Sudan University of Science and Technology

College of Graduate Studies

Phyto-chemical Screeningof Seedsand Physicochemical Characterization of the Oil of *Citrullus Colocynthis*

المسح الفيتوكيميائي للبذور والتوصيف الفيزيوكيميائي لزيت نبات الحنظل

A Dissertation submitted in partial fulfillment for an M Sc. Degree in chemistry

By

Nana Mohamed Khier ElZibair (B Sc. P G D,chemistry)

Supervisor:Dr. Mohamed Elmubark4 Osman

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بسم الله الرحمن الرحيم

قال عز وجل :

إِنَّا كُلَّ(شَيْءٍ خَلَقْنَاهُ بِقَدَرٍ)

سورة القمر 49

Dedication

I dedicate this research to my…

Mother

Daughters …… **Elaf, Abrar, Ethar, Anwar, Noor Al Ain.**

Husband and Friends

Without their understanding, support and love the completion of this research would not have been possible.

Acknowledgements

Praise is due to Allah, the most Gracious, the most Merciful, upon completion of this work.

I wish to express my gratitude and thanks to Dr. Mohammed El Mubark Osman for his suggestion, guidance, encouragement and support throughout the period of study.

Thank are due to the staff of the National Research Centre for technical support.

Abstract

A sample of *Citrullus colocynthis* fruits were collected from Alamab Area South Khartoum state, the seeds were isolated after dryness of the fruits.

Phytochemical screening of the seeds indicate that they contain of flavonoids, tannins, cumarins , sterol, triterpene, saponins and Alkaloids.

The oil of *Citrullus colocynthis* was extracted from the seeds. The average yield was 20%.

Physico-chemical characterization result show that the density of the oil was 0.9728 g/cm^3, Iodine value was 94.68, Saponification value was 223.763, Acid value was 1.29, Peroxide value was trace and Ester value was 222.473.

المستخلص

تم جمع ثمار الحنظل من منطقة اللاماب جنوب الخرطوم وتم استخرج البذور بعد تجفيف الثمار.

الفحص الفيتوكيميائي لبذور الحنظل تشير إلى أنها تحتوي على مركبات الفلافونويد، التاينين والكومارين،الستيرول، تربينات ، الصابونين وقلويدات.

الزيت المستخلاص من بذور الحنظل نسبته 20% .

الخواص الفيزوكيميائية لزيت الحنظل هى كثافة الزيت $0.9728\ g/cm^3$ ، قيمة اليود 94.68 ، قيمة التصبن 223.763 ، قيمة الحموضة 1.29 ، قيمة البيروكسيد مقدار ضئيل ، رقم الاستر 222.473 .

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Chapter One
Introduction

Literature review

(1.1) natural products:-

Natural products chemistry is the chemistry of metabolite products of plant, animals, insects , marine organisms and microorganisms. The metabolic products include alkaloids, flavonids, terpenoids, glycosids, amino acids, protein, carbohydrates etc. the application of natural products range from medicines, to sweeteners and pigments. (Rensheng' et al , 2010)

(1.1.1)Alkaloids:-

Alkaloids are defined as natural plant compounds having a basic character and containing at least one nitrogen atom in a heterocyclic ring. The alkaloids are usually colorless, crystalline, non-volatile solids which are insoluble in water , but are soluble in ethanol, ether, chloroform, etc. Some alkaloids are liquids which are soluble in water. Most alkaloids have a bitter taste and are optical active. They are generally tertiary nitrogen compounds and contain one or two nitrogen atoms usually in the tertiary state in a ring system, most of the alkaloids also contain oxygen. (Saxen, 2007). Figure (1.1) show structure of some common alkaloids. (Harborne,1998)

(1.1.2)Flavonoids:-

Flavonoids are plant secondary metabolites, aromatic and belong to the group of plant phenols. Concerning the plant phenol, and consequently the flavonoids, it may be considered as those compounds originating in the shikimate and phenyplpropanoid pathways. Notwithstanding, the flavonoids, as a differentiate subgroup inside the phenolic compound, show a characteristic metabolic intermediate, the naringeninchalcone, from which all the bioflavonoids originate. Exclusively from a chemical point of view the flavonoids are characterized by a skeleton of three units, $C_6 - C_3 - C_6$, that forms a cyclic structure in most cases. In this skeleton tow aromatic ring, referred to as A and B (in chalcones) , can be distinguished, and an additional third ring C , in the rest of the flavonoids. This last ring appears as a cyclation of chalcones with hydroxyl in 6 position figure (1.2). A and B ring have a different metabolic source. The B ring is formed in the shikimate pathway, while the A ring comes from the condensation of three units of malonil

Co-A {3,4}(Atta-ur-Raham, 2002). Figure (1.3) show Chemical structures of the various classes of flavonopids(Atta-ur-Raham, 2002).

(1.1.3)Terpenoids:-

Terpenoids are defined as materials with molecular structures containing carbon backbones made up of isoprene (2-methylbuta-1,3-diene) units. Isoprene contains five carbon atoms and therefore, the number of carbon atoms in any terpenoids is a multiple of five. Degradation products of terpenoids in which carbon atoms have been lost through chemical or biochemical processes may contain different number of carbon atoms, but their overall structure will indicate their terpenoid origin and they will still be considered as terpenoid. The generic name “ terpene ” was originally applied to the hydrocarbons found in turpentine, the suffix “ ene ” indicating the presence of olefinic bonds. Each of these materials containing 20 carbon atoms are named as diterpenes. Figure (1-4) show how the isoprene unit and the original backbone can be traced out in three simple terpenoids. (Charle., 2003)

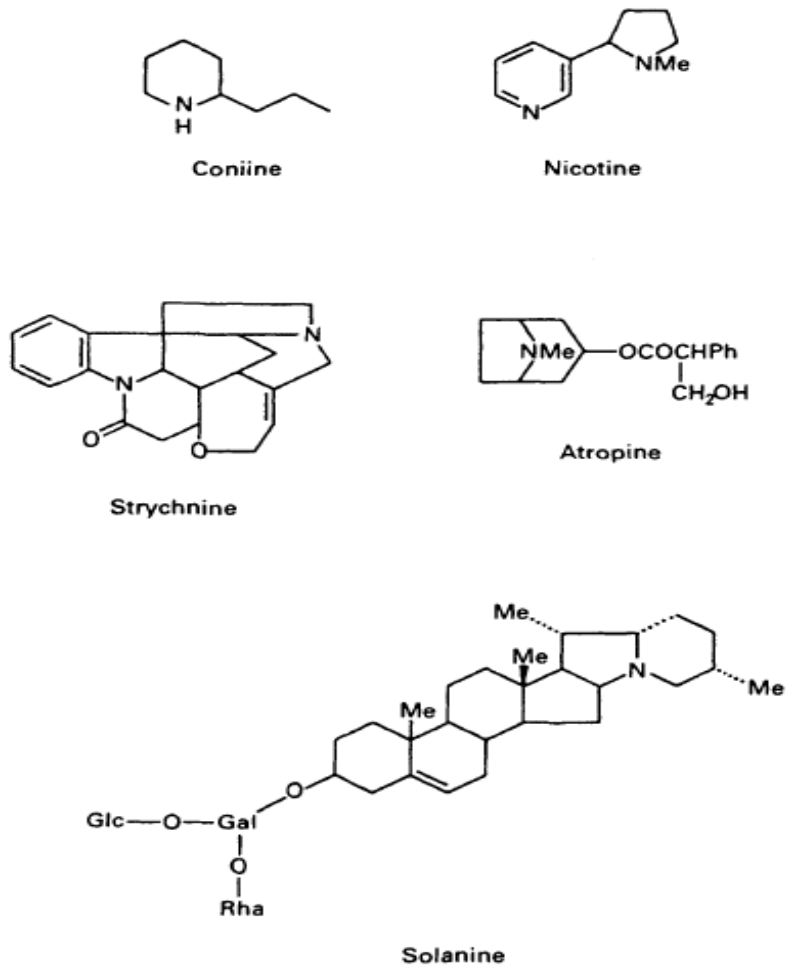


Figure (1-1):Structure of some common alkaloids.(J.B. Harborne,1998).

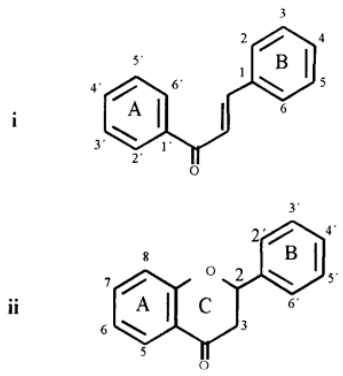
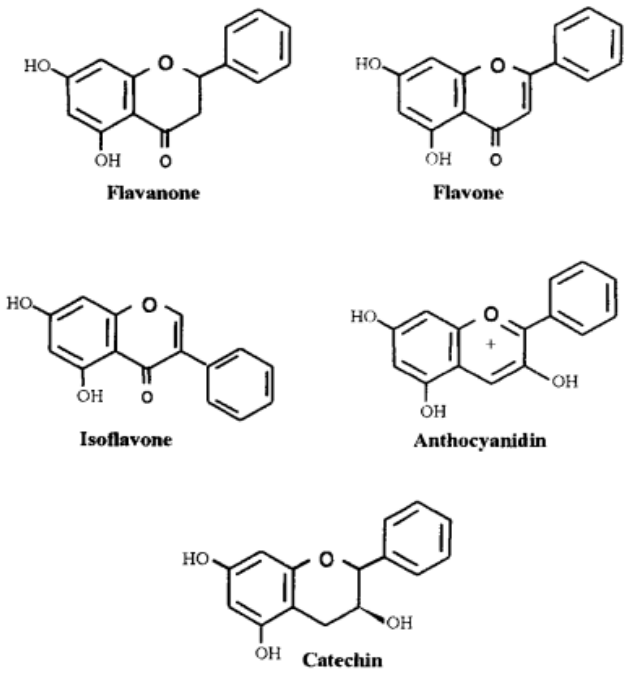


Figure (1-2)Basic skeleton of the flavonoids.(i)Chalcones(ii)Phenylbzopiran-4-one. (Atta-ur-Raham, 2002).



Figure(1.3) Chemical structures of the various classes of flavonopids(Atta-ur-Raham, 2002).

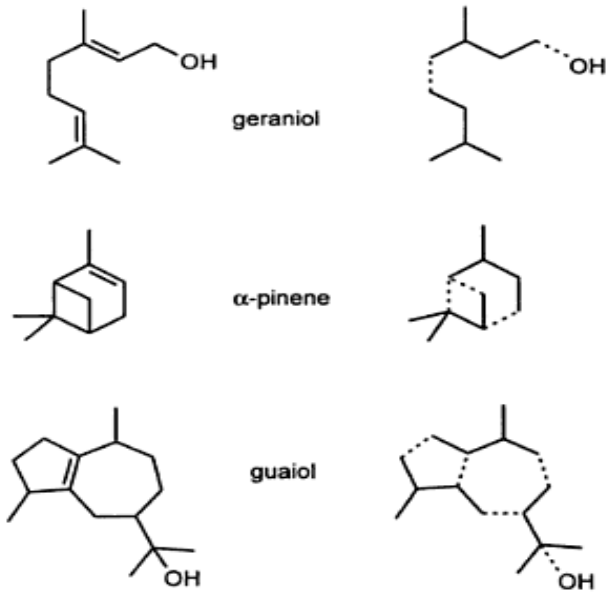


Figure (1-4): isoprene unit and the original backbone in three simple terpenoids (Charle., 2003)

(1.2)*Citrullus colocynthis* :-

Citrullus colocynthis commonly known as bitter apple(figure1.5), colocynth , or wild-gourd , is a tropical plant that grows abundantly in many place in the world. Originally from Asia and Africa, it is now widely distributed in the Saharo-Arabian phytogeographic region in Africa and the Mediteranean region.(J. Nat,et al, 2011).

Figure(1-5). *Citrullus colocynthis* (Abbah, et al, 2014)

(1-2-1) Botanical Classification of *Citrullus colocynthis*:-

Kingdom : *Plantae*

Division : *Magnoliophyta*

Class: *Magnoliopsida*

Order : *Cucurbitales*

Family : *Cucurbitaceae*

Genus : *Citrullus*

Species : *C. colocynthis* (Abbah,et al, 2014)

(1.2.2) Morphology :-

The angular leaves are altemately located on long petioles. Each leaf is almost 5 to 10 centimeters in length and has around 3 to 7 lobes. Sometimes, the middle lobe may have an ovate structure. The leaves have a triangular shape with many clefts. The leaves have a rough, hairy texture with open sinuses. The upper surface of the leaves are fine green in color and the lower surface is comparatively pale. Each bitter apple plant produces around 15 to 30 globular fruits having a diameter of almost 7 to 10 centimeters. The outer portion of the fruit is covered with a green skin having yellow stripes. The fruits may also be yellow in color. The ripe fruits are characterized by a thin but hard rind. The fruits have a soft, white pulp which is filled with numerous ovate seeds. The yellow-colored flowers appear singly at leaf axils. They are monoecious, the pistils and stamens are present in different flowers of the same plant. They have long peduncles. Each flower is also comprised of a yellow campanulate. The corolla has five lobes and the calyx is parted five ways. The female flowers are easily identified from the males by their villous, hairy ovary. The seed are around 6 mm in size, smooth, compressed and ovoid-shaped. They are located on the parietal placenta. The seeds are light yellowish-orange to dark brown in color. The Bitter Apple plant has a large perennial root that sends out long and slender, angular, tough, rough vine-like stems. The stems are normally spread on the ground and have a tendency to climb over herbs and shrubs by their axillary branching tendrils.(Borhade,et al, 2013)

(1.2.3) Chemical contents :-

Chemical contents of *Citrullus colocynthis* are showing in Table (1.1), Physical and chemical properties of *Citrullus colocynthis* seed oil are showing in Table (1.2) and table (1.3) show photochemical screening of *Citrullus colocynthis* seed.

Table (1.1) Chemical content of *Citrullus colocynthis* (Borhade, et al, 2013)

Seed	1-fatty acid like Stearic, Myristic, Palmitic, Oleic, Linolenic, Linoleic acid 2-protein 8.25 % , rich content in lysine, leucin and sulfo amino acid like methionine 3-vitamin B_1 , B_2 and Niacin 4-Mineral like Ca, Mg, K, Mn, Fe, P, and Zn
Aerial part and fruit	Flavonoid glycoside quercetin, Flavone- 3- glucoside viz iso-orentine and iso-orentine -3-methly ether
fruit	1-Cucurbitane type triterpen glycoside viz colocynthoside Aand B. 2- Cucurbitane type triterpen glycoside viz cucurbitacin E 2-O-beta-D-glcoside and its aglycone Cucurbitation E 3- 2-O-beta-D-glucopyranosyl-16alpha-20R-dihyroxy-cucurbita-1,5.23E(26)-teraen-3,11,22-trione. 4- 2-O-beta-D-glcopyranosyi-cucurbitacin B and 2, 25-di-o-beta-D-glucopyranosyl-cucurbitacin L .

Table (1.2) :- Physical and chemical properties of *Citrullus colocynthis* seed oil :-

Physico - chemical properties	Ref (Mirjana,2005)	Ref (Hiba,2015)	Ref (Kulkarni,2012)
Specific gravity	0.914 (20° C kg/dm^3)	0.886 (g/cm^3)	0.919 (15° C)
Refractive index	1.4733	1.4873 (20° C)	1.4730 (25° C)
Acid value (mgKOH/g)	1.00	3.91	1.2
Saponification value(mKOH/g)	188	196.66	189
Iodine value (g/1oog)	119	119.53	122
Peroxide value (mmolO_2/Kg)	7.9	6.97	-----
Unsaponifiable matter (%)	1.02	1.44	1.4
Ester number	187	----	-----

Table (1.3):- photochemical screening of *Citrullus colocynthis* seed (Lakshmi,2013)

phytoconstituents	Inference
alkaloids	+
Flavonoids	+
Carbohydrates	+
Glycosides	+
Saponins	+
Tannins	+
Phytosterol	+
Triterpenoids	+
Anthraquinones	-
phenols	+

(1.2.4) Uses :-

Citrullus colocynthis seed oil is used for frying and cooking in some African and Middle Eastern American countries owning to its unique flavor. (Mirjana, et al,2005)

More recent ethnopharmacological studies show that *Citrullus colocynthis* is used widely in different part of world for the treatment of a number of diseases , e.g. intestinal disorder, constipation, hypertension, anti-diabetic medication in many tropical and subtropical countries, as a remedy for sore throat and skin infection. The simply leaves of *Citrullus colocynthis* showed considerable anti-microbial, anti-diabetic, antioxidant, regional pain-killer, and anti-inflammatory action.(Hiba 2015) . Table (1-4) show Medicinal uses of different parts of *Citrullus colocynthis.*

Table (1-4) Medicinal uses of different parts of *Citrullus colocynthis* (Mahesh,et al, 2014)

Part used	Use
Seed oil	Bowel complaints
Seed oil	Epilepsy
Small fruits are collected during rainy season, stuffed with salt and ajwain	Acute stomach ache
(a)Seed oil (b)Seed powder	Cooking purpose Soup thickener and flavouring agent
Extract of pulp	Ant-bacterial
(a)Juice of fruit mixed with sugar (b)A poultice of root	(a)Dropsy (b)Inflammation of the breasts
(a)powder of roots mixed with ginger (b)Oil from seeds	(a)Inflammation of joints (b)useful hair growth and maintaining them blak
(a)fruit and root paste with water (b)Equal parts of the root with long pepper are given as pill. (c)A paste	(a)Boils and pimples (b)Rheumatism (c)Enlarged abdomen
Juice of the fresh fruit is made. Cotton dipped in juice is placed over the mouth of uterus.	Timely and easy delivery
Decoction is drunk	Hepatitis
(a)Root paste (b)Fruit and root-antidote	(a)Ascites, jaundice, urinary disease and rheumatism (b)Snake poison
Fruits in low doses	Urticaria, constipation and toxemia
Seed oil apply on hair	Blackness grey hair
Pulp of seeds	Malaria
Fruit pulp dried and powdered and taken orally	Cause abortion
Jam prepared from pulp of fruits and seeds	Effective in curing biliousness in animals
Root base mixed with cow milk is applied on hypogastrium	Easy delivery
Glycosidic extract (50mg/kg)	Lowering glucose level

(1.3)Apparatus for Extraction Methods of Oil Content Analysis :-

Several extractors are used to perform fat or oil extraction. The use of some extractor is recommended by some official methods whereas others are strictly proscribed. The most common and unavoidable systems used for oil extraction in an analytical laboratory are Butt Tube, Soxhlet Extractor and Immersion Extractors.(Luthria, 2004).

(1.3.1)Butt Tube:-

In the Butt Tube system, the sample is ground and a weighed portion is placed into a porous thimble or folded into filter paper. The thimble or filter paper is then placed into the Butt tube and the solvent is placed in flask. The apparatus is assembled as shown in figure (1-6) and the solvent is boiled, vapors rise to the condenser where they condense and drip down though the sample back into the boiling solvent below. The extraction process is continuous and can be completed within a few hours, although exhaustive methods call for regrinding of the sample after the initial oil has been removed and again near the end of the extraction. Once the extraction is finished, the solvent is removed from the extracted oil by distillation followed by vacuum drying. The extracted oil is weighed. The Goldfisch Fat Extractor (Labconco) has long been recognized as a commercial version of the Butt tube. An improvement on the Butt tube is the Twisselman extractor, which add a stopcock between the sample container and the condenser. When the stopcock is closed, it is possible to reclaim the solvent from the extracted material. The Twisselman extractor is recommended in the German standard method. (Luthria, 2004).

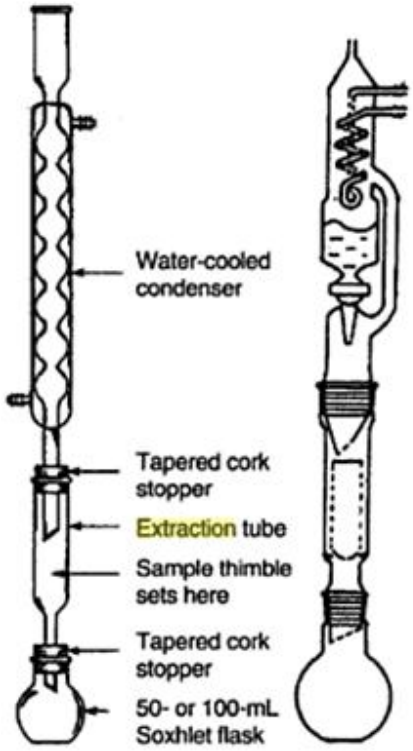


Figure (1.6):left: Butt tube extractor. Right: Twisselman modification.

(1.3.2) Soxhlet Extractor:-

In a Soxhlet extractor (figure1.7) the solvent is heated in a boiler, the pure vapor rise up through a by-pass and into the top part the Soxhlet container where the sample to extract is contained. In the condenser , the vapors are condensed and drip into the sample-containing thimble. When the level of liquid reaches the same level as the top of the top of the siphon, the liquid containing the extracted material is siphoned back into the boiler. Soxhlet extraction is not a continuous procedure, but a batch system with repeated extractions. The temperature of the solvent in the solvent vessel rise during the extraction due to the presence of higher concentration of oil in the solvent. (Luthria, 2004).

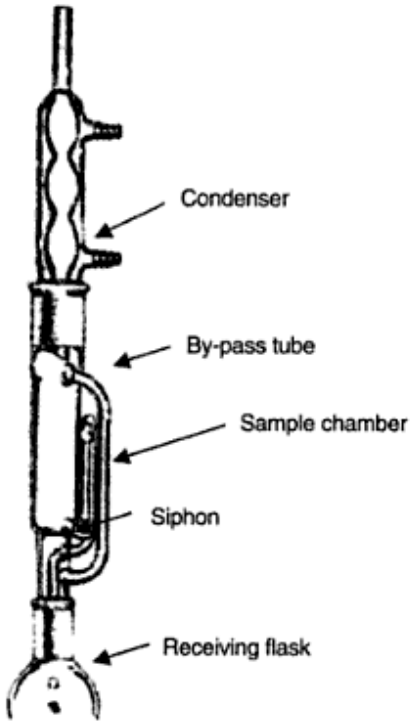


Figure (1.7): Soxhlet extractor

(1.3.3)Immersion Extractors:-

Immersion Extractors (figure 1.8) include the soxtec^{TM} system manufactured by Foss-Tecatur. The method is a two- step procedure based on the best parts of Soxhlet and Twisselman methods. At the beginning of extraction, the ground sample is placed in a thimble and lowered into the boiling solvent where the extractable material passes rapidly into the solvent. During the second phase of the extraction , the sample is raised from the solvent and the condensed vapors of the boiling solvent drip through the thimble containing the sample. In this rinse position of apparatus, there is a continuous supply of fresh solvent, allowing the continuous increase of the concentration of extracted material in the solvent recovered in the cup. Once the extraction is completed, the valve on the drip tube may be closed so that the solvent can be recovered from the extract. (Luthria, 2004).

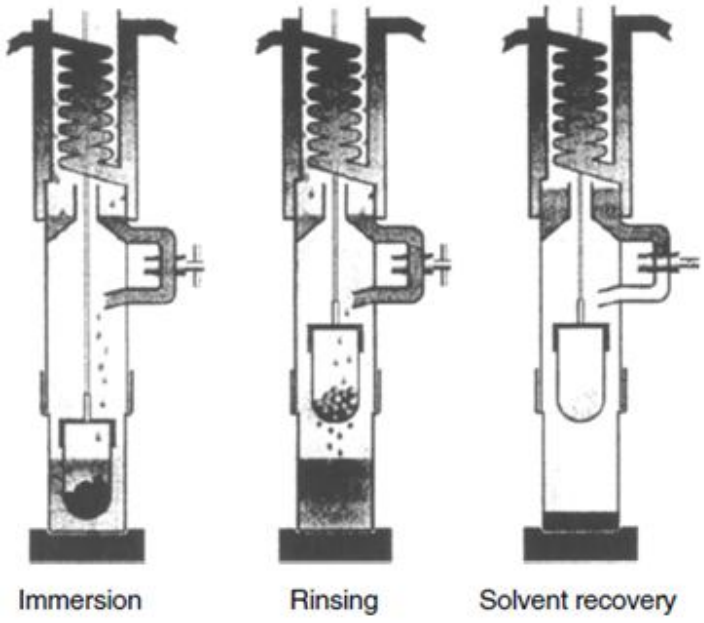


Figure (1.8): Immersion Extractors, stage of operation of the immersion extractor.

(1.4)General chemical methods of the oil:-

(1.4.1)The acid value :-

Acid value is measures of the free fatty acid content of fats and oils(Richard, 2009).

The acid value Is the number that expresses, in milligrams the quantity of potassium hydroxide required to neutralize the free acid present in 1g of the substance.(British pharmacopeia,2007).

(1.4.2)Peroxide value :-

Oxidation of lipids is a major cause of their deterioration, and hydroperoxides formed by the reaction between oxygen and the unsaturated fatty acid are the primary products of this reaction. Hydroperoxides have no flavor or odor but break down rapidly to form aldehydes, which have a strong, disagreeable flavor and odor. The peroxide concentration, usually expressed as peroxide value, is a measures of oxidation or rancidity in its early stage. Peroxide value is one of the

most widely used chemical test for determination of fats and oils quality, Peroxide value has show good correlation with organoleptic flavor score. A peroxide determination does not provide a full and unqualified evaluation of fats and oils flavor because of the transitory nature of peroxides and their breakdown to nonperoxide materials. Although a linear relationship has been observed between peroxide value and flavor score during the initial stages of lipid oxidation. (Richard pharmacopeia, 2009)

The peroxide value is the number that expresses in milliequivalents of active oxygen the quantity of peroxide contained in 1000 g of the substance. (British pharmacopeia,2007)

(1.4.3)Saponification value :-

Saponification value, a measures of the alkali-reactive groups in fats and oils, was used to predict the type glycerdes in sample. Glycerdes containing short-chain fatty acids have higher saponification value than those with longer chain fatty acids.(Richard, 2009)

The saponification value is the number of mg of potassium hydroxide required to neutralize the free acids and to saponify the ester in 1g of the substance. (British pharmacopeia,2007).

(1.4.4)Iodine value:-

The iodine value is a chemical constant for a fat or oil. It a valuable characteristic in fat analysis that measures unsaturation, but does define the specific fatty acids. Iodine value analyses are very accurate and provide nearly theoretical values, except in the case of conjugated double bond is or when the double bond is near a carboxyl group.(Richard, 2009)

The iodine value is the number that expresses in grams the quantity of halogen, calculated as iodine, that can be fixed in the prescribed condition by 100 g the substance. (British pharmacopeia ,2007).

(1.4.5)The ester value :-

Is the number that expresses in milligrams the quantity of potassium hydroxide required to saponify the ester present in 1 g of the substance. It is calculated from the saponification value and the acid value. (British pharmacopeia,2007).

Chapter Two
Materials and Methods

(2.1)Materials :-

(2.1.1) Sample collection:-

Representative sample of *Citrullus Colouynthis* was collected from Khartoum, Alamab Nasser near the White Nile River. Figure (2.1) and figure (2.2)

Figure (2.1) : plant of Citrullus Colocynthis near the White Nile River

Figure (2.2): Fruit of Citrullus Colocynthis

(2.1.2)Preparation of the sample:-

The fruits were cut into mpieces, placed in the shade until dry and then seeds were isolated. The seeds weighted 400g. The dry seeds of Citrullus Colouynthis were crushed by Moulinex grinder into powder. figure(2.1) and figure(2.2)

Figure (2.3) : fruit slices.

Figure (2.3) : Seeds of Citrullus Colocynthis

(2.2)Methods:-

(2.2.1)Extraction of the oil:-

200g of the crushed seeds were placed in a soxhlet apparatus figure (2.5), using 400 ml of petroleum ether (40-60)˚ C, then heated in 40˚ C for 5 to 6 hours. The extract solution was evaporated . Petroleum ether was removed, and the oil % yield was calculated as follows:-

$$\% \text{ yield} = \frac{weight\ of\ oil \times 100}{weight\ of\ seed}$$

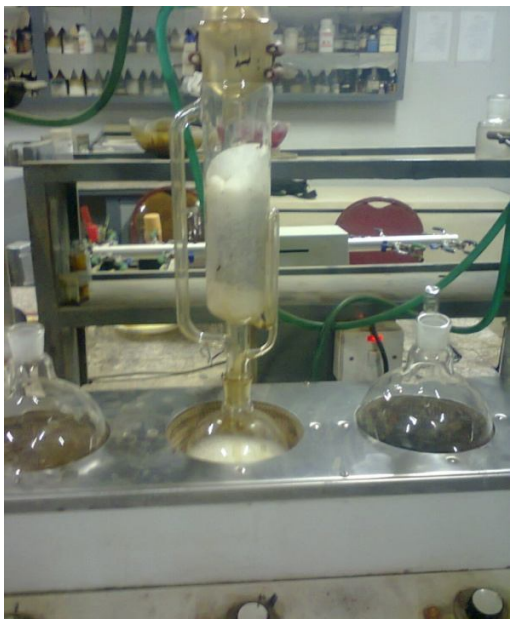


Figure (2.5) :The soxhlet apparatus

(2.2.2)General chemical analytical methods for the *Citrulls Colocynthis* seeds oil:-

(2.2.2.1) Acid Value:

25ml of ethanol (96%) were mixed with 25ml of petroleum ether then 10.02g of oil `was dissolved in the mixed solvent and titrated with 0.1M sodium hydroxide using 0.5 ml of phenolphthalein solution as indicator, with constant shaking until a pink color persists for 15 second was obtained.

$$\text{Acid value} = \frac{\text{titration (ml)} \times 5.61}{Wt\ of\ oil}$$

(2.2.2.2) The Saponification Value:-

3.5 g of potassium hydroxide were dissolved in 2 ml of water and completed to 100 ml with ethanol. 2.039 g the oil were added to 25 ml of the ethanolic solution of potassium hydroxide and boiled under reflux for 1 hour. While the solution was still hot, excess alkali was titrated with 0.5 M hydrochloric acid used 1 ml phenolphthalein solution as indicator. The operation was repeated without the oil.

The saponification value was calculated from the expression:-

$$\frac{28.05V}{w}$$

Where V is the difference, in ml, between the titrations and W is the weight, in g, of the oil.

(2.2.2.3)The Iodine Value :-

0.256 g of the oil were weighed and dissolved in 10 ml dichloromethane in dry iodine flask. 20 ml of iodine monochloride solution was added and inserted the stopper, previously moistened with dilute potassium iodide solution. The mixture allowed to stand in the dark at 15˚ to 25˚ for 30 minutes. 15 ml of dilute potassium iodide solution (10%) were placed in the top cup, the stopper was removed carefully, the stopper and the sides of the flask were rinsed with 100ml of water, shaked and titrated was with 0.1M sodium thiosulphate. Starch mucilage was added, towards the end of titration, as indicator. The same operation was repeated using a blank.

The iodine value was calculated from the expression:-

$$\frac{1.269V}{w}$$

Where V is the difference , in ml, between the titrations and W is the weight ,in g, of the oil.

(2.2.2.4) The Peroxide Value:-

5.040g of the oil were placed in a 250 ml conical flask fitted with a ground-glass stopper. 30 ml of a mixture of 2 volumes of chloroform and 3 volumes of glacial acetic acid was added and shaked to dissolve the oil. 0.5 ml of saturated potassium iodide solution was added. Shaken for, exactly, 1 minute then 30 ml of water were added. Titrated with 0.01M sodium thiosulphate, adding the titrant slowly with continuous, vigorous, shaking, until the yellow color is almost discharged. 5ml of starch solution were added and the titration continued, shaking vigorously, until the color is discharged. The blank test was carried out under the same conditions. The volume of 0.01M sodium thiosulphate used in the blank titration must not exceed 0.1 ml.

The peroxide value was calculated from the expression:-

$$\frac{10\times v)}{m}$$

Where v is the difference , in ml, between the titrations and m is the weight ,in g, of the oil.

(2.2.3) Phytochemical screening of the seeds of *Citrullus Colocynthis:-*

Preparation of the extract :-

10 g of the crushed seeds were refluxed with 100 ml of ethanol (80%) for 4 hours. The cool solution was filtered and divided into 4 equally parts , than each part were evaporated to dryness on water bath. The 4 extracts residues were used for various tests.

(2.2.3.1) Tannins:-

The extract residue was dissolved in 10 ml of hot saline solution and divided in two tests tubes. To one tube 2-3 drops of ferric chloride added and to other one 2-3 drops of gelatin salts reagent added. The occurrence of a blackish blue color in the first tube and turbidity in the second denotes the presences of tannins, figure (2.6)

Figure (2.6): Test for tannins

(2.2.3.2) Sterols and Triterpenes:-

The extract residue was defatted by several extractions with petroleum ether and the defatted residue was dissolved in 10ml of chloroform. To 50 of the solution 0.5 ml acetic anhydride was added and then 3 drops of conc. Sulphuric acid at the bottom of the test tube. The gradual appearance of green, blue pink to purple color was taken as an evidence of the presence of sterols (green to blue) and or triterpenses (pink to purple) in the sample, figure(2.7).

Figure (2.7) : Test for sterols and triterpenes

(2.2.3.3) A lkaloids:-

The extract residue was defatted by several extractions with petroleum ether and the defatted residue was dissolved in 2M HCL in water bath and stirred while heating for 10 minutes, cooled, filtered and divided into 3 test tubes. To one test tube few drops of Mayer's reagent were added while to the other tube few drops of valers reagent was added. A slight turbidity or heavy precipitate in either one the two test tubes was tanked as, presumptive, evidence for the presence of alkaloids, figure(2.8)

Figure (2.8) : Test for alkaloids

(2.2.3.4) Flavonoids :-

The extract residue was defatted by several extractions with petroleum ether and the defatted residue was dissolved in 30 ml of 80% ethanol and filtered. The filtrate was used for following tests :-

A- To 3 ml of filtrate in a test tube 1 ml of 1% aluminum chloride was added. Formation of a yellow color indicates the presence of flvonoids

B- To 3 ml of the filtrate in a test tube 1 ml of 1% potassium hydroxide solution was added. A dark yellow color indicates the presence of flavonoids compounds (flavones or flavonenes)

C- To 2ml of the filtrate 0.5ml of magnesium turnings were added. Producing of pink or red was taken as presumptive evidence that flavonenes were present in the plant sample, figure(2.9).

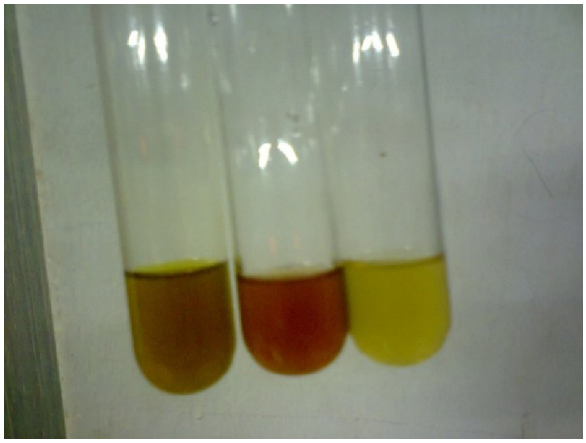


Figure (2.9) : Test for flavonoids

(2.2.3.5) Saponins:-

1 g of crushed sample was placed in test tube. 10 ml of distilled water were added, the tube stoppered and vigorously shaken for about 30 second. The tube was, then, allowed to stand and the formation of foam, which persisted for least , hour, was taken as evidence for presence of saponins.

(2.2.3.6) Cumarins:-

1g of crushed sample was dissolved in 10 ml distilled water in test tube and filter paper attached to the test tube to be saturated with the vapor after a spot of 0.5M KOH, put on it. Then the filter paper was inspected under UV light, the presence of cumarins was indicated if the purple colour appear under UV light.

(2.2.3.7) Anthraquinone glycoside:-

1g of crushed sample was boiled with 10 ml of 0.5M KOH containing 1ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of chloroform. 5 ml of the chloroform solution were shacken with 3ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of anthraquinones was indicated if the alkaline layer was found to have assumed pink or red colour.

(2.2.3.8) Cyanogenic glycosides: -

3g of the powdered plant sample were placed in an erlenmeyer flask and sufficient water was added to moisten the sample, followed by 1ml of chloroform (to enhance activity). A piece of freshly prepared sodium picrate paper was carefully inserted between a split crock which was used to stopper the flask, a change in color of the sodium picrate paper from yellow to various shades of red was taken as an indication of the presence of cyanogenic glycosides.

Chapter Three
Results and discussion

Results and Discussion

Table (3.1) show the results of general phytochemical screening tests of *Citrullus Colocynthis* seeds.

Table (3.1) shows phytochemical screening of the seeds of Citrullus Colocynthis results showing almost similar as compared to Table (1.3)(Lakshmi,2013), it contain of flavonoids, tannins, ,triterpeneses , alkaloids and saponins. Absence of anthraquinones.

Table (3.2) show the results of physico -chemical properties of the *Citrullus Colocynthis* seeds oil.

Physiochemical properties of Citrullus Colocynthis seeds oil results Table (3.2) compared to Table (1.2) previous studies table (3.3).

Acid value was 1,29 showing good agreement with Kulkarni,2012 studies. Saponification value 223,76 and Ester value 222,4 were higher than that of previous studies. Iodine value 94.68 was quite lower. Peroxide value trace showing differences as compared with previous studies. .

Table (3.1) : Results of general phytochemical screening tests:

Constituent	Results
Flavonoids	+++
Sterols and Triterpenses	+++
Cumarins	+++
Tannins	+++
Saponins	+
Alkaloids	+
Anthraquinones glycoside	absent
Cyanogenic glycoside	absent

+ low concentration , ++ medium concentration , +++ high concentration

Table (3.2):Resuts physico -chemical properties of the Citrullus Colocynthis seeds oil:-

Physic-chemical properties	result
Percent yield	20.26
Density (30°C)	0.9728 (g/cm^3)
The acid value	1.29 (mg KOH/g of oil)
The saponification value	223.76 (mg KOH/g of oil)
The iodine value	94.68 (g of I_2 /100g of oil)
The ester value	222.47 (mg KOH/g of oil)
The peroxide value	trace

Table (3.3): Physiochemical properties of Citrullus Colocynthis seeds oil

Physico - chemical properties	Ref (Mirjana ,2005)	Ref (Kulkarni,2012)	Ref (Hiba,2015)	Results this studies
Specific gravity	0.914 (20° C kg/dm^3)	0.919 (15° C)	0.886 (g/cm^3)	0.9728
Acid value (mgKOH/g)	1.00	1.2	3.91	1.29
Saponification value(mKOH/g)	188	189	196.66	223.76
Iodine value (g/1oog)	119	122	119.53	94.86
Peroxide value (mmolO_2/Kg)	7.9	-----	6.97	trace
Ester number	187	-----	----	222.47

Conclusion

Phytochemecal screening of *Citrullus Colocynthis* seeds contian high concentration of Flavonoids, Sterols, Triterpenses, Cumarins and Tannins. Low concentration of Saponins and Alkaloids. Absence of Anthraquinones glycoside and Cyanogenic glycoside.

Physicochemical properties of the *Citrullus Colocynthis* seeds oil are Acid value showing good agreement with previous studies . Saponification value and Ester value were higher , Iodine value was quite lower. Peroxide value trace showing differences as compared with previous studies. .

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