (in kcal/g) by multiplying the percentages of crude protein, crude fat and carbohydrate with the recommended factors (2.44, 8.37 and 3.57, respectively) as proposed by Martin and Coolidge (1978).

3.2.3.5.2. Nutritional Quality of the Samples

Nutritional quality of the samples was determined on the basis of the amino acid profiles. The Essential Amino Acid Index [EAAI] was calculated using the method of Labuda *et al*. (1982) according to the following equation:

$$EAAI = \sqrt[9]{\frac{[\text{Lys } x \text{ Threo x Val x Meth x Isoleu x leu x Phynylal x Histi x Trypt}]a}{[\text{Lys } x \text{ Threo x Val x Meth x Isoleu x leu x Phynylal x Histi x Trypt}]b}}$$

Where: [lysine, tryptophan, isoleucine, valine, threonine, leucine, phenylalanine, histidine and methionine][a] in test sample and [lysine, tryptophan, isoleucine, valine, threonine, leucine, phenylalanine, histidine and the sum of methionine and cystine][b] content of the same amino acids in standard protein [%] [wheat flour] respectively.

Nutritional index of the food samples were calculated using the following formula:

$$\text{Nutritional Index [\%]} = \frac{\text{EAAI} \times \%\ \text{protein}}{100}$$

3.2.3.5.3. Biological value (BV)

Biological value is the capacity of the protein to maintain the N balance or to promote growth and it was calculated according to Mune-Mune *et al*., (2011) using the following equation:

BV = 1.09 × Essential amino acid index [EAAI] - 11.7

The Protein Efficiency Ratio [PER] is the weight gain per weight of protein eaten and it was estimated according to the regression equations as given below (Mune-Mune *et al*., 2011):

PER = - 0.468 + 0.454 (LEU) - 0.105(TYR)

3.2.3.5.4. Amino Acid Scores

A chemical grading of the quality of a protein can be made by comparing its amino acid content with that of reference protein (Passmore and Eastwood, 1986).

Amino acid scores (%) was calculated using the following formula:

$$\text{Amino acid scores (\%)} = \frac{\text{Value of essential amino acid in diet } (\frac{g}{100}\text{g protein})}{\text{FAO Ref.value for essential amino acids}} \text{X100.}$$

3.2.3.6. Preparation of *Chrozophora brocchiana* Seed Protein Concentrate *(CSPC)*

Protein was extracted from CSPC using alkali solution with isoelectric precipitatiton and freeze drying .The dried defatted seed was weighed and suspended in distilled water in 1:10 (w/v) ratio using magnetic stirrer, the mixture was stirred for 1 hour while adjusting the pH at 9.0 using sodium hydroxide NaOH solution (4M). Then, the mixture was centrifuged at 3500 rpm for 15 minutes at room temperature. The supernatant was transferred into a beaker and stirred for another 30 minutes and the pH was adjusted into 4.5. The supernatant was left undisturbed for cold precipitation overnight in 4°C freezer. After that, the supernatant was carefully siphoned off and the protein slurry was washed 3 times with distilled water by adding in some distilled water and centrifuging at 3500rpm for 10 minutes at 4°C. The pellet was then mixed together and some distilled water added in. The pH was adjusted at 7.0. The slurry was kept overnight inside -80°C freezer before it was freeze dried (Chandi and Sogi 2007). The sample inside the freeze dryer took 2 to 3 days before it was completely dried. The protein concentrates obtained from ungerminated and germinated seeds were weighed using analytical balance.

3.2.3.6.1.Protein Concentrate Properties

3.2.3.6.1.1.Water Absorption Capacity (WAC*)*

The method of Jyothirmayi *et al*., (2006) was followed , where 0.1 g of **CSPC** sample was taken and mixed with 1 ml of distilled water. and the slurry was centrifuged at 3000rpm for 15 minutes. The supernatant was removed. and the pellet was drained for 30 min and the gain in weight per unit weight was reported as water absorption capacity (g/g).

3.2.3.6.1.2. Oil Absorption Capacity (OAC)

One gram of **CSPC** sample was taken and mixed with 10 ml of refined sunflower oil, vortexed thoroughly, and centrifuged at 3000rpm for 15 minutes. The oil absorbed by the samples was noted and expressed as oil absorption capacity (g/g) (Beuchat 1977).

3.2.3.6.1.3.Foaming Capacity and Foam Stability (FC& FS)

Foaming capacity (FC) of **CSPC** was determined by measuring the volume of foam immediately after the introduction of air (90cm^3/min) for 15s into 5ml of 0.2% protein solution in 0.05M phosphate buffer (PH 7.4) in a glass tube (2.4 x 30cm). Foam stability (FS) was calculated from the following equation:

$$FS = V0\ (\Delta t/\Delta V)$$

Here F: foaming , S: stability, ΔV is the change in the volume of foam (V), occurring during the time interval, Δt (30 min), and $V0$ is the volume of foam at 0 time (Kato, 1989).

3.2.3.6.1.4. pH system (5, 7 and 9)

0.25 g of **CSPC** was dispersed in 25 ml of citrate buffer PH 5, 7, and 9. The dispersion was whipped in a blender for 2 minutes and immediately transferred to a measuring cylinder. The foam height was noted with time till it collapsed and the half life was calculated.

3.2.3.6.1.5. Salt system (0.5%, 1.0%, and 1.5%)

0.25 g of protein concentrate was dispersed in 25ml of citrate buffer PH 7 with chloride, NaCl 0.5%, 1.0% and 1.5%. The dispersed was whipped in a blender for 2 minutes and immediately transferred to a measuring cylinder. The foam height was noted with time till it collapsed half life.

3.2.3.6.1.6. Sugar system (5%, 10%, and 15%)

0.25g of protein concentrate was dispersed in 25ml of buffer PH 7 with sucrose 0.5%.10% and 1.5%. The dispersion was whipped in a blender for 2 minutes and immediately transferred to a measuring cylinder. The foam height was noted with time till it collapsed half life.

3.2.3.6.2. Supplemented Food

3.2.3.6.2.1.Preparation of Kisra (Control) and Supplemented Sample**s**

In the control sample 500 grams of sorghum flour were mixed with 1000ml of water in a round earthenware container (Khumara)., and fermented dough (150g) was added to the mixture of flour and water to act as starter . In supplemented sample, 5%,10% and 15% of CSPC from ungerminated and germinated seeds were used separately to supplement sorghum flour in such a way that dough contained a total of 500 gram sorghum and CSPC. One thousand ml of water and 150 g of starter were then added. The various samples were allowed to ferment for 24 hours at 32C°

3.2.3.6.2.2. Kisra baking method

Kisra was baked traditionally according to the method described by Dirar (1993) with slight modifications. An electrical plate (Saj) was heated to 150°C and 80 g of fermented dough was spread on the hot plate into a thin sheet which was peeled off the plate after the time between 10-15 seconds of baking. The produced kisra

were stacked one over the other and was ready for further analysis

3.2.3.6.2.3. Sensory Evaluation of the Supplemented Kisra.

The sensory panels can measure most aspects of food quality. Food quality and acceptance can be indicated more tightly by correlating the quantitative and qualitative measurement of the various sensory characteristics contributing to the overall appreciation of the food with the consumer assessment or preference ratings (Williams, 1970; Larmond, 1982).

Sensory evaluation is defined as a scientific discipline used to measure, evoke, analyze, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing. Hedonic tests decide on the attribute to judge the quality e.g. texture and flavor, and they allow tasters to evaluate samples using scale of 1 to 9 as follows: 1, extremely bad; 2, very bad; 3, bad; 4, fairly bad;5, satisfactory; 6, fairly good;7, good;8, very good; 9, excellent.

The produced Kisra (A1, A2, A3, B1, B2, B3, C) was subjected to panel tests where color, texture, flavor and overall acceptance assessments were obtained by using hedonic test. The results obtained by the panelists were statistically analyzed.

Twenty semi-trained panelists were selected among the students of the Department of Food Science and Technology, University of Gazira. The procedures for the samples preparation, the training of the panelists, and the establishment of the sensory attributes were based on Meilgaard *et al*. (1999).

The tests were conducted in the laboratory of the Department of Food Science and Technology, University of the Gazira.

3.2.3.7. Antimicrobial Activity

3.2.3.7.1. Phenolic Compounds Extraction

3.2.3.7.1.1. Phenolic Compounds of Leaves and Stem

The phenolic compounds of leaves and stem were extracted following the method of Parekh *et al*,(2005) .In brief: 100 g of the dried ground leaves and stem of *C. brocchiana* was taken in 500 ml of methanol 80% (v/v), ethyl acetate and hexane, respectively and separately in conical flask, plugged with cotton wool and then kept on a rotary shaker at 220 ppm for 16 h, then the yield was collected using a filter paper. This procedure was repeated twice. The solvents were removed using a rotary evaporator and the final volume was taken then the final yield was stored at -8°C in a dark bottle, till analysis.

3.2.3.7.1.2. Phenolic Compounds-Free Oil

Phenolic compounds-free oil was obtained by extracting phenolic compounds from the crude oil following the method of Tsimidou *et al*. (1996). In brief, 50 g oil was dissolved in 50 mL petroleum ether using a 250-mL extraction funnel, and then was extracted three times with 30 mL of a mixture consisting of methanol: water (60:40, v/v). The lower layers containing phenolic compounds were removed. The petroleum ether was evaporated in a rotary evaporator at 40°C to obtain phenolic compounds-free oil. Samples of phenolic compounds-free oil were weighed and kept at -8°C for further analysis

3.2.2.7.1.3. Preparation of Different Concentrates of the Phenolic Extracts

One gram of each crude extract was dissolved in 10ml sterile distilled water to prepare four concentrations of the test extracts (0.1- 0.01- 0.001- 0.0001).

3.2.3.7.1.4. Test Strain and Culture Media

Bacterial and fungal strains were obtained from ATCC (American Type Culture Collection) obtained from the stock of the Department of food Hygiene and Safety Laboratory at University of Khartoum. Antimicrobial activity of the plant extracts against *B. subtilis'* DSM618, *B. cereus* MK 131, *S. aurous* ATCC 29213, *E.coli* (ATCC 25922), *P. aeruginosa* ATCC 27853, *S. typhi* laboratory isolates, *C. albians* (ATCC90028) and *A. flavus*- laboratory isolates-was studied.

The species of bacteria were grown in nutrient agar (HIMEDIA M001) and fungal species in Sabouraud dextrose (HIMEDIA M063). The concentration of bacteria suspensions was adjusted by using
Macfrland SHD turbidity Tube. All bacterial strains were cultured aerobically at 37°C in nutrient broth and agar medium. Before the susceptibility test, cultures from solid mediums were sub-cultured in liquid media, incubated for 18 h and used as the source of inoculums for each experiment.

A standard antibiotic disc of Streptomycin was used as control at concentration of 106 cfu/mL for inoculating nutrient agar plates for antibiotic sensitivity disc. All tests were carried out induplicate.

3.2.3.7.1.5. Assay of Antimicrobial Activity Using Agar Well Diffusion Method

The antimicrobial activity of the plant extracts to the test organisms was screened by using the agar well diffusion method (Perez *et al*., 1990) with a slight modification. The 25 ml of sterilized nutrient agar was poured into sterile petriplate, after solidification (28 g of nutrient agar in 1000ml sterile distilled water for bacterial and 65g of Sabouraud dextrose in 1000 ml sterile distilled water for fungi). 100µl of the fresh culture microorganism were swabbed on the respective plats. The well was prepared in the plats with the help of a cork-borer (1cm) then 100µl of the test extracts introduced into the well and were allowed

to stand on the bench for 1 h for proper diffusion. The plats were incubated overnight at 37°C for bacterial, and 48 h at 30°C for fungi (memmert). Microbial growth was determined by measuring the diameter of zone inhibition. The results were obtained by measuring the diameter.

4.0 Results and Discussion

4.1.Physical Characteristics of *Chrozophor brocchiana* Seeds

As shown in **Fig 4.**1*C.brocchiana* seeds are similar to those of sorghum in shape and size, yet are somewhat darker in colour.

Table4.1. shown that the weight of 100 seeds (kernel+ hull) was 3.27 g which is similar to that reported by Hussein *et al*.,2006. The weight of 100 kernels was 2.07 g which was higher than the weight of 100 hulls 1.2 g. The ratio of the hull to the kernel was 43:57. The percentage of kernel to whole seed was 63.3 while it was 36.7 for hull to whole seed.

4.2.Effect of Germination on Seed, Chemical Analysis and Oil Physicochemical Properties

Table 4.2 demonstrates the proximate chemical analysis of ungerminated and germinated *C.brocchiana* seed .

The results of moisture, protein, fiber and ash contents of ungerminated seed were 4.87±0.05, 18.05±0.07, and 21.7±0.28 and 1.05±0.08%, respectively. These results were increased by germination process to 6.75± 0.07, 18.6±0.14, 27.2±0.14, and 1.95±0.21 respectively, while fat and carbohydrates before germination were 42.9±0.14 and 11.28±0.70%, respectively, and were decreased to 37.85± 0.07 and 7.65± 0.49% after germination process. The increase of protein content improves the nutritional value of the germinated seeds.

Table 4.1. Description of *Chrozophora brocchiana* Seeds*

Sample	Length (mm)	Weight of whole seed (100 seeds/g)	Weight of kernel (100 kernels/g)	Weight of hull (100 hulls/g)	Kernel /whole seed (%)	Hull/whole seed (%)	Hull: kernel ratio
seeds	0.039 ±0	3.27±0.02	2.07	1.2	63.30	36.7	40:60

Measurements of the seeds were carried out in triplicate and mean value ± standard deviations (SD) were reported.

Table 4.2. Proximate Analysis of Ungerminated and Germinated *Chrozophora brocchiana* Seeds*

Parameter	Ungerminated seeds	Germinated seeds
Moisture of seed, (%)	4.87± 0.05	6.75± 0.07
Oil Content, %	42.9± 0.14	37.8± 0.07
Protein in cake, (%)	18.0±0.07	18.6±0.14
Fiber (%)	21.7±0.28	27.2±0.14
Ash (%)	1.05±0.08	1.95±0.21
Soluble carbohydrates + starch (%)	11.4± 0.35	7.6± 0.49

*All determinations were carried out in triplicate and mean value ± standard deviation (SD) was reported.

The increase of protein content by germination might be due to the net synthesis of enzyme protein, which might have resulted in the production of some amino acids during protein synthesis (Kim *et al*., 2012). The results show that *C.brocchiana* seed is a good source of oil (42.9%) and protein (18.2%), the values of protein and oil were compared favorably with those reported in previous studies (Hussein *et al*., 2006 and Mirghani *et al*., 1996). The slight increment in protein is chiefly because of the utilization of the seed components and degradation of the high molecules of the protein to simple peptides during germination process. This result agrees with Tian *et al.,(*2010) who reported that the protein concentration in oat seeds gradually expanded as a result of germination.

From **Table 4.2**, the reduction in fat and carbohydrate contents could be ascribed to their utilization as a source of energy for the germination process. These results are in good agreement with Akpapunam *et al*., (1997) who reported that the germination process reduced fat and starch contents and increased the protein and fiber content of jack beans. The obtained results are in contrast with Mariod *et al*., (2012) who reported that germination of black cumin seeds increased both oil and protein contents while other constituents were decreased. Colmenares and Bressani, (1990) reported a marked increase in moisture content during germination of Amaranth species. And they reported that protein, ether extract, crude fiber, and ash contents did not change significantly.

Table 4.3. Physicochemical Properties of *Chrozophora brocchiana* seed Oil

Parameter	**A**	**B**	**C**
Refractive Index, (25°C)	1.4720±0.00	1.4718±0.00	1.4720±0.00
Free Fatty Acids (%)	2.66±0.01	6.30±0.14	3.3± 0.07
Unsaponifiable matter (%)	0.022±0.00	0.040±0.00	0.0.24±0.00
Color	2.2 R, 2.8 Y	8.9R,7.0Y	7.5 R,7.1Y
Peroxide Value, meq/kg oil	1.4±0.07	2.2±0.14	1.1±0.14

A:Ungerminated seeds oil **B**:Germinated seeds oil **C**:Cold press oil R:red Y:yellow. *All determinations (except color) were carried out in duplicate and mean value ± standard deviation (SD) was reported

4.3. Physicochemical Properties of *Chrozophora brocchiana* Seed Oil

Table 4.3 shows that the oil extracted from ungerminated seeds had 2.66% of free fatty acids; this value was increased significantly
($P < 0.05$) to 6.30±0.14 as affected by germination process. The reading of its color was red 2.2, yellow 2.8, and blue 0.0, these values were changed to 8.9 ,7.0 and 0.0, respectively, indicated a clear intensive in yellow color while red color was decreased. The free fatty acids were high when compared with Hussein *et al*.,(2006) who reported that *C. brocchiana* freshly extracted oil had low free fatty acid that increased due to long storage.

From **Table 4.3** the unsaponifiable matter of oil extracted from *C.brocchiana* ungerminated seed was 0.224%, while the refractive index was 1.4720 at 25°C, which agrees with results reported by Hussein *et al*., (2006). And is in the range of sunflower, groundnut and soybean oils (Al-Kahtani, 1983). Unsaponifiable matter was different from that reported by Mirghani *et al*.,(1996).The free fatty acids and unsaponifiable matters increased to 33.3 and 0.40%, respectively, while the refractive index decreased by the germination process to 1.4718. The oil extracted mechanically (cold press) had 3.3±0.07 free fatty acids, 0.024% unsaponifiable matter,1.4720 at 25C refractive index ,1.1±0.14 peroxide value and red, yellow color 7.5, 7.1 respectively.

4.4. Minerals Composition and Effect of Germination Process

Table 4.4 shown the mineral content of ungerminated and germinated *Chrozophora brocchiana* seed cake.

The results from **Table 4.4** shows that the ungerminated seeds had a high content of potassium, magnesium and sodium, which were 32.01±0.01, 9.77±0.01 and 1.58±0.00, respectively. These amounts were highly increased significantly ($P < 0.05$) to 58.70±0.14 for potassium, and to 5.80±0.014 for

sodium, while magnesium showed a little decrease to 9.52±0.14. The content of calcium, manganese, iron and Copper were 0.34±0.0,0.304±0.0, 0.55±0.0 and 0.15±0.0, respectively in ungerminated seeds and it was increased slightly to 1.2±0.0, 0.31±0.0, 0.53±0.0 in geminated seeds, with the exception of iron which was decreased to 0.50±0.0 by germination process. Potassium was the predominant element in the seeds followed by magnesium and sodium then calcium, manganese and iron. Germinated seeds of *C.brocchiana* showed a noticeable increase in the contents of Na, K, Ca and Zn. These results are sensibly great in light of the fact that potassium assumes an essential part in human physiology, and a sufficient content of it will lessen the danger of heart stroke, while calcium assumes a vital part in building stronger, denser bones early in life and keeping bones solid and healthy later in life (Dawson-Hughes *et al*., 1997).

Table 4.4. Minerals Composition of Ungerminated and Germinated *Chrozophora brocchiana* seedcake*

Mineral mg (100g)	Ungerminated seeds	Germinated seeds
Sodium (Na)	1.58 ± 0.00	5.80 ± 0.01
Potassium (K)	32.01 ± 0.01	58.70 ± 0.14
Calcium (Ca)	0.34 ± 0.00	1.20 ± 0.00
Magnesium (Mg)	9.77 ± 0.01	9.52 ± 0.01
Copper (Cu)	0.15 ± 0.0	0.53 ± 0.0
Iron (Fe)	0.55 ± 0.0	0.50 ± 0.0
Manganese (Mn)	0.30 ± 0.0	0.31 ± 0.0
Zinc (Zn)	0.83 ± 0.0	1.57 ± 0.0
Cobalt (Co)	0.02 ± 0.0	0.12 ± 0.0
Lead (Pb)	tr	tr
Chromium (Cr)	tr	tr
Cadmium (Cd)	tr	tr

*Values are means ± standard deviation (SD) of two replicates. tr = trace (detected limit for pb=0.003, Cr= 0.003, Cd=0.001

Table4.5. Fatty Acids Composition and of *Chrozophora brocchiana* seeds oil (%) and effect of germination process

Fatty acid (%)	A	B	C
Myristic 14:0	0.11	0.12	0.18
Palmitic 16:0	8.0	8.2	9.6
Palmitoleic 16:1	0.2	0.0	0.1
Stearic 18:0	16.0	15.8	12.5
Oleic 18:1	24.9	25.0	31.9
Linoleic 18:2	49.3	50.1	42.6
Linolenic 18:3	0.5	0.5	0.3
Gadoleic 20:1	0.1	0.0	0.3
Total of FA	99.11	99.72	97.48
Saturated fatty acid	24.11	24.12	22.28
Unsaturated fatty acids	75.0	75.5	75.1
Mono- unsaturated	25.2	25.0	32.2
Di- unsaturated	49.3	50.1	42.6
Tri- unsaturated	00.5	00.5	00.3

A& B oil extracted by solvent **A**:ungerminated seeds oil; **B**: germinated seeds oil; **C**: oil extracted by cold press

4.5. Fatty Acids Composition of *Chrozophora brocchiana* oil before and after Germination Process

Fatty acids are very important sources of energy, and most of them have wide range of physiological effects.

Table 4.5 shows the fatty acid composition of oil from ungerminated and germinated *Chrozophora brocchiana* seeds

As shown in **Table 4.5** the major fatty acids in *C.brocchiana* seed oil are linoleic ~50% and oleic acid ~25%, with no difference between solvent extracted ungerminated and germinated seeds. These results are in good agreement with that reported by Hussein *et al*.,(2006) and Mirgani *et al*., (1996) who reported linoleic acid as the most dominant fatty acid followed by oleic, stearic, and palmitic acids.

Table 4.6 shown the changes in the vitamin E (tocopherols) composition of oils of *C.brocchiana* seeds before and after germination process. The amounts of these tocopherols were compared with three commercial oils that are mainly used in Sudanese diet (CODEX-STAN 210-1999).

From this table the amount of 8-t and y-t were 73.6 and 13.5 mg/100g, respectively, these amounts decreased to 60.0 and 4.3 mg/100g due to germination process.

The main tocopherols of the ungerminated sample was delta –tocopherols, which represents 84.6% of the total tocopherols, followed by gamma-tocopherol which represents up to 15.4%. These percentages were 93.3 and 6.7 in oil extracted from germinated seeds. The other tocopherols in the oil of the two samples were below 0.05 mg/ 100 g each. The total vitamin E contents were 87.1 and 64.3 mg/100g in oil from ungerminated and germinated seeds, respectively.

Table 4.6 Vitamin E (tocopherols) Composition of Oils of *Chrozophora brocchiana* Seeds before and after germination process.

Tocopherols mg/100g	A	B	C
α-t	0.0	0.0	3.8
β-T	0.0	0.0	0.2
P8	0.0	0.0	0.7
8-t	73.6	60.0	52.4
y-t	13.5	4.3	14.9
Sum of tocopherols	87.1	64.3	72.1

A& B oil extracted by solvent **A**:ungerminated seeds oil; **B**: germinated seeds oil; **C**: oil extracted by cold press

These amounts were higher than those reported by Codex diet (CODEX-STAN 210-1999). for sunflower oil (44.0 mg/100g), for sesame (33.0 mg/100g) and groundnut oil (17.0 mg/100g). Tocopherols induce a protective effect against oxidative stress linked to metabolic syndrome and are also essential for normal neurological function (Dias, 2012).

The oil extracted by cold press contained α, β-, P8, 8- and y-tocopherols and their amount were found to be 3.8, 0.2, 0.7, 52.4 and 14.9 respectively. This result suggests that *C.brocchiana* seed oil had α- tocopherol when extracted by cold press. This may account for its lower peroxide value in tables 4.3 and 4.8, and may suggest a greater stability.

4.6. Oxidative Stability of *Chrozophora brocchiana* Oil and Effect of Germination Process

The oxidative stability of the crude *C.brocchiana* oil and the effect of the germination process was monitored using peroxide value and FTIR analysis as is shown in **Table 4.8**, **Fig 4.2** to **Fig 4.4**.

The studied samples were subjected to successive heating at 70°C for three days.

Table 4.8. Peroxide values of oil extracted from germinated and ungerminated *Chrozophora brocchiana* seeds and stored for 0 –3 days under oxidative conditions (meq O_2/kg oil)

Sample	Zero time	6 hr	12 hr	24 hr	28 hr	72 hr
Ungerminated oil seed	1.4±0.07	2.4±0.14	3.6± 0.14	10.1±0.21	18.9±0.07	23± 0.14
Germinated oil seed	2.2±0.14	2.9±0.07	3.9±0.14	12.2±0.14	22.9±0.14	24.2±0.14
Cold press extract	1.1±0.14	2.2± 0.14	3.8±0.07	10.3±0.21	17.7±0.14	22.2±0.14

*All determinations were carried out in replicate and mean value ± standard

From **Table 4.8** the Peroxide values of ungerminaed *C. brocchiana* oil increased gradually from 1.1±0.14 meq O_2/kg oil at zero time to 23.2±0.14 after 3 days of storage at 70°C. While peroxide values of the oils obtained from germinated *C.brocchiana* seeds increased from 2.2±0.14 meq O_2/kg oil at zero time to 24.2±0.14 meq O_2/kg oil after 72hrs of storage at 70°C. The initial peroxide value of the oil obtained from germinated seed was slightly higher than that of oil obtained from ungerminated seed.

To study the oxidative stability of *C.brocchiana* oil, the extracted oils from ungerminated and germinated seeds were kept in force- drafted air oven at 70ºc for 72 hrs then examined by FTIR**.**

Fig 4.2 to **4.4** shown the FTIR spectra of the fresh oils extracted from germinated (A) and ungerminated (B) *C. brocchiana* seeds. The prevailing groups in these oils are the same as in other edible oils. From **Fig 4.2** there were fourteen visible peaks in ungerminated *C.brocchiana* seed oil at frequencies of 3473, 3008, 2923, 2854, 2677, 1745, 1649, 1463, 1377, 1236, 1163, 1099, 914, and 723 cm-1. Henna and Tan (2009) reported that absorption peaks at 3600–2800 and 1800–700 cm-1 are the dominant bands in vegetable oils. When the results of germinated seed oils were compared with those of oils from ungerminated seeds, there were several changes in the peak intensities (absorbances). For example, (i) the band at frequency 723 cm−1, associated to bending of –(CH2)n-, HC=CH- (cis) , was changed to 721 cm−1 and its intensity was increased to 56, (ii) the band near 1163 cm−1 experienced an increased in wavenumber and intensity and gave a broad band as affected by germination, while (iii) the band near 1236 cm−1 disappeared as a result of germination (Figure 4.2).

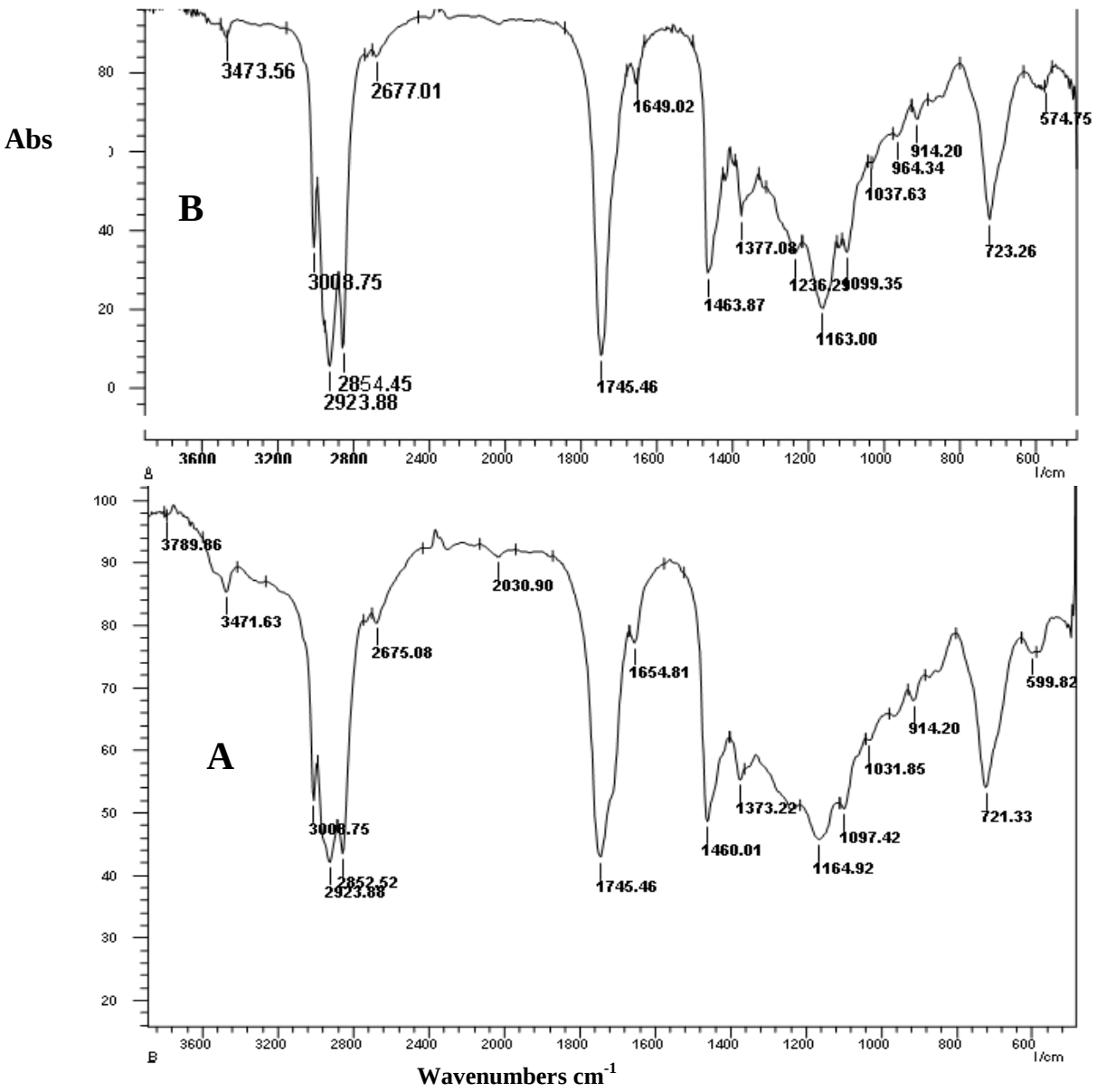

Figure 4.2. Fourier transform infrared spectra of oil extracted from germinated **(A)** and ungerminated **(B)** *Chrozohpora brocchiana* seeds storaged at 70°C for zero hour

Fig 4.3 shows FTIR spectra of the oil extracted from germinated and ungerminated *C. brochiana* seeds stored at 70°C for 24hrs. The peak intensities of oil extracted from ungerminated seeds were changed in comparison with oil extracted from germinated seeds. An increase in the band absorbing at 3473, corresponding to an increase in the concentration of hydroperoxides as a consequence of oxidation of the oil extracted from germinated seeds is quite clear. Upon progressive heating, increments in absorbances of bands corresponding to carbonylic compounds such as aldehydes, esters, ketones, and lactones should be evident Rohman *et al*., (2011).

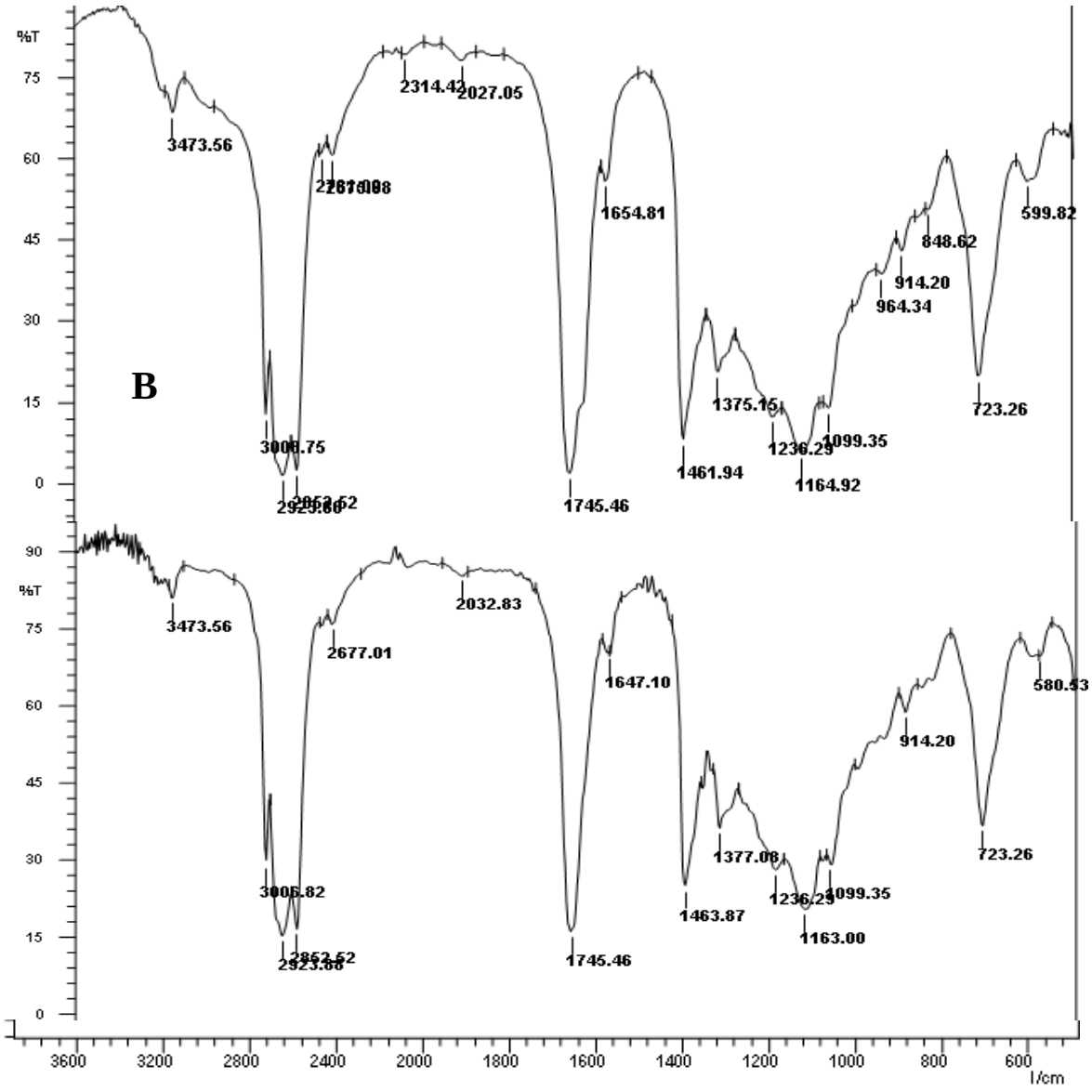

Figure 4.3. Fourier transform infrared spectra of oil extracted from germinated **(A)** and ungerminated **(B)** *Chrozohpora brochiana* seeds storaged at 70°C for 24 hours

Figure 4.4 shows the FTIR spectra of oils extracted from germinated and ungerminated *C. brochiana* seeds incubated at 70°C for 72 hrs. The fourteen peaks were visible at the abobe-mentioned frequencies (3473, 3008, 2923, 2854, 2678, 2028, 1745, 1654, 1460, 1373, 1164, 1099, 914, and 723cm^{-1}). After 72 hrs of incubation, there were sharp changes in the intensities (absorbances) of other peaks such as the peak at 1164 cm^{-1} corresponding to the –C–O, –CH2 – of extending vibration and the band at 1745 cm^{-1} related to C=O of extending vibration, for which the intensity is influenced by germination and by progressive warming at 70°C. The absorbance in the region 2800-3200 cm^{-1} was expanded showing that the bands 2854, 2923 and 3008 cm^{-1} accomplished a sharp increasing in intensity. The band at 3008 cm^{-1} was related to the extending vibration of the CH groups of *cis* double bonds while the other two bands indicated stretching vibration of carbon-carbon double bonds. Hence, the progressions of oxidation was accompanied by reduction in the number of cis double-bonds and a decline in the degree of unsaturation. From **Figures (4.2 - 4.4),** germination caused a more adverse influence on the oxidative stability of the oil extracted from germinated *Chrozohpora brocchiana* as indicated by changes in the intensities and absorbance of most bands.

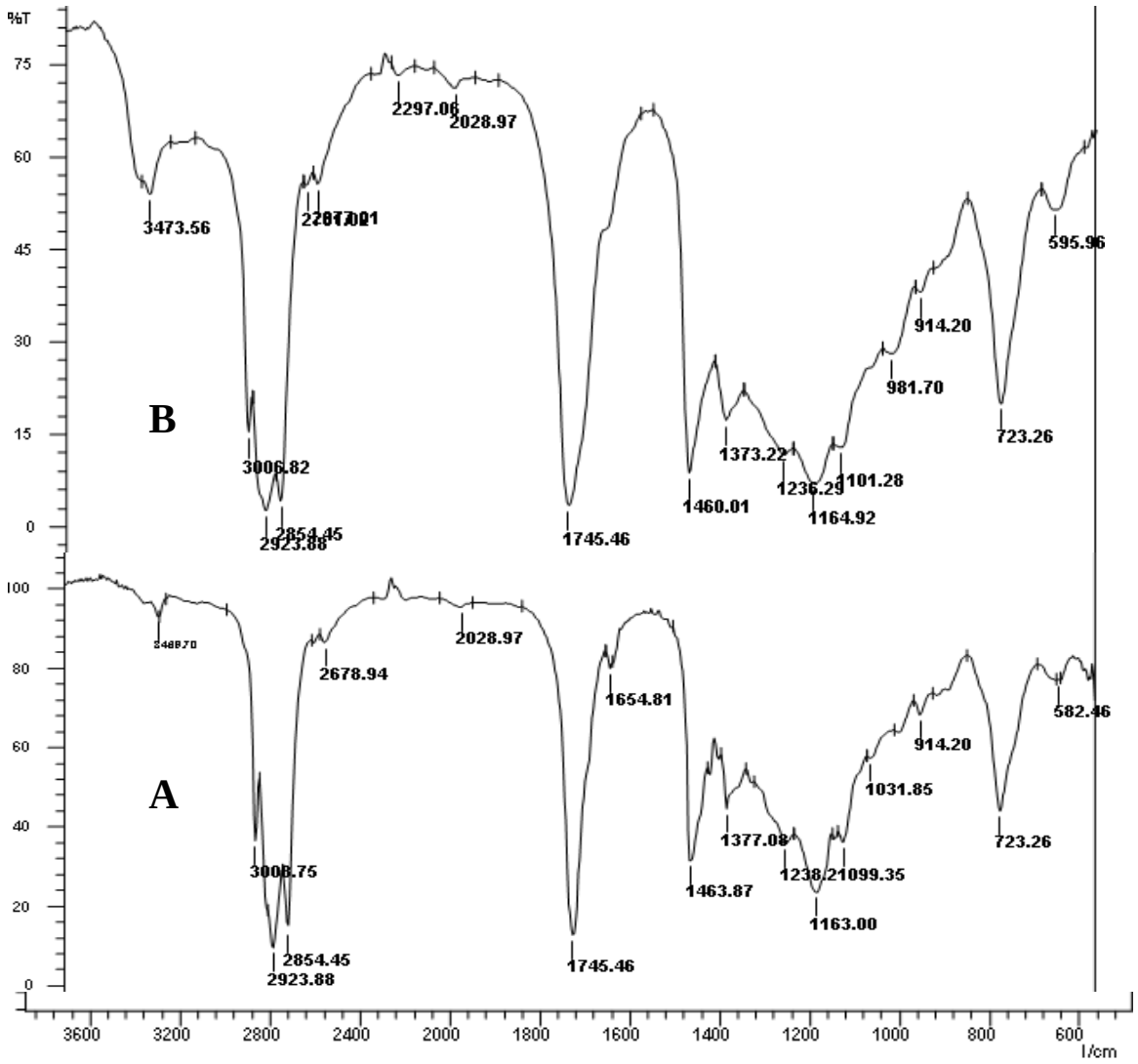

Figure 4.4. Fourier transform infrared spectra of oil extracted from germinated **(A)** and ungerminated **(B)** *Chrozohpora brochiana* seeds storaged at 70°C for 72 hours

4.5. Amino Acids Composition of Ungerminated *Chrozophora brocchiana* seeds

The amino acid profile and chemical scores of the protein are given in **Table4.7**.

Eight amino acids are generally regarded as essential for humans; these are valine, threonine, phenylalanine, tryptophan, isoleucine, methionine, leucine and lysine (Young 1994). Also the deficiency of cysteine (or sulphur amino acids) and tyrosine (or aromatic amino acids) may hinder the healing recovery process (Mat *et al*., 1994).The human bodies do not synthesis these essential amino acids, but can obtain them from food. All of these amino acids are found to be present in ungerminated *C.brocchiana* seed samples investigated except tryptophan, which was not determined.

C.brocchiana seed is rich in the essential amino acids; especially valine (548mg/100g) which is the main essential amino acid followed by Leucine (437mg/100g) and isoleucine (394mg100/g).

Table 4.7 Amino Acids Composition in mg/100g of Ungerminated *Chrozophora brocchiana* seeds Compared with WHO reference protein (FAO, 1981)

Amino acid mg/100g		Reference pattern, g/100g protein	Chemical score%
TEA A			
Lysine	118	5.17	22.8
Histidine	124	1.77	70.05
Thereonine	170	3.47	48.99
Valine	548	4.81	113.72
Methonine	53	1.53	33.98
Isoluecine	394	4.19	94.03
Leucine	437	7.03	62.16
Phenylalanine	218	3.01	72.05
TNEA			
Aspartic Acid	355		
Alanine	780		
Serine	106		
Tyrosine	64		
Glutamic Acid	299		
Glycine	82		
Ammonia	353		
Arginine	666		
TAA	4767		
TEAA	2062		
TSAA	54		
TAAA	282		

* TAA= Total Amino Acid. TEAA= Total Essential Amino Acid. TNAA= Total 1Nonessential Amino Acid. TSCAA= Total Sulphur-Containing Amino Acid. TAAA= Total Aromatic Amino Acid.

Every 100g of this protein contains 20.6g of essential amino acids, 2.82g of sulphur-containing amino acids and 5.4g of aromatic amino acids.

From **Table 4.***7 C.brocchiana* seed contains the essential amino acids but will not meet the minimum daily requirements [FAO/WHO/UNU, 1991; McGilvery and Goldstein, 1983].

As shows in **Table 4.7** in comparison to the WHO reference protein pattern (McGilvery and Goldstein, 1983; FAO/WHO/UNU, 1991),the limiting amino acid of the protein lysine, giving it a protein score of 22.8 %. This proteins chemical score is lower than that of maize and whole wheat, soya bean, peanuts, polished rice, human milk, egg, cow milk, and beef (FAO, 1981; McGilvery and Goldstein, 1983).

4.8. *Chrozophora brocchiana* seed protein concentrate (CSPC)*

As shows in **Table 4.12**, the highest protein concentrates yield was obtained from germinated seeds which is 15.06% followed by ungerminated seeds with 14.66%. Meanwhile *C.brocchiana* defatted seed was reported to consist of 26% protein (Hussein *et al*., 1994). The difference might be due to the diversity in the different strains of the plant itself. From the protein concentrates obtained, the protein contents were 79.8±0.3 for ungerminated and 82.2 ±0.0 for germinated, therefore *C.brocchiana* seeds have a potential as a suitable source for animal feed for its high protein content.

Table 4.12 Functional properties of *Chrozophora brocchiana* seed protein concentrate*

Sample	PY%	PC%	OAC(g/g)	WAC(g/g)	FC%	FS%
A	14.6	79.8±0.3	2.8±0.0	2.7±0.0	160	40
B	15.0	82.1±0.0	3.2±0.0	2.1±0.0	167	41.5

A**:** Protein concentrate from ungerminated seeds, B**:** Protein concentrate from geminated seeds, PC: protein content% in protein concentrate ,PY: percentages of protein in 100g defatted seeds, FC: foaming capacity, FS: foam stability, OAC: oil absorption capacity and WAC : water absorption capacity

4.8.1. *Chrozophora brocchiana* seeds protein concentrates

4.10.1.1.Water and Oil Absorption Capacity

High water absorption of proteins helps to reduce moisture loss in food products. Also it is required to maintain freshness and moist mouth feel of these foods.

From **Table 4.12** *C.brocchiana* seeds protein concentrates CSPC obtained from ungerminated seed exhibited water absorption capacity (WAC) of 2.68 g/g; whereas the CSPC obtained from germinated (CGSPC) absorbed 2.98 g/g. The two types showed low water absorption and this may be due to their chemical composition. However these values were higher than 1.74 reported for cashew nut protein concentrate (Ogunwolu., 2009), and 1.45 for peanut powder reported by (Monteiro and Prakash 1994).

WAC values ranging from 1.49 to 4.72g/g were considered critical in viscous foods such as soups (Chandi, and Sogi, 2007). Therefore the protein concentrates obtained from defatted *C.brocchiana* seeds have low water absorption but are still considered high in the range of WAC of glutinous food. Oil absorption capacity (OAC) of protein concentrate was found to be 2.96 g/g for ungerminated seeds and 3.15g/g for germinated seeds. This is in good agreement with Campell., *et al* (1992) ; Ogunwolu *et al*., (2009) who reported that OAC increased as protein content increased in cashew nut and soya protein products, respectively.

4.10.1.2. Foaming properties

Protein in dispersions causes lowering of surface tension at water-air interface, thus creating foaming capacity (FC) (Guan *et al*, 2007). Therfore to exhibit good foaming , protein must be capable of migrating at the air-water interface, unfolding and rearranging at the interface (Halling, 1981).

Foaming capacity of CSPC of ungerminated and germinated seed is shown in **Fig 4.4 and Table 4.12** The foaming capacity of ungerminated and germinated

seed protein concentrates reached a maximum at pH 9. Germinated seed protein concentrate showed higher foaming capacity than ungerminated seed**.** The foaming capacity of both types of protein concentrate was affected by pH and it tended to decrease at pH 5. From **Fig 4.5, 4.6** and **4.7** in salt conditions FC of the two types of protein concentrate decreases with salt concentration while it increases with sugar concentration. The same situation was observed in foaming stability (FS). The two type of CSPC have the same foaming stability (40 and 41.5). The foaming capacity and stability were enhanced by greater protein concentration (Damodaran, 1997).

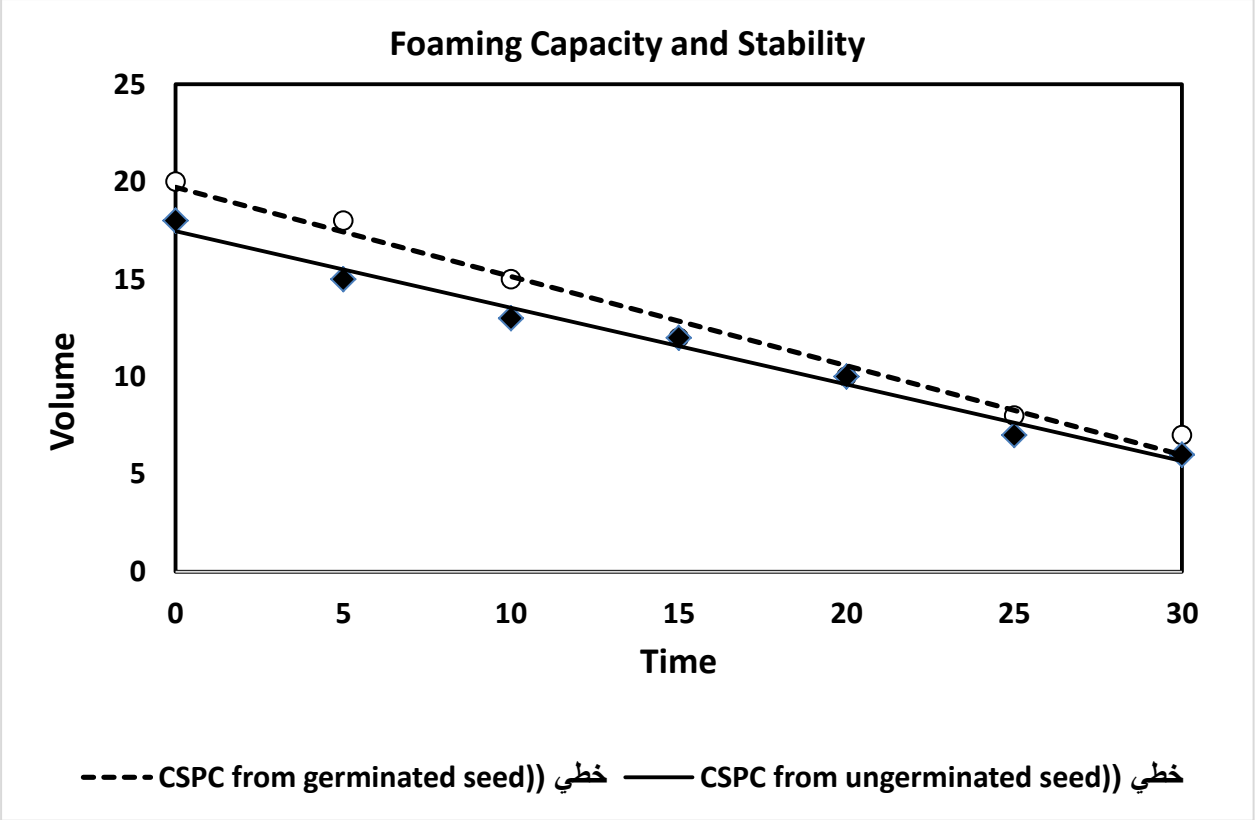
Foaming Capacity and Stability
25
20
15
10
5
0
Volume
0
5
10
15
20
25
30
Time
CSPC from germinated seed)) خطي
CSPC from ungerminated seed)) خطي

Fig 4.5

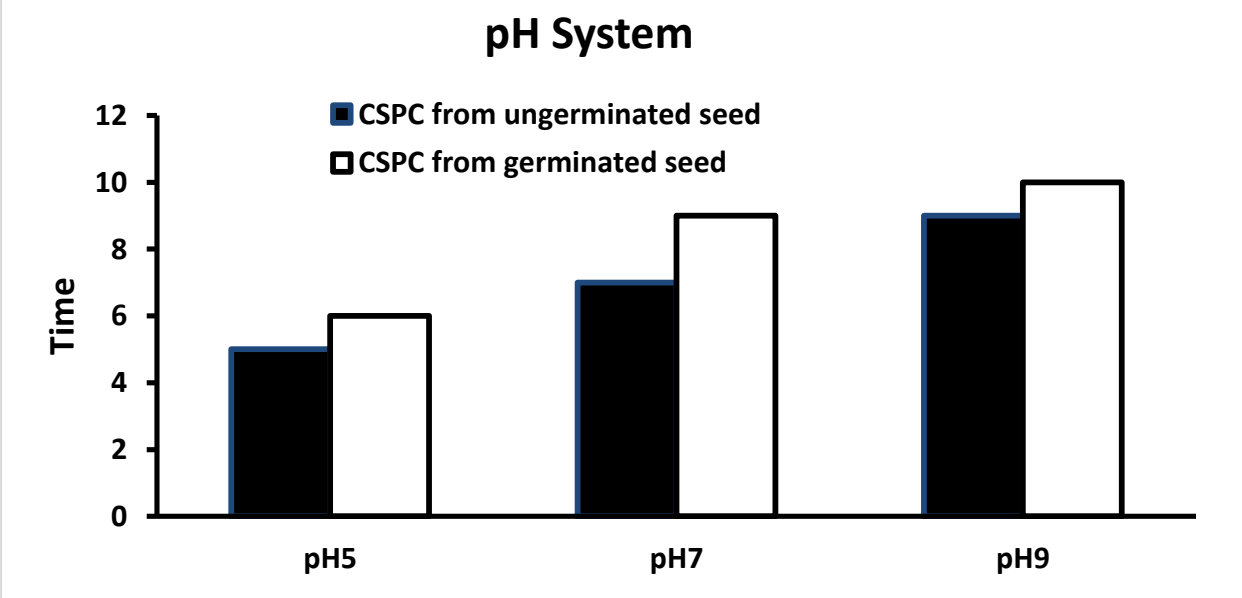
pH System
CSPC from ungerminated seed
CSPC from germinated seed
12
10
8
6
4
2
0
Time
pH5
pH7
pH9

Fig 4.6

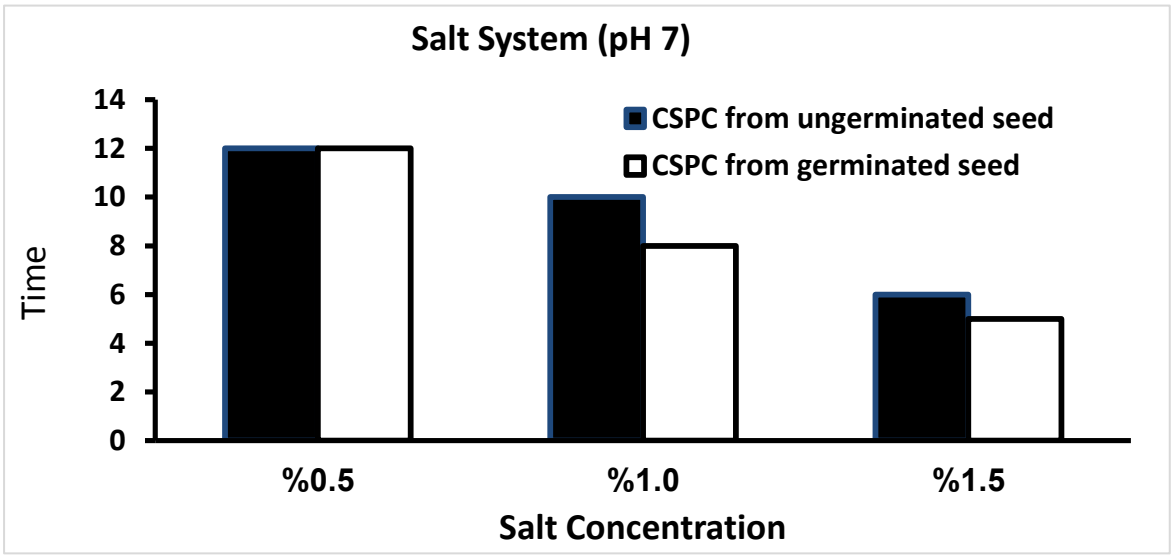
Salt System (pH 7)
Time
14
12
10
8
6
4
2
0
CSPC from ungerminated seed
CSPC from germinated seed
%0.5
%1.0
%1.5
Salt Concentration

Fig 4.7

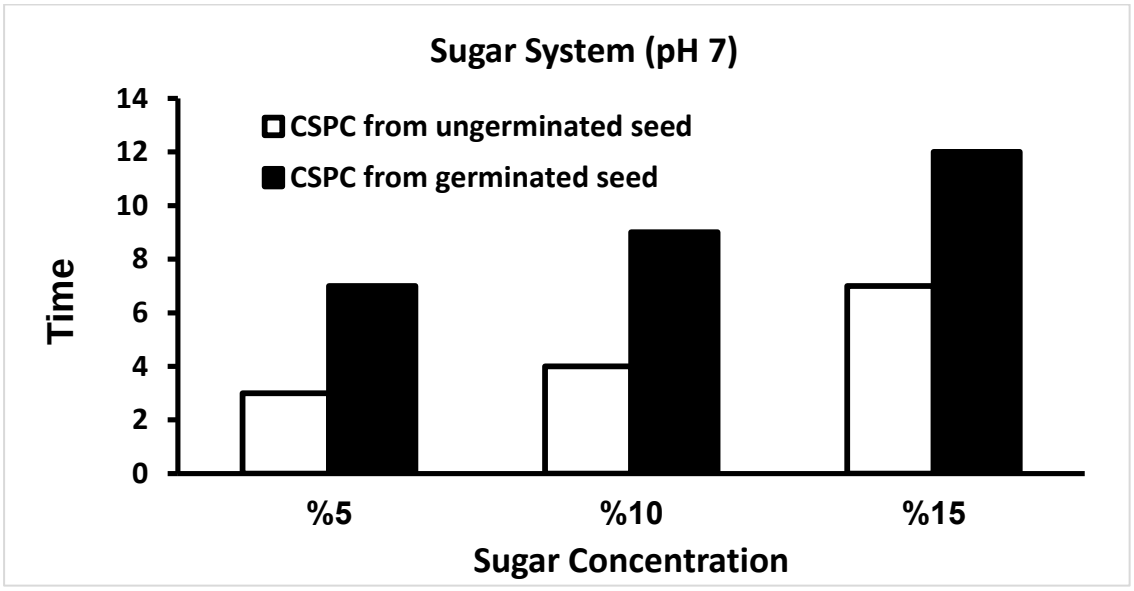
Sugar System (pH 7)
Time
14
12
10
8
6
4
2
0
CSPC from ungerminated seed
CSPC from germinated seed
%5
%10
%15
Sugar Concentration

Fig 4.8

4.10.1.3.Proximate Analysis of Control *kisra* and Supplemented with CSPC

The analysis of kisra as a control sample and formulated supplemented kisra (A1, A2 and A3) and (B1, B2 and B3) are given in **Table 4.13**.The most significant effect of *C.brocchiana* seeds protein concentrate (CSPC) supplementation of kisra was the improvement in protein and increase in fiber. As shown in Table **4.13**,The crude protein, crude fiber and ash were 12.3±0.02,3.3±0.01 and 1.5±0.01 respectively, in the control kisra, and increased to 14.6±0.14, 6.3±0.0 and 2.1±0.03 in supplemented kisra sample (A3) which was formulated by addition of 15% CUSPC (ugerminated seeds). While moisture contents, oil contents and carbohydrates were 49.2±0.02, 4.2±0.14 and 29.7±0 respectively in control kisra and decreased to 47.1±0.0, 3.5±0.01 and 26.4±0.01in supplemented kisra (A3) and there was a slight increase in the sample (B3) which was supplemented with 15% CGSPC (germinated seeds). However the formulated kisra were good source of high protein and high fiber while low in fat; and nutritionally they have a potential to be better than control kisra in case of diabetic patients and protein deficiency disease.

4.10.1.4. Sensory evaluation of supplemented Kisra

The mean scores for sensory attributes of kisra which was supplemented by the various percentages of the protein concentrate from (ungerminated and germinated) *C.brocchiana* seeds are given in Table **4.14**

Table 4.13 Chemical composition of Kisra and formulated samples*

Content%	C	A1	A2	A3	B1	B2	B3
Moisture	49.2±0.02	48.9±0.0	47.8± 0.01	47.1±0.0	48.1±0.00	47.8±0.0	47.10±0.05
Protein	12.3±0.02	12.6±0.1	13.9±0.02	14.6±0.14	12.7±0.02	13.1±0.02	14.7±0.01
Oil	4.2±0.14	3.9±0.02	3.7±0.0	3.5±0.01	3.9±0.0	3.7±0.02	3.4±0.02
Crude fiber	3.3±0.01	4.2±0.01	5.2±0.02	6.3±0.0	4.2±0.01	5.3±0.01	6.3±0.02
Ash	1.5±0.01	1.6±0.02	1.9±0.02	2.1±0.03	1.7±0.02	1.9±0.0	2.2±0.02
Carbohydrate	29.6±0.0	28.8±0.	27.5±0.	26.6±0.	28.8±0.	29.2±0.0	26.7±0.01
Energy value	170.5±.0.01	166.10.1	162.7 ±.0.02	160.2±0.01	165.8±0.0	162.4±0.02	158.6±0.03

C: Kisra(Control)- **A**:ungerminated seed and **B**: germinated seed
A1: (Kisra with 5% CUSPC);**A2** (Kisra with 10 %CSPC); **A3**(Kisra with 15%CSPC)
B1: (Kisra with 5% CSPC);**B2** (Kisra with 10CSPC);**B3** (Kisra with15%CSPC)

As shown in this table the data indicated that there were no significant differences between treatments in all the sensory attributes measured; thereby the preference of control Kisra was similar to that of Kisra supplemented with 5%,10% and 15% defatted seed protein concentrate. Hence, for nutritional purposes, it is advisable to add CSPC flour at a level of up to 15% in the manufacture of Kisra.

Table (14). Mean scores for sensory attributes of control unsupplemented *kisra* and *kisra* supplemented with different levels of *Chrozophora brocchiana* seed protein concentrate

Kind of`*kisra*	C	A1	A2	A3	B1	B2	B3
Colour	7.8	8.05	7.2	7.5	7.7	7.75	7.6
Texture	7.85	6.92	7.85	6.89	6.85	7.02	6.86
Flavour	7.05	7.1	6.95	7.65	6.91	7.3	7.05
Overall acceptance	7.9	7.3	7.1	7.1	7.4	7.6	7.3

Means based on 9 points hedonic scale (1, extremely bad; 2, very bad; 3, bad; 4, fairly bad; 5, satisfactory; 6, fairly good;7, good;8, very good; 9, excellent.).

There are no significant differences between treatment means in all the sensory attributes measured and hence the lettering system is not used to indicate significance level

4.7. Antimicrobial Activity of *Chrozophora brocchiana* phenolic extracts

The antimicrobial activity of methanol, ethyl acetate and hexane, extracts of leaves and stem, and oil of *C.borcchiana* plant were observed using agar well diffusion method by measuring the diameter of the growth inhibition zone.

Table 4.9. The zone of inhibition diameter of *Chrozophora brocchiana* leaves extract*

Microorganism	Extract concentration	Zone of inhibition A	Zone of inhibition B	Zone of inhibition C	STR
Bacillus cereus	0.1	2.1	1.5	R	3.0
	0.01	1.6	R	R	
	0.001	R	R	R	
	0.0001	R	R	R	
Bacillus subtitles	0.1	1.8	R	2.0	NT
	0.01	1.6	R	1.1	
	0.001	R	R	R	
	0.0001	R	R	R	
Staphylococcus aureus	0.1	2.5	3.0	2.5	1.8
	0.01	1.9	2.3	1.5	
	0.001	1.6	1.8	R	
	0.0001	1.5	1.5	R	
Escherichia coli	0.1	1.2	R	R	1.9
	0.01	R	R	R	
	0.001	R	R	R	
	0.0001	R	R	R	
Pseudomonas aeruginosa	0.1	1.8	R	R	1.8
	0.01	1.3	R	R	
	0.001	R	R	R	
	0.0001	R	R	R	
Salmonella	0.1	1.6	R	R	NT
	0.01	1.2	R	R	
	0.001	R	R	R	
	0.0001	R	R	R	
Candida albicans	0.1	1.5	R	R	NT
	0.01	R	R	R	
	0.001	R	R	R	
	0.0001	R	R	R	
Aspergillus flavus	0.1	1.5	R	R	NT
	0.01	R	R	R	
	0.001	R	R	R	
	0.0001	R	R	R	

**A =Methanol extract, B=Ethyl acetate extract, C=Hexane extract, STR Streptomycin NT: Not Tested, R= resistance*

From **Table 4.9** the methanol extract of leaves showed a positive significant ($p \leq 0.05$) antibacterial and antifungal activity against all the tested organisms with inhibition zone between (1.5-2.5cm) at 0.1-.0.01concentration, and showed highest significant at all concentrations against *S. aureus*. The ethyl acetate extract of leaves showed a positive significance against *B. cereus* with inhibition zone of 1.5 cm at concentration 0.1mg and *S. aureus* with inhibition zone between (3.0-2.3) at all concentrations and resistance to fungi.

The hexane extract of leaves showed appositive significant effect against *B.subtitile* with inhibition zone of 1.1-2.0 cm at the concentration of 0.1- 0.01 mg of extract and *S. aureus* with inhibition zone between1.5 and 2.5 in the same concentration and resistant to fungi.

From **Table 4.10** the methanol extract of stem showed a positive significant antibacterial and antifungal activity against the entire test organisms with inhibition zone between 1.5 and 2.5 cm at 0.1mg concentration. The ethyl acetate extract of stem showed a positive significance against *S. aureus* with inhibition zone of 1.8 at 0.1 concentration and resistance to other organisms. The hexane extract of stem showed a positive significance against *S. aureus* with inhibition zone 1.5 at 0.1 concentration and resistance to other organisms.

Table 4.10. The zone of inhibition diameter of *Chrozophora brorcchiana* stem extract*

Microorganism	Extract concentration(mg/ml)	Zone of inhibition A	Zone of inhibition B	Zone of inhibition C	STR zone
	0.1	2.	R	R	3.1
Bacillus cereus	0.01	R	R	R	
	0.001	R	R	R	
	0.0001	R	R	R	
	0.1	2.3	R	R	NT
Bacillus subtitles	0.01	R	R	R	
	0.001	R	R	R	
	0.0001	R	R	R	
	0.1	2.5	1.8	1.5	1.8
staphylococcus	0.01	R	R	R	
aureus	0.001	R	R	R	
	0.0001	R	R	R	
	0.1	2.0	R	R	1.9
Escherichia coli	0.01	R	R	R	
	0.001	R	R	R	
	0.0001	R	R	R	
	0.1	2.5	R	R	1.8
Pseudomonas	0.01	R	R	R	
aeruginosa	0.001	R	R	R	
	0.0001	R	R	R	
	0.1	2.0	R	R	NT
Salmonella	0.01	R	R	R	
	0.001	R	R	R	
	0.0001	R	R	R	
	0.1	1.5	R	R	NT
Candida albicans	0.01	R	R	R	
	0.001	R	R	R	
	0.0001	R	R	R	
	0.1	2.0	R	R	NT
Aspergillus flavus	0.01	R	R	R	
	0.001	R	R	R	
	0.0001	R	R	R	

*A =Methanol extract, B=Ethyl acetate extract, C=Hexane extract, STR Streptomycin NT: Not Tested, R = resistance

Table 4.11. The zone of inhibition diameter of *Chrozophora brorcchiana* oil extract*

Microorganisms	Extract concentration	Zone of inhibition A	STR zone
	0.1	1.2	3.0
Bacillus cereus	0.01	1.0	
	0.001	R	
	0.0001	R	
	0.1	R	NT
Bacillus subtitles	0.01	R	
	0.001	R	
	0.0001	R	
	0.1	1.5	1.8
Staphylococcus aureus	0.01	1.5	
	0.001	1.2	
	0.0001	1.2	
	0.1	R	1.9
Escherichia coli	0.01	R	
	0.001	R	
	0.0001	R	
	0.1	R	1.8
Pseudomonas	0.01	R	
aeruginosa	0.001	R	
	0.0001	R	
	0.1	R	NT
Salmonella	0.01	R	
	0.001	R	
	0.0001	R	
	0.1	R	NT
Candida albicans	0.01	R	
	0.001	R	
	0.0001	R	
	0.1	R	NT
Aspergillus flavus	0.01	R	
	0.001	R	
	0.0001	R	

***A =Methanol extract, STR Streptomycin NT: Not Tested, R = resistance*

As shown in **Table 4.11** the methanol extract of oil showed a positive significant antimicrobial activity against *B. subtitles* with inhibition zone between 1.0 and 1.2 cm at 0.1- 0.01 mg concentration, and *S.aureus* with inhibition zone 1.2 and 1.5 at all concentrations and resistance to other organisms.

The antimicrobial screenings are recorded in **Table 4.9 to Table 4.11** expressing the zones of inhibition of bacterial and fungal growths. It is interesting to note that the leaves and stem extracts are more effective against bacteria than fungal, especially methanol extracts. While the oil extract was found to be effective against pathogenic bacteria.

Successful prediction of antibacterial activity from plant material is largely dependent on the type of solvent used in the extraction procedure. The methanol, followed by ethyl acetate and hexane, extracts showed considerable amount of inhibition against *B. subtilis, B. cereus, E. coli, S. typhi, and P. euroginosa* respectively; with much activity on *S. aureus*. Many *S. seureus* strain are well known for their high antibiotic resistance against different antibacterial agents (Smith and Palumbo 1980). The activity also targeted non-pathogenic strains *B. subtilis*. This may be due to better solubility of the active components in organic solvents (De Boer *et al*., 2005).

5.0 Conclusions and Recommendations

5.1.Conclusions

Argessi plant, *Chrozophora brocchiana*, of the family Euphorbiaceae, is widely scattered in the poor savanna areas in western Sudan as a range crop. Many traditional uses of plants as food, feed and medicine show its importance as a good new oil and protein source. All parts of this plant are edible and valuable for nutritional and medicinal purposes.

This study investigated the nutritional value and potential of *C.brocchiana* seed as a source for edible oil. The results demonstrated that *C.brocchiana* seeds contain high amounts of protein, oil and carbohydrates; thus it has the potential to be utilized as new vegetable oil of high unsaturated fatty acids.

The germination procedure prompted an increment in moisture, and protein substances of the germinated seeds while fat and carbohydrate substances were decreased.

Successive heating at 70°C has significantly decreased the stability of ungerminated and germinated *C. brocchiana* seed oils. The occurrence of oil oxidation was proven lower in ungerminated *C. brocchiana* oil through FTIR, which appears suitable for studying the oxidative stability of this oil.

C.brocchiana seed as a new source is necessary for supplying some elements. Therefore, seeds protein concentrate has a great probability to serve as cheap source of edible protein

The methanol, followed by ethyl acetate and hexane, extracts showed considerable amount of inhibition against *B. subtilis, B. cereus, E. coli, S. typhi, and P. euroginosa* respectively; with much activity on *S. aureus* . The activity also targeted non-pathogenic strains *B. subtilis*. The leaves methanolic extract was highly active against all bacterial and fungal strains at high dose, while the ethyl acetate extract was active against *Staphylococcus aureus* strain. The stem

extract showed medium antimicrobial activity when compared with other extracts.

Characterization of the properties of protein concentrates obtained from *C.brocchiana* seeds has improved our knowledge, thus assisting in the efforts for its industrial application.

Formulated kisra were good source of high protein and high fiber while low in fat; and nutritionally they have a potential to be better than control kisra in case of diabetic patients and protein deficiency disease.

The oil produced by expellers showed a higher tocopherol content, especially alpha , than the oil produced by other routes .

5.2.Recommendations

The leaves, stem and oil methanolic extracts of *C.brocchiana* plants should further be studied for its phytochemical constituents in order to elucidate the active principle within the extract which can turn out to be a novel antimicrobial agent of the future.

Further studies are needed on protein concentrates and phenolic compounds, their identification, and determination of their technological suitability as ingredients in food and as antibiotic agent. Further study is needed for knowing the role of germination in lowering the oxidative stability of the oil .

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Appendix

Fig 4.1 (**A**), whole *C.brocchiana* fruit; (**B**), *C. brocchiana* Seeds; (**C**), defatted *C. brocchiana* seed flour (DSSF); (**D**), freeze dried *C. brocchiana* seed protein concentrate