Chemical Composition and Antioxidant Activity of *Ceratonia-siliqua* L. pods Extracts

A Thesis Submitted in Partial Fulfillment of the Requirements for the M.Sc Degree in Chemistry

By

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(B.Sc.Honors in Chemistry, University of Zalingei 2015)

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Sudan
September 2018
1. Introduction and literature review

1.1 General Introduction

The Kharrub/Algarrob is name of *Ceratonia siliqua* L. which belong to family (fabaceae) is a native evergreen plant of the Mediterranean area (Batlle and Tous, 1997). The nonfleshy and bean-like fruits, called “carob pods,” are a traditional part of the diet in the Mediterranean region, and the plant has been cultivated in the region for centuries for its edible fruits, the pod is light to dark brown, oblong, flattened, straight or slightly curved, with a thickened margin, and ranges from 10 to 20 cm in length and 1.5–2 cm in width, Current world production of *C.siliqua* pod has been estimated at about 310,000 tons per year (Mitrakos, 1988). *C.siliqua* pod is widely used in the food industry to product *C.siliqua* bean gum and locust bean gum, which are polysaccharides, (Batlle and Tous, 1997).

The pod consists mainly of pulp (90%), which is rich in sugars (48–72%), but also may contain a large amount of condensed tannin (16–20%), protein and low level of fat. The crude fiber content recorded 7.30%. *C.siliqua* powder was rich source of minerals (Fe, Ca, Na, K, P, Cu, Zn,) and vitamins A, B, B₁₂, E, D, C, Niacin, B₆ and folic acid (Hiramo, 2001).

*C.siliqua* powder is consisted of eleven phenolic compounds which are considering natural antioxidant. *C.siliqua* pods have been used in many countries as an antioxidant in different foods, as a thickener, stabiliser or flavourant in food applications, in ethanol production, in the production of cosmetics, in animal nutrition, in lactic acid production and in medical applications etc. The use of *C.siliqua* pods in food dates back to ancient times, where the pods are reported that in raw form have been consumed, (Brand, 1995).
1.1.1 Objectives

The objectives of this work can be summarized as follows:

1- To provide information on the secondary metabolites and other phytochemicals constituents of *C. siliqua* L grown in Sudan

2- To determine the chemical constituency of *C. siliqua* L.

3- To evaluate the minerals content in *C. siliqua* L.

4- To assess the antioxidants activity of *C. siliqua* L. extracts

5- To fractionate the active antioxidant extract using chromatographic methods

6- To analyze the structure of the isolated pure fraction (s), using spectroscopic techniques

1.2 Literature review

1.2.1 Taxonomy:

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1.2.2. Botanical description

The *C.siliqua* tree grows as an evergreen shrub or tree up to 10 m high, with a broad crown and a thick trunk with brown rough bark and sturdy branches. Leaves are 10-20 cm long, alternate, pinnate, with or without a terminal leaflet. Leaflets are 3-7 cm long, ovate to elliptic, in 4-10 normally opposite pairs, coriaceous, dark green and shiny above, pale green beneath and finely veined with margins slightly undulate, and tiny stipules. (Mitrakos, 1988).

*C.siliqua* does not shed its leaves in the autumn but only in July every second year, and it only partially renews leaves in spring April and May (Diamantoglou and Mitrakos, 1981).

The *C.siliqua* is a dioecious species with some hermaphroditic forms; thus male, female and hermaphrodite flowers are generally borne on different trees. Unisexual and bisexual flowers are rare in the inflorescence. The flowers are initially bisexual, but usually one sex is suppressed during late development of functionally male or female flowers (Tucker, 1992).

Hermaphrodite flowers are a combination of both types, containing a pistil and a complement of 5 stamens. Pollen grains released from the anthers are of spheroidal shape and are tetracolpate, pollen diameter is 28-29 μm at the poles and 25-28 μm at the equator (Ferguson, 1980)

The fruit is an indehiscent pod, elongated, compressed, straight or curved, thickened at the sutures, 10-30 cm long, 1.5-3.5 cm wide and about 1 cm thick with blunt or sub acute apex. Pods are brown with a wrinkled surface and are leathery when ripe (Hammer *et.al.*, 1992).
Figure (1.1) The *C.siliqua* bean pod (a) the pulp (b) and the seeds (c)

1.2.3. Distribution

The original distribution of *C.siliqua* is not clear as it has undergone extensive cultivation since ancient times.

Hillcoat *et al.* 1980 suggested its range in the wild included Turkey, Cyprus, Syria, Lebanon, Israel, southern Jordan, Egypt, Arabia, Tunisia and Libya and that it moved westward at an early stage. *C.siliqua* is believed to have been spread by the Greeks to Greece and Italy and then by the Arabs along the coast of northern Africa into the south and east of Spain, from where it migrated to the south of Portugal and the southeast of France. Its wild occurrence in the western
Mediterranean is doubtful according to (Zohary and Spiegel, 1975). Seedling trees grown for shade on the streets of cities in Southern California and Arizona were selected for commercial production on the basis of their floral and fruit characteristics. In Mediterranean countries, the distribution of the evergreen sclerophyllous species like, *C. siliqua* is controlled by winter cold stress (Mitrakos, 1981).

*C. siliqua* is one of the most characteristic and dominant trees in the lower zone (0-500 m and rarely up to 900 m) of the Mediterranean evergreen (Zohary and Orshan, 1959).

**Figure (1.2): The global distribution of the Carob.**

Stars in the map indicate to the area in which the (*C. siliqua* L.) grow.
1.2.4 Ecology

The *C. siliqua* is a long-lived evergreen tree thriving in habitats with mild Mediterranean climates; it grows well in warm temperate and subtropical areas, and tolerates hot and humid coastal areas. *C. siliqua* and orange trees have similar temperature requirements but *C. siliqua* tolerates poorer soils and needs much less water. The *C. siliqua* tree is tenderer than the olive. For resistance to dry environments it is surpassed only by pistachio (Evreinoff, 1955).

It is also axerophytic species well adapted to the ecological conditions of the Mediterranean region, by virtue of its efficient hydric regulation by stomatic adjustment and its foliar structure and anatomy. Leaf wax synthesis increases in dry conditions reducing cuticular permeability and thus protecting the plant from excess transpiration (Baker and Procopiu, 1980).

However in some very warm places and under favourable conditions, *C. siliqua* grows without becoming dormant either in winter or summer (Liphschitz and Lev-Yadun, 1988).

The cambium can be active throughout most of the year or all year with low rates of activity in January (Arzee et al., 1977).

The rooting habit of *C. siliqua* is similar to pistachio; its extensive root system penetrates the soil deeply. *C. siliqua* develops roots under stressful conditions to explore deeper soil layers where water may be available (Christodoulakis, 1992). It can thus survive long periods of drought. In addition, (Nunes et al, 1989).

Although the *C. siliqua* tree is a legume, like most Caesalpinioideae it does not nodulate and thus is unable to fix nitrogen (Martins and Rodriguez, 1982).
1.2.5 Chemistry of (*C. siliqua* L):
The *C.siliqua* pulp is the fruit of an evergreen (*C.siliqua* L.) cultivated in the Mediterranean area. The pulp represents 90% of the fruit. It has a high content of sugars and tannins and low contents of protein and fat. *C.siliqua* powder or syrup is used as an ingredient in cakes and cookies and chocolate substitute (Carlson, 1986).

1.2.5.1 Minerals content

*C.siliqua* powder is considered as a rich source of the minerals as (Fe, Ca, Na, K, P, S) and trace element as (Cu, Zn, Se) this act as cofactors of antioxidant enzymes to protect the body from oxygen free radical that are produced during oxidative stress (Bouzouita,N. et al., 2007).

1.2.5.2 Vitamins content.

The *C.siliqua* powder is good source of vitamins E, D, C, B6 (1) and folic acid

*C.siliqua* powder also contained lower levels of vitamins A, B (Hiramo, 2001).

![Chemical structure of folic acid](image)
1.2.5.3. Phenolic compound content:-

Phenolics are compounds with an aromatic ring bearing one or more hydroxyl groups. Polyphenols occur in foods of plant origin and because of their antioxidative properties ability to modulate several proteins; polyphenols generally have beneficial effects on human health once consumed. *C.siliqua* powder consisted of eleven phenolic compound. Pyrogallol,(2) catechol, chlorogenic (3) and protocatechuic(4) recorded the highest values, while coumarin cinnamic, ferulic, gallic acid (5) and vanillic ,caffeine and catechin (6) recorded the least values of the phenolic compounds (Tahir  *et al.*, 2009).

![Image of phenolic compounds](2)

![Image of phenolic compounds](3)
1.2.5.4 Fatty acid composition

Maza, et al. 1989 reported that the *C.siligua* pods oil consisted of 17 fatty acids, but the mainly four fatty acids namely: oleic(7), linoleic(8), palmitic (9) and stearic(10).
1.2.6 *C.siliqua* in traditional uses:

*C.siliqua* fiber-containing foods cause slower emptying of the stomach, being slowly digestible, thus resulting in more satiety, which is very useful for reducing obesity problems. The use of *C.siliqua* flour has been of considerable value as a dietetic therapy for infantile diarrheal disturbance and bacterial dysentery in terms of shortening the duration of the disease. The fruits are also traditionally used as an antitussive and against warts (Fortier, 1953).

1.2.7. Medicinal uses and pharmacological effects:

*C.siliqua* has numerous pharmacological uses in the various parts of tree are detailed as follows:-

1.2.7.1. Antioxidant activity:

The *C.siliqua* pods has considerable amounts of dietary fiber and polyphenols (hydrolyzable tannins, derived from gallic acid and condensed tannins, derived from flavan-3-ol,anthocyanidines, and flavan-3,4-diol). (Custodio, *et al.*, 2011). Moreover, the *C.siliqua* flour was evaluated as an ingredient with a marked nutritional value due to its high levels of phenol compounds. The level of total flavonoids was 8.13±0.34 mg, the polyphenols possessed antioxidant activity evaluated by DPPH and FRAP assay which is important for the prevention or delay the oxidative damage. Consequently, polyphenols are involved in protection against several diseases (cardiovascular and neuronal, among others). (Custodio, *et al.*, 2011).

1.2.7.2. Antibiotic activity:

Alzoreky and Nakahara, 2003 reported that the extracts of edible parts of *C.siliqua* showed the ability as antibacterial activity against *Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli* and *Salmonella infantis* also buffered methanol (80% methanol and 20% PBS) and acetone extracted exhibited inhibitory effect against tested for bacteria using disc assay.

1.2.7.3. Anti carcinogenic activity:

There have been advances in cancer research during the past decade. However, the rate of cancer incidence is increasing at an alarming rate. Many studies have suggested a protective role of tannins and other polyphenols against cancers.

*C. siliqua* fiber extract has been found to have protective effects against oxidative stress in adenoma cells that might defend against or slow down the carcinogenic process in colorectal cancer. Colorectal cancer is one of the most prevalent cancers in Western societies *C. siliqua* fiber, consisting of polyphenols, strongly inhibits proliferation (due to inhibition of DNA synthesis) of both adenoma and adenocarcinoma cells of the human colon (El-Shatnawi, *et al.*, 2001).

Aqueous extracts of *C. siliqua* pods showed a marked alteration in cell proliferation of a mouseheaptocellular carcinoma cell line and also induced apoptosis in the cells. This demonstrated that *C. siliqua* pods contain antiproliferative agents that could be of practical importance in the development of functional foods and/or chemopreventive drugs. Studies have revealed that an extract *C. siliqua* of germ has considerable antioxidant activity, and it reduced the viability of cervical cancer (HeLa) cells (Custodio, *et al.*, 2011).
1.2.7.4. Anti diabetic activities:
Administration of crude polyphenol extract of *C.siliqua* pods to male rats showed a significant reduction in blood glucose after 30, 60, and 120 min when compared with the control group. (Croze and Soulage, 2013). Compounds in *C.siliqua* may be reducing the blood glucose response by inhibiting the enzyme activity (amylases) resulting in the slow rate of starch digestion.
*C.siliqua* flour, particularly *C.siliqua* fiber, being rich in polyphenols and tannins, has a high potential to be incorporate into diabetic-friendly foods. Both animal experiments and human clinical trials showed significant antidiabetic effects. (Croze and Soulage, 2013).

1.2.7.5. Anti diarrheal

*C.siliqua* bean juice when used in combination with ORS (oral rehydration solution of WHO) for the treatment of acute diarrhea in children resulted in reduction of the disease duration by 45% and stool output by 44% as compared to using ORS (Abdullahia *et al*, 2001). The fruits are also traditionally used as an antitussive and against warts

1.2.8. Other uses:

1.2.8.1 Animal nutrition

*C.siliqua* pods and seed seem to be promising as non-conventional feed resource that can be used for small ruminants feeding. Silanikove *et al.*, 2006 evaluated the possibility of feeding carob pulp to livestock and mainly focused on ruminants. *C.siliqua* meal obtained more than 200 g and decreased the time needed to reach significant weight. For centuries, due to the high sugar content, *C.siliqua* pods have been used as animal feed. When fed to animals in feeding trials, *C.siliqua* pods have been shown to give results similar to those reported for barley. Cattle, horses, goats and sheep have also been reported to feed on the lower leaves and branches of the
C.siliqua tree. The C.siliqua tree is highly recommended for use as feed supplement for animal farming in drought stricken regions (Battle and Tous, 1997).

C.siliqua pulp as a favourable fatty acid composition due to the presence of essential fatty acids, such as linoleic and alpha-linolenic acids and might represent a natural source of desirable fatty acids in the diets of concentrate-fed animals.

1.2.8.2. Food technology

From the seed of C.siliqua, the endosperm is extracted to produce a galactomanna, which forms locust bean gum, a valuable natural food additive for its strong gel characteristics, which are useful in products such as canned pet food, since they are maintained after heating (Carlson, 1986).

The main use is the production of C.siliqua bean gum from the seed endosperm which is used as the food additive (stabilizer and thickener) in food- and pharmaceutical industry. In addition, C.siliqua fruits are used in food industry as a source of many products such as gum, sugar and alcohol (Carlson, 1986).

C.siliqua is used in many Arab countries to make a popular drink which is consumed mainly in the month of Ramadan; C.siliqua is also used in preparation of special traditional types of arabic confectionery (Owen et al., 2003).

In western Countries, C.siliqua powder is produced by deseeding of C.siliqua pods, yielding of kibbled C.siliqua followed by roasting and milling of the kibbled. C.siliqua juice concentrate is produced by boiling, C.siliqua juices without any added ingredients and technological or scientific techniques. Due to its high sugar content, C.siliqua was consumed as a food especially in ancient times, as a sweet for children or in emergency situations such as war (Owen et al., 2003). throughout the Mediterranean region including Turkey, gently milled C.siliqua pods are processed to cocoa-like flour which is sold as a “carob cocoa” in big stores and local markets. The milled flour is often added to hot or cold milk for drinking.
2. Materials and methods

2.1 Materials:

2.1.1 Plant material:

*C.siliqua* were collected from trees growing naturally in centre Darfur state, locality of Wadisaleh, Garcilla, Western of Sudan. The randomly selected sample was harvested during March 2018, and washed carefully; then separated the pods (whole pod with seed). The pods were ground in a mill. The grounded and milled sample was stored at room temperature for further extraction and for subsequent analyses.

2.1.2 Equipment's:

Weighing analytical balance, oven, separatory and heater.

Atomic absorption spectrometer (XPLORAA - GBC Scientific equipment Victoria, Australia.

The UV- Spectra were recorded on (UV/1800 Spectrophotometer Shimadzu-Japan), UV lamp Model /34015 LA (U.S.A).

The IR Spectra were recorded on Fourier Transform Infra-Red Spectrophotometer. (FT/IR 8400s-Shimadzu.Japan).

Soxhlet extractor

Water bath.

2.1.3 Chemicals and Reagents:

2.1.3.1 Chemicals

Chloroform, toluene, petroleum ether (60-80°C), ethyl acetate, ethanol, hexane, methanol acetic acid (glacial) ferric chloride, magnesium turning, sodium chloride, potassium iodide, picric acid, aluminum trichloride, butanol, sodium hydroxide, ammonium hydroxide, and 2-naphthol.
2.1.3.2 Reagents:

**Baljects reagents:** solution A: 1gm picric acid was dissolved in 100 ml 95% ethanol, solution B: 10 gm sodium hydroxide in 100 ml water solution A and B: were mixed before use.

**Mayers reagent:** solution A: 1.36 gm of mercuric chloride were dissolved in 60 ml distilled water solution B: 5 gm of potassium iodide were dissolved in 10 ml distilled water solution A and B were mixed and diluted to 100 with distilled water.

**Wagner’s reagent:** 1gm of iodine and 2gm potassium iodide was dissolved 5ml of distilled water and the solution was diluted to 100 ml.

**Molishs reagent:** To 10 gm of 2-naphthol, 50 ml of ethanol were added and the volume was completed to 100 ml.

**Vanillin reagent:** 0.1gm of vanillin dissolved in 8.5 ml methanol and 1.5 ml concentrated sulfuric acid.

**Aluminum trichloride solution:** 1% AlCl₃ in distilled water

**Ammonia test solution** 37.5ml of strong ammonia were diluted to 100 ml with distilled water.

**Ferric chloride solution:** 5% w/v of anhydrous ferric chloride in distilled water.

**Gelatin solution:** 50 ml of distilled water were added to 1 gm of gelatin and allowed to settle for one hour shaking frequently then the distilled water was decanted and a fresh portion of 60 ml of distilled water were added to gelatin and the later was dissolved with shaking and warming to 60°C, 10 gm of NaCl were added to solution, mixed, cooled filtered and completed to 100 ml with distilled water.

2N HCl: 129 of concentration HCl were diluted to 1000 ml with distilled water.

Hydrogen peroxide: 10% of w/v of 30% H₂O₂ solution in distilled water.
2.2 Methods of analysis

2.2.1 Determination of moisture content

Moisture content of the *C.siliqua* pod samples was determined according to (AOAC, 1990) method. 5 gm of sample was accurately weighed in a clean, dry reweighed shallow weighing dish. The weighted dish and its contents were dried in an oven at 105°C for five hours, cooled in desiccators and reweighed. The loss on drying was calculated as follows:

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

\( W_1 = \) Original weight of sample (g).
\( W_2 = \) Weight of sample after drying (g).

2.2.2 Determination of ash content

Accurately 5 gm of the dried samples were weighed in dry porcelain crucible and ignited at 550°C in a muffle furnace until free from carbon, cooled in desiccators and weighed. Then the total ash % was calculated as follows: (FAO, 1998)

\[
\text{Ash (\%) = } \frac{W_3 - W_1}{W_2 - W_1} \times 100
\]

Where:
\( W_1 = \) Weight of the empty crucible (g).
\( W_2 = \) Weight of the crucible + the sample (g).
\( W_3 = \) Weight of the crucible + ash (g).
2.2.3. Determination of elements composition

1 gm of sample was placed in a well-glazed porcelain dish. Stored in a cold furnace, and then heated for 550 C° the temperature was maintained for 4 hours. The sample was cooled and 10 ml of hydrofluoric acid (HF), 3ml of nitric acid (HNO₃) and 5ml perchloric acid (HClO₄) were added. The sample was dried and concentrated HCl was added. The sample was dropped into a 100 ml volumetric flask, and diluted to the volume with deionized water, 10 ml of LaCl₂ was added as masking agent to determine (Ca, Mg and Fe) and for (Na, K) the (Ce Cl₃) was added to mask another elements. Atomic absorption spectrometer was used to determine the elements. (Kitson and Mellon, 1944).

2.2.4. Extraction methods:

2.2.4.1. Preparation of ethanol extracts:

100 gm of dried pods powder of *C. siliqua* L were transferred into beaker and solution of 95% ethanol (500 ml) was added the contents of the beaker were left at room temperature for three days with shaking; the extract was filtered through filter paper, left to dry at room temperature. The residual weight was recorded and % yield was calculated.

2.2.4.2. Preparation of Water extract:

50gm of *C. siliqua* powder were transferred into a beaker 250 ml of water was added and two drops of acetic acid were added the contents of the beaker were left at room temperature for three days the extract was filtered through filter paper and left to dry at room temperature. The residual weight was recorded and % yield was calculated.

2.2.4.3 Preparation of Soxhlet extracts.

40 gms of *C. siliqua* pod powder were successively extracted with n-hexane, chloroform, and methanol. The solvent was carefully evaporated from each extract and the extractability of each solvent was determined.
2.2.5. Phytochemical screening of *C. siliqua* L. Extracts:
The 95% ethanol and water extracts of pod were used for the following tests according to methods described by (Harbone, 1984).

2.2.5.1 Screening for sterols and triterpenes:
Using the prepared extract the equivalent of 10 gm of plant material were evaporated to dryness on water bath 10 ml of pet-ether were added and stirred for a few minutes and allowed to settle, the supernatant liquid was decanted and discarded. The above treatment was repeated several times to remove the most of the pigments. 10 ml of chloroform were added to the residue and stirred thoroughly for 5 minutes, and were then decanted into a clean dry test tube 100 gm of (anhydrous Na₂SO₄) were added to the chloroform extract, shaken gently, filtered and divided into two clean dry test tubes.

(a) The Liebermann Burchard for saturated sterols:
To the first portion of chloroform extract 3 drops of acetic anhydride were added and mixed gently by swirling the tube, one drops of conc. H₂SO₄ was added and mixed gently, the gradual appearance of a green to blue colour was taken as an indication for possible presence of sterols, while a pink to purple colour indicates the possible presence of triterpenes.

(b) The Salkouaski test for unsaturated sterols:
To the second portion of the chloroform extract 1-2 ml of concentrated sulphuric acid were added by allowing it to run gently down the side of test tube, any immediate colour change at junction of the extract and the sulphuric acid is an indication of possible presence of unsaturated sterols the sulphuric acid and the extract were mixed and observed for an immediate and a gradual colour change over a period of one hour. A cherry colour is taken also as a presumptive evidence for the presence of unsaturated sterols.
2.2.5.2. Screening for tannins:
A volume equivalent to 10 gm plant material was evaporated to dryness on a water bath 25 ml of hot distilled water were added to the residue and stirred well and allowed to cool 2-4 drops of 10% NaCl solution were added to salt out any non-tannin compounds. The solution was filtered and to 3 ml of filtrat, 4-5 drops of 1% gelatin solution were added. Formation of an immediate precipitate was taken as presumptive evidence for the presence of tannins.

2.2.5.3 Screening for saponins (Forth test)
50 gm of the powder sample were placed in to a clean dry test tube. 10 ml of distilled water were added and vigorously shaken for about 30 seconds the tube was allowed to stand in a vertical position and observe to for 3 minutes, the formation of Froth persisted after 30 minutes the sample is presumed to contain saponins.

2.2.5.4 Screening for flavonoids and leucoanthocyanins:
An equivalent of 3% of the extract was evaporated to dryness on a water bath, the residue was cooled and triturated with 15 ml of pet ether and filtered titration of the residue was prepared with additional volume of pet ether until the last volume of pet-ether was essentially colorless.
The defatted residue was dissolved in 30 ml of 80% ethanol and filtered:
(a) To 3 ml of the filtrate in a test tube 1ml of 1% AlCl$_3$ solution in methanol was added formation of a yellow color indicates to the possible presence of flavonoid compounds (flavones, flavonones, chalcones and flavonols)
(b) To 3ml of the filtrate in a test tube, 1 ml of KOH solution was added dark yellow colour indicates the possible presence of flavonoid compounds (flavones, flavonones, chalcones and flavonols).
(c) To 2 ml of the filtrate 0.5 ml concentrated HCl and few magnesium turnings were added, production of a definite colur change to pink or red was taken as presumptive evidence that flavonols or flavonones were present in the plant sample.
(d) To 5 ml of the filtrate in a test tube 0.5 ml of concentrated HCl was added and warmed on a water bath for 5 minutes red violet colour development is an indication of the possible presence of leucoanthocyanins.

2.2.5.5. Screening for Alkaloids:
A volume of the prepared extract equivalent to 50 gm of plant material was taken and evaporated to a syrupy consistency on a water bath 10 ml of 2N HCl were added to the extraction an evaporating dish and stirred while heating on a water bath for 5 minutes the dish was removed from the water bath and cooled to room temperature.0.5 gm of NaCl were added, stirred and then filtered. The residue was washed with sufficient volume of additional HCl to final volume of 10 ml was divided in to two equal portions in dry test tubes.
1- To one of the test tube a few drops of mayers reagent were added.
2- To another test tube a few drops of wagners reagent were added.
Any precipitate or turbidity is an indication of the alkaloid.

2.2.5.6 Screening for coumarins:
The extract 3 ml was evaporated to dryness, the residue was dissolved in hot water, After cooling the solution was divided in to two test tubes, one tube contained the reference and the aqueous solution of the second tube was made alkaline with 0.5 ml of ammonia solution 10%, the occurrence of an intense florescence under UV light indicates the presence of coumarins.

2.2.5.7. Screening for 2-Deoxygen sugar:
10 ml of Alcoholic (95%) extract were placed in an evaporating dish and evaporated to dryness on water bath, the dried extract defatted triturated with pet-ether to remove as much pigments as possible, the pet-ether was decanted and the defeating process was repeated twice and the residue was dried by evaporating the residual pet-ether 3ml of FeCl₃ reagent were added the mixture was well stirred and then transferred to small test tube and 2ml of concentrated H₂SO₄ were added by
allowing them to run down side wall of the test tube. A purple ring at the interference indicates to the possible presence of 2-Deoxy sugar.

2.2.5.8. Screening for carbohydrates:
To 1ml of molish reagent 2.5 ml of crude extract and 3ml concentrated H₂SO₄ were added and mixture was heated at 45 °C to formation of a violet ring between two solution surfaces present which spread by shaking.

2.2.5.9 Screening for reducing compounds:
The methanol extract 1ml was diluted with distilled water 2 ml and Fehling’s solution 1 ml was added and heated, a brick-red precipitate indicates the presence of reducing compounds.

2.2.6 Quantitative determination of chemical constituency: (Edeoga, 2005)

2.2.6.1. Alkaloid content:
5 gm of plant sample were weighed in to 250 ml beaker and 100 ml of 10% acetic acid in ethanol were added and the beaker was covered and allowed to stand for 4 hours the solution was filtered and the extract was concentrated on a water bath to one quarter of the original volume than concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed, the whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residual alkaloid was dried and weighed and the alkaloid content was calculated as percentage yield.

2.2.6.2 Flavonoid content
10 gm of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature the whole solution was filtered through whatmann filter paper. The filtrate was later transferred in to 250 ml beaker and evaporated to dryness over water bath and weighed to constant weight and the flavonoids content was calculated as percentage yield.
2.2.6.3. **Terpenes content**

10 gm of plant sample were transferred into beaker and dissolved in 100 ml chloroform. The contents of the beaker were left at room temperature for three days with frequent shaking the extract was filtered under pressure and the residue was dissolved in cold ethanol (95%), the produced solution was treated by hydrous lead acetate solution (5%) to precipitate fatty acids and phenolic compounds. The solution was filtered and evaporated to minimum volume over water bath the concentrate was cooled and transferred in to 250 ml reparator funnel and 10 ml of chloroform were added and shacked vigorously the aqueous layer was discarded while the chloroform layer was recovered, the process was repeated two times the filtrate was collected, dried, weighed to constant weight and the terpenes content was calculated as percentage.

2.2.6.4. **Saponins content**

5 gm of plant sample was transferred in to conical flask and 100 ml of 20% aqueous ethanol were added, the sample were heated over a hot water bath for 3 hours with continuous stirring at about 65°C the mixture was filtered and the residue was extracted with another 100 ml of 20% ethanol the combined extracts were reduced to 40ml over water bath about 90°C the concentrate was transferred into 250ml reparator funnel and 20 ml of diethyl ether were added and shaken vigorously, the aqueous layer was recovered 60 ml of n-butanol were added and the combined n-butanol extracts drop wise with 10 ml of 5% aqueous sodium chloride, the remaining solution was heated in water bath after evaporation, the residue was dried, weighed to constant weight and the alkaloid content was calculated as percentage yield.
2.2.6.5. Tannins content

10 gm of plant sample were transferred into a 250ml beaker and dissolved in 100 ml distilled water. The sample was heated on a water bath at 100°C and then cooled. The mixture was filtered and the residue was transferred into a 250 ml separatory funnel and 20 ml of chloroform were added and shaken vigorously. The chloroform layer was discarded while the aqueous layer was recovered. After that, the ethyl acetate was added and shaken. The ethyl acetate layer was discarded, and the aqueous layer was transferred to a beaker and evaporated and weighed to constant weight. The tannin content was calculated as percentage yield.

2.2.7. Biological activity:

2.2.7.1. Antioxidant activity:

DPPH radical scavenging was determined according to the method of Shimada et al. (1992) with some modification in 96-wells plate. The test samples were allowed to react with 2,2-Di(4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as 300 um. The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using a multiplate reader spectrophotometer. Percentage radical scavenging activity of samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

2.2.7.2. IC₅₀ Calculations:

The IC₅₀ (the concentration of test material, which possess 50% inhibition of free radical) of all the extracts and their fractions was determined by monitoring the effect of different concentrations ranging from 0.5-0.03 mg/ml, the extracts and their fractions were calculated using EZ-Fit Enzyme Kinetic program.
2.2.8. Chromatographic methods:

2.2.8.1 Thin layer chromatography (TLC):

The active methanol (Soxhlet) extract was spotted using capillary tube on the one end of the thin layer plate at above 1 cm. Plate was allowed for air dry, then it was placed in a tank containing solvent Chloroform: Methanol in the ratio of 8:2. The samples were allowed to run towards the other end of the plate, the sheet was removed and allowed it to air dry for 10 minutes. The plate was then visualized under the UV light and with vanillin- sulfuric acid reagent.

2.2.8.2 Preparative thin-layer chromatography:

A concentrated active methanolic extract examined with TLC were chromatographed on s preparative TLC plates. The plates were twice developed in a mixture of (8:2 Chloroform: Methanol). By examining plates (under UV light), detection of the plates by 1% vanillin H₂SO₄ spray reagents, different zones were located. Each was scrapped off in a separate container, and each zone was removed by washing the dry Silica gel obtained with (chloroform: methanol) (80:20) several times, decanting the solvents and removing the excess Silica gel by centrifugation. The extracts were concentrated and the resulting solutions of different zones were passed separately through a sintered glass (porosity no3) to remove any trace of Silica gel. The filtered extracts were then concentrated, and air dried.

2.2.9. Infrared spectroscopy (IR):-

A few milligram of fraction was dissolved into chloroform placed in the machine and the spectrum were recorded the finished spectrum consist of a chart showing down-ward peaks corresponding to absorption plotted against wave length or frequency. Fourier Transform -IR spectrometer,( FT/IR 8400s-Shimadzu, Japan) was used.
2.2.10. Ultra violet spectroscopy (UV):-

UV/vis. double beam recording Spectrophotometer model: /UV/1800 Shimadzu (Japan) was used for scanning of fractions in the rage of (200-600 nm). The eight fractions were dissolved in chloroform made up to 100 ml. A portion of this was transfer to quartz of 1cm thickness. A matched cell containing pure chloroform was also prepared and each cell was placed in appropriate place in the spectrophotometer, bank test was carried.
3. Results and Discussion

3.1 Chemical composition of C. siliqua L pods:

The proximate analysis (moisture, ash content and mineral composition) of C. siliqua L. pods are presented in Table (3.1).

Table (3.1): proximate analyses of the C.siliqua pods

<table>
<thead>
<tr>
<th>Items</th>
<th>Percent/ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>4.4%</td>
</tr>
<tr>
<td>Ash content</td>
<td>19.47%</td>
</tr>
<tr>
<td>Calcium(Ca)</td>
<td>0.134</td>
</tr>
<tr>
<td>Manganese(Mn)</td>
<td>11.6</td>
</tr>
<tr>
<td>Potassium(k)</td>
<td>0.755</td>
</tr>
<tr>
<td>Sodium(Na)</td>
<td>172</td>
</tr>
<tr>
<td>Iron(Fe)</td>
<td>0.133</td>
</tr>
<tr>
<td>Copper(Cu)</td>
<td>1.2</td>
</tr>
<tr>
<td>Zinc(Zn)</td>
<td>117.1</td>
</tr>
</tbody>
</table>

Moisture and total solids content are important in the food industry as they influence food preservation, food quality and resistance to deterioration. The present study showed that the moisture content of C. siliqua L. pods (4.4%). The ash content represents the inorganic materials found in the plant, for C. siliqua L. pods ash content (19.47%). Table (3.1)

The contents of copper, lead, zinc, calcium, manganese, potassium, sodium and iron were established by the atomic absorption spectroscopy; The mineral composition, expressed in gm per 100 gm, the C.siliqua pods contain considerable amount of sodium(172), zinc (117), and Mn 11.6 ppm, and trace amount of K (0.755), Cu (1.2 ), Ca (0.134), and finally Fe(0.133) Table (3.1).
Table (3.1) showed that the pods of the studied plant would represent potential sources in mineral and notably in sodium and zinc the scarcity of which constitutes a problem in public health. The Sodium and Zinc content were similar to the one we previously reported but the iron content was lower. It is interesting to note that the level in sodium was relatively much higher than those reported in the Shatnawi, et al., 2001 study. Iron is an essential trace element for haemoglobin formation, normal functioning of the central nervous system and in the oxidation of carbohydrates, protein and fats, iron concentration was (0.755ppm) and this result was inconsistent with the findings with that reported by (FAIK et al., (2007). Fe (1.88 ), K (970 ), P (71) Ca( 300 ), Mn (1.29 ), Zn( 0.75) and Cu (0.85) ppm.

3.2. Extraction of C. siliqua L. pods:
The C. siliqua L. pods were extracted using different solvent and various methods (soxhelt and maceration). The yield% and properties of extracts are described in table (3.2) and table (3.3) respectively.

**Table 3.2 Yield percentage and properties of C. siliqua L. pods**

<table>
<thead>
<tr>
<th>Extract</th>
<th>Yield%</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>2.27</td>
<td>Yellow sticky-paste</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.42</td>
<td>Green –black sticky-paste</td>
</tr>
<tr>
<td>Methanol</td>
<td>25.55</td>
<td>Brown-Vague solid</td>
</tr>
</tbody>
</table>

**Table 3.3 yield percentage and properties of C. siliqua L. pods extracts**

<table>
<thead>
<tr>
<th>Extract</th>
<th>Yield%</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>18.32</td>
<td>Brown - solid</td>
</tr>
<tr>
<td>Water</td>
<td>10.70</td>
<td>Brown -solid</td>
</tr>
</tbody>
</table>

Tables (3.2) and (3.3) show that methanol is by far is a good solvent for soxhlet extraction of carob pods gave (25.55%), hexane (2.27%) and finally chloroform
(0.42 %) have lowest ratio of extraction so the soxhlet extraction is considered better comparative with maceration. Our study on contrary with study by (Ydjedd et.al, 2017), the lowest yield was recorded with the chloroform extract (0.50%) at the unripe stage and in disagreement in yield of hexane extract (0.28%). In this study, the extractive yield of *C.siliqua pods* varied depending on the different solvents used and the recovery percentage of extractable compounds ranged from 0.42% to 25.55% (Table 3. 2 & Table 3.3).

The yields of polar extracts (methanol 25.55%, ethanol 18.32%, and water 10.17%) of *C.siliqua pods* are higher than the less-polar hexane (2.27%), chlorform (0.42%) extracts. Differences of yield extracts can be explained by the difference in the polarity of solvent used and the chemical nature of the sample. Indeed, our previous studies indicated that the extraction yield depends on solvent, time and temperature as well as chemical composition of the sample (Benchikh and Louaileche, 2014). Moreover, under the same time and temperature conditions, the solvent used and the chemical property of sample are the two most important factors (Baravalia et al., 2009). It can consequently be argued that the *C.siliqua pods* are constituted mainly with polar extractable compounds at both ripe and unripe stages.
3.3. Phytochemical screening of *C. siliqua* L. pods:

The results of phytochemical screening of *C.siliqua* L. are shown in table (3.3).

**Table 3.4: Phytochemical screening of *C. siliqua* L pod extracts**

<table>
<thead>
<tr>
<th>Tests</th>
<th>(95%)Ethanol extracts</th>
<th>Water extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triterpenes</td>
<td>+ +</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+ +</td>
<td>+</td>
</tr>
<tr>
<td>Saturated sterol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Unsaturated sterol</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leucoanthocyanins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>2-deoxy sugars</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Reducing compounds</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+++) = relatively higher amounts, (++) = low amounts, (+) = lowest amounts

(-) = not detectable.

Table (3.4) show the results of phytochemical screening for *C.siliqua* pod extracts the results show that, *C.siliqua* pods is contained relatively higher amounts of tannin, carbohydrates, alkaloids and flavonoids and lower amounts of reducing compounds and coumarins these results are in agreement with that reported (Kabesh *et al*, 2015) in amounts of tannins, carbohydrates and flavonoids and disagreement in amount of alkaloids and coumarins.
3.4. Quantitative determination of chemical constituency of *C. siliqua* pods:

The result of chemical constituency of *C. siliqua* L pods is show in table (3.5)

Table (3.5): chemical constituency of *C. siliqua* L pods

<table>
<thead>
<tr>
<th>NO</th>
<th>Chemical content</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>6.18</td>
</tr>
<tr>
<td>2</td>
<td>Falvonoid</td>
<td>18.10</td>
</tr>
<tr>
<td>3</td>
<td>Trepenes</td>
<td>3.03</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>4.50</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>14.40</td>
</tr>
</tbody>
</table>

The results in table (3.4) show that *C. siliqua* pods is rich source of flavonoids (18.1%), tannins (14.4%) and contain a low amount of terpenes (3.03%), this study is in good agreement with (Strnad et al., 2007) in amount of flavonoids (17.55%) and tannins (15.9%), but disagree in amount of saponins (8.5%) and alkaloids (10.34%).

3.5. Evaluation of antioxidant activity of *C. siliqua* L pod extracts

The results of antioxidant activity for *C. siliqua* L pod extracts were shown in table (3.6) below.

Table (3.6): Antioxidant activity for *C. siliqua* L pod extracts

<table>
<thead>
<tr>
<th>No</th>
<th>Extract</th>
<th>%RSA±SD (DPPH)</th>
<th>IC 50± SD mg/ml (DPPH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>63+ - 0.01</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Hexane</td>
<td>87+- 0.01</td>
<td>0.189± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform</td>
<td>19+- 0.04</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Methanol</td>
<td>92+- 0.01</td>
<td>0.060±0.03</td>
</tr>
<tr>
<td>5</td>
<td>Tritrepenes</td>
<td>44.+ 0.02</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Ethanol</td>
<td>85+- 0.01</td>
<td>0.117±0.01</td>
</tr>
<tr>
<td>Standard</td>
<td>Propyl Gallate</td>
<td>95+- 0.01</td>
<td>0.077 ±0.01</td>
</tr>
</tbody>
</table>

DPPH = 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl
The results of the DPPH radical-scavenging activity of bioassay extracts shown in table (3.5) above revealed that all extracts quenched DPPH radical and the scavenging activity increases with the solvent polarity. Among all the tested extracts, the methanol, and ethanol extracts from the carob pods show stronger activity than with the other solvents. The DPPH radical scavenging activity of the methanol extract was 92±0.01 μg/mL and hexane extract was 87±0.01 μg/mL followed by ethanol extract with values of 85±0.01 μg/mL respectively. The IC\textsubscript{50} values are presented in Table (3.5) A high IC\textsubscript{50} value corresponds to a low antioxidant activity. The IC\textsubscript{50} values for methanol extract the 0.060±0.03 and for standard propyl gallate were found to be 0.077 ±0.01. These differences in DPPH activity between different solvents extracts could be certainly due to the phenolic contents.

### 3.6. Thin layer chromatography of C. siliqua L pod methanolic extract:

Based on the antioxidant activity results in Table (3-6) the methanol (sox) extract was subjected to analytical thin layer chromatography using various solvent systems. Phytochemical screening presented in table (3.8) revealed that the methanol extract contains tannin, carbohydrates, alkaloids and flavonoids as the main natural components. In a keen and active attempt, to isolate the active component(s), the active methanol extract was subjected to various analytical thin layer trials using various solvent systems. The active methanol extract was fractionated in preparative thin layer chromatography (PTLC) using solvents mixture (Chloroform: Methanol (8:2). Finally eight fractions were separated by PTLC chromatography. The characteristic colours and RF values of these fractions are tabulated in table (3.7).and plate (3-1) below.
Table (3.7): Thin layer chromatography of *C. siliqua* L pod methanolic extract:

<table>
<thead>
<tr>
<th>TLC fraction</th>
<th>RF value</th>
<th>Colors</th>
<th>Visible light</th>
<th>UV $\lambda$ 254</th>
<th>UV $\lambda$ 366</th>
<th>Spray reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.088</td>
<td></td>
<td>-</td>
<td>Blue</td>
<td>-</td>
<td>Green</td>
</tr>
<tr>
<td>F2</td>
<td>0.152</td>
<td></td>
<td>-</td>
<td>Green</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F3</td>
<td>0.235</td>
<td></td>
<td>-</td>
<td>Blue</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F4</td>
<td>0.405</td>
<td></td>
<td>Pale Yellow</td>
<td>Pale Green</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F5</td>
<td>0.5</td>
<td></td>
<td>-</td>
<td>Blue</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F6</td>
<td>0.623</td>
<td></td>
<td>Pink</td>
<td>Green</td>
<td>-</td>
<td>Pink</td>
</tr>
<tr>
<td>F7</td>
<td>0.882</td>
<td></td>
<td>-</td>
<td>Green</td>
<td></td>
<td>Grey</td>
</tr>
<tr>
<td>F8</td>
<td>0.970</td>
<td></td>
<td>-</td>
<td>Red</td>
<td>-</td>
<td>Brown</td>
</tr>
</tbody>
</table>
Plate (3.1): PTLC of methanolic extract

**Mobile phase:** (Chloroform: Methanol (8:2))

**Detection:** spraying with 1% vanillin / sulphuric acid
3.7 Characterization and analysis of isolated fractions:
The component eluted from the preparative TLC chromatography ended up with the isolation of eight pure fractions from active methanol extract using mobile phase Chloroform: Methanol (8:2)). Analysis of fractions was based on different spectroscopic techniques such as UV, IR spectroscopy. The IR–spectra Table (3-8), and the UV-spectra Table (3-9) respectively.

Table (3.8): Infrared Spectroscopy for C. siliqua L pod methanolic extract

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Wave Number</th>
<th>Bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>3369.00</td>
<td>O-H</td>
</tr>
<tr>
<td></td>
<td>2931.13</td>
<td>C-H</td>
</tr>
<tr>
<td></td>
<td>1732.05</td>
<td>C=O</td>
</tr>
<tr>
<td>F2</td>
<td>2928.52</td>
<td>C-H</td>
</tr>
<tr>
<td></td>
<td>1732.22</td>
<td>C=O</td>
</tr>
<tr>
<td>F3</td>
<td>2930.58</td>
<td>C-H</td>
</tr>
<tr>
<td></td>
<td>1731.04</td>
<td>C=O</td>
</tr>
<tr>
<td>F4</td>
<td>3430.20</td>
<td>O-H</td>
</tr>
<tr>
<td></td>
<td>2083.91</td>
<td>C-H</td>
</tr>
<tr>
<td></td>
<td>1643.23</td>
<td>C=O</td>
</tr>
<tr>
<td>F5</td>
<td>2932.56</td>
<td>C-H</td>
</tr>
<tr>
<td></td>
<td>1731.35</td>
<td>C=O</td>
</tr>
<tr>
<td>F6</td>
<td>2935.13</td>
<td>C-H</td>
</tr>
<tr>
<td></td>
<td>1731.66</td>
<td>C=O</td>
</tr>
<tr>
<td>F7</td>
<td>2928.47</td>
<td>C-H</td>
</tr>
<tr>
<td></td>
<td>1731.20</td>
<td>C=O</td>
</tr>
<tr>
<td>F8</td>
<td>2929.78</td>
<td>C-H</td>
</tr>
<tr>
<td></td>
<td>1731.54</td>
<td>C=O</td>
</tr>
</tbody>
</table>
Table (3.9): Ultraviolet visible spectroscopy (UV) for *C. siligua* L pod methanolic extract

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Wavelength(nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>300.00</td>
</tr>
<tr>
<td>F2</td>
<td>300.00</td>
</tr>
<tr>
<td>F3</td>
<td>302.00</td>
</tr>
<tr>
<td>F4</td>
<td>298.00</td>
</tr>
<tr>
<td>F5</td>
<td>302.00</td>
</tr>
<tr>
<td>F6</td>
<td>298.00</td>
</tr>
<tr>
<td>F7</td>
<td>214.00</td>
</tr>
<tr>
<td>F8</td>
<td>224.00</td>
</tr>
</tbody>
</table>

Based on the close similarity of the spectral characteristics (IR) functional group and the UV Wavelength (nm) of isolated fractions in tables (3.8) and (3.9) above, in addition to the positive result of phytochemical tests done to characterize the class to which fractions belong to, all those factors give an indication that isolated fractions are isomers and may be phenolics compounds (flavonoids, or tannins).
4. Conclusion and Recommendation

4.1 Conclusion:

Phytochemical study on *C.siliqua* pods extracts illustrated the presence of tannin, alkaloid, saponins, deoxy sugars, saturated sterols, unsaturated sterols, flavonoids, carbohydrates, reducing compound, and leuco-anthocyanins.

The results obtained in this study demonstrated the activity of *C.siliqua* as antioxidant more importantly the result indicated that methanolic extract of *C.siliqua* are more effective as antioxidant than the water extracts.

The result is shown that *C.siliqua* pods is important source of element, and phenollic compound for that it’s uses in wide range as antioxidant, antitamoral, anti-diarrheal, antimicrobials, antibiotic and anti diabetic. Methanolic fractions from thin layer chromatography were analyzed using IR and UV spectroscopy confirmed the presence of pure eight fractions.

4.2 Recommendation:

According to the results it recommended that:

- The results of this study are promising because methanolic, ethanolic and hexane extracts of pods have considerable antioxidant activity.
- The study also supports the use of *C.siliqua* is not only as dietary supplement but also as agent to prevent or control the some diseases.
- A *C.siliqua* pods extracts warranting more investigations, fractionations and purification for active antioxidant compounds.
- It is need for modern equipment to extract, purify of plant constituent to enable researcher to accomplish their goal successfully and obtain reliable results.
- This study is to be developed in pharmacology to give antioxidant drugs for treatment the multiple infections.
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