Chapter one

1.1. Introduction

Although the oil and fat business is based almost entirely on a limited number of commodity oils differing in fatty acid composition, there are many other plant oils with fatty acid composition not too dissimilar from the commodity oils, these could be used as food lipids but unless they were sufficiently differentiated, it would be difficult for them to compete with the commodity oils. There are also plants that produce uncommon fatty acids such as epoxy acids, acids with conjugated unsaturation, or oils with very high levels (>80%) of a single fatty acid. Identification of new oil sources from a large number of commonly grown vegetables, fruits, and wild plants in Sudan has been of recent research interest. One of these wild plants is citrullus colocynthis**.**

1.1.1. Citrullus colocynthis

Citrullus colocynthis [family: cucurbitacease]. This is perennial herbs usually trailing. Commonly found wild in the sandy lands of North West, the Punjab, Sind, and central and southern India, and coromandal coast.

Also found indigenous in Arabia, west Asia. And Tropical Africa and in the Mediterranean region..

It originally bore the scientific name Colocynthis citrullus, but is now classified as Citrullus colocynthis. Tendrils are simple, 2 – 3 fits slender and hairy.

Leaves are very variable in size. Wild leaf is 3.8 to 6.3 cm in length and 2.5 cm in width while cultivated are large size. Lea show deltoid margin, pale green color above and ashy color beneath, scab rid on both surface, 5 – 7 lobed.

Citrullus colocynthis shows presence of male and female flowers. Fruit are globular, slightly depressed. 5– 7.5cm in diameter, green in color and get white glabrous when ripe. Fruit filled with a dry spongy very bitter pulp. Seeds are 4 – 6 mm long and pale brown.

1.1.1.1. Botanical Description:

1.1.1.1.1. Leaf:

The angular leaves are alternately located on long petioles. Each leaf is almost 5 to 10 centimeters in length and has around 3 to 7 lobs.

Sometimes the middle lobe might 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.

1.1.1.1.2 Fruit:

Each bitter apple pant produces around 15 to 30 globular fruits having a diameter of almost 7 to 10 centimeters. The outer potion of the fruit is covered with a green skin having yellow in color.

The ripe fruits are characterized by a thin but hard rind. The fruits it have a soft, white pup which is filled with numerous ovate compressed seeds.

1.1.1.1.3. Flowers:

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 yellow campanulas. The female flowers are easily identified from the males by their villous, hairy ovary.

1.1.1.1.4. Seed:

The seeds are around 6 mm in size, smooth, compressed and ovoid-shaped. They are located on the partial placenta. The seeds are light yellowish – orange to dark brown in color.

1.1.1.1.5. Root:

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 tendril.

1.1.1.2. Medical value of Citrullus colocynthis:

1- The fruit are bitter bunged cooling purgative anthelmintic antipyretic carminative cures tumors ascites leucoderma ulcers asthma bronchitis urinary chicharages jaundice enlargement of spleen tuberculosis gland of the neck dyspepsia constipation anemia throat diseases elephantiasis joints pain.

2- Root is useful in jaundice, ascites urinary diseases rheumatism and given in abdominal enlargements and in cough and asthmatic attacks of children. A poultice of root useful in inflammation of the breast.
3- Fruit of root without nux-vomica is rubbed into a paste with water and applied to bails and pimples.
4- Past of the root is applied to the enlargement of abdomen of children.

1.1.1.3. Phytochemical constituents:

The main chemical contain of fruit pulp colocynth in (the bitter principle up to 14%)

Colocynthis (resin) colocynth tin pectin gum seed contain a fixed oil (17%) and albuminiods (64%) investigated chemical content is mentioned.

1.1.1.4. Pharmacological activity:

1.1.1.4.1. nti-iAnflammatory:

Colocynthis fruit and seed at immature state for anti-inflammatory activity using the Carrageen an induced paw edema assay in rats. The best anti-inflammatory activities were obtained with immature fruits from south Tunisia therefore; colocynthis shard could be a potential useful product suitable for further evolution for inflammatory diseases "*Balsam – mar3ouk and – et – Al*".

1.1.1.4.2. Anticandidal and antibacterial:

In vitro antibacterial and Anticandidal activity of aqueous and diluted acetone extracts of colocynthis chard mice and MBC/MFC were determined for plant argavs of different malfunction stage. Were screened for activity against gram. Negative and gram. Positive bacteria (Escherichia coli – pseudomonas aeruginosn.

Staphylococcus auras and Enterococcus faceless) and Candida (Candida glabrate – Candida alb cans) "*RasoolKhatibi and –et*"

1.1.1.4.3. Hypoglycemic:

The effect of root of colocynthis ant biochemical parameters of normal and alloxan-induced diabetic rats. Diabetes mellitus was induced by intreperittneal (120 mg/kg b.w.) injection of alloxan monolychrate for 3 days and the animals 3 hawing blood glucose level in the range of 175-300 mg/cal were selected for study "*Agarwal and et-al*".

1.1.1.4.4. Anti-inflammatory and analgesic activities immature fruit and seed:

Screen the analgesic and anti-inflammatory activities of aqueous extracts Citrullus colocynthis from roots and stems of the plant and from fruits and seeds at different maturation stage. Method uses respectively the acetic acid uerithing test

in mice and the carragneenan – induced paw edema assay in rats "*Mar3ouk B-and et-al*".

1.1.1.4.5. Hypolipidemia:

Investigate the lypolichemic effect of citrullus colocynthis beyond the lypolichemic impact on lwmau. One landed dislipidemic patients were randomly divided into two groups namely treated. Colocynthis can lower the triglyceride and cholesterol concentration sign ificantly in vondiabetic lypolichemic patierts

1.1.1.4.6. Anti-alopecia:

C.coloynthis for hair growth activity in androgen. Induced alopecic petroleum ether extract of c.coloynthis was applied topically for its hair groth-promotiny. Activity. Alopecia was induced in albino mice by testosterone administration intramuscularly for 21 days.

"Dhanotia R and et al"

1.1.1.4.7. Antioxidant and free radical scaverying:

Methanalic fruit extract of c.coloynthis was screened to evaluate ints free radical scavenging effect the highest anticlant and free radical scavenging ability at the fruit extract was observed at a concentration of 2500 mirgmal (-1)

Table (1.1) chemical content of citrullus colocynthis

S.NO	Part	Chemical content (reported / investigated)
1	Seed	1- Fatty acid like (Stearic_Myristic_palmitic oleic_ lain oleic acid). 2- Protein 8.25% and rich content rich in lysim-leucin and sulfa amino acid like methionine. 3- Mineral like Ca-Mg-k-Mn-Fe-P and Zn. 4- Vitamin B.B_2 and niacin.
2	Aerial part and fruit	Flavonoid – glycoside querctin-flavon: -3-glucside viz iso-vilexin, iso-orentine and iso-orentine-3- methyl ether.
3	Fruit	1- Cucurbit one type triterpen glycoside viz colocynth side A and B. 2- Cucurbit ate type triterpen glycoside viz cucurbitacin E 2-D beta –glycoside. 3- 2-0 beta –D-gluopyromosyl-16 alpha – 20 R dihydroxy – cucurbits – 1, 5, 23 E 25(u). 4- 2- 0 beta – D – glcopyranosyl –glcopyranosyl cucurbitacin.

1.2 Objective:

1. Extraction of oil in colocynths citrullus seed.

2. Determination the percentage of oil extract by two method:

a. Solvent extraction (n_hexane, petroleum ether).

b. press extraction.

Chapter two

Experimental and methods

2.1. Experimental

2.1.1. Extraction by solvent

2.1.1.1. Extraction definition:

Extraction are many to separate a desired substant when it is mixed with others The mixture is brought into contact with a solvent in which the substance of interest is soluble, but the other substances present are insoluble.

Essential oils are volatile oils material volatiles in the leares and flowers and leaves vegetable citrutus which is responsible for argetable Smell of many of these plants oils task in of medical and industrial and biomedical materials eontaining in the pharmaceutical industry or as material for the formation of primitive synthesis zeros and others than the chemically the aromatic oils mixture of mono terpiens (sesquie terpiens often the parts with aromatic rolatile acids' and dissolved with any organic polar shower .

2.1.1.2. Objective

- Extraction of oil in colocynthis citrullus using by Solvent.
- Determination of the percentage of oil in seed.
- Determination of physical and chemical properties

2.1.1.3. Apparatus

- Refractive light
- Viscometer
- Peppite
- Conical flask
- Beaker (1000 and 500 ml)
- Sensitive Balance
- Filter paper
- Funnel.

2.1.1.4. Chemicals

- Seed of colocynthis citrullus
- n_hexane
- petroleum ether
- potassium iodide
- Chloroform.

2.1.1.5. Procedure

We obtained from areas of the fruit the Banks of river ATBRA and west Omdurman and were drying pools and that exposure to sunlight.

The sunlight dry for a weak and then was crushing grain extract then and crush grain mill then weight accurately 500g in beaker (1000 ml) and were immersion sample in solution of Hexane normal (1150 ml) with continues stirring for 24 hours the sample covered and the filtering process and leave the solution for a week so has the entire process of cooperating of Hexane and weight the oil abstract and determination physical properties and chemical and percentage of the oil in the sample.

$$\% = \frac{weight\ of\ oil}{weight\ of\ sample}\ x\ 100$$

2.1.2. Physical properties:

2.1.2.1. Refractive light:

The refractive index (RI) was determined by 60 refract meter as described by AOAC method. Prism was opened few drops of oil were placed on the prism and determined the refractive of oil 1.478 volume of refractive

2.1.2.2. Viscosity:-

The viscosity of the oil sample was recorded using on Ostwald -4- tube viscometer. The viscometer was suspended in constant temperature bath (32+3c) so that the capillary was vertical (the instrument) was filled to the mark and determined compared by water

$$\text{Viscosity} = \frac{time\ of\ oil - time\ of\ water}{time\ of\ water}$$

$$= \frac{5.8 - 0.4}{0.4} = 13.5 \text{ sec}$$

2.1.3. Chemical properties:

2.1.3.1. Peroxide value (PV).

2.1.3.1.1. Procedure.

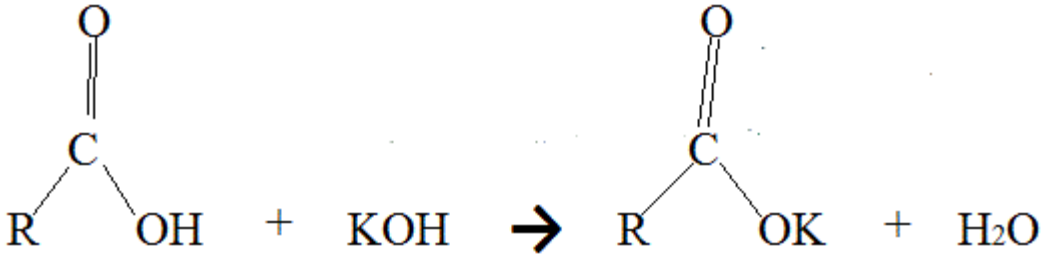
The (PV) of the oil sample was determined according to the AOAC method (1984), (1.01)g of oil was a accurately weighted into 100 ml conical flack and glacial acetic acid and chloroform (3:2) was added and the solution was swirled gently to dissolve the oil and were left to stand for one minute before added (D-W) 30 ml and titrated the solution with 0.01N sodium thiosulphate until the yellow color added 2 drops of starch and titration continued until the blue color completely and the volume recorded.

$$(PV) = \frac{(b-a)x\ Nx\ 1000}{s}$$

2.1.3.2. Free Fatty Acid value (FFA)

In chemistry, free fatty acid value (or "neutralization number" or "acid number" or "acidity") is the mass of potassium hydroxide (KOH) in milligrams that is required to neutralize one gram of chemical substance. The acid number is a measure of the amount of carboxylic acid groups in a chemical compound, such as a fatty acid, or in a mixture of compounds. As oil-fats

rancidify triglycerides are converted into fatty acids and glycerol, causing an increase in acid number.



2.1.3.2.1. Procedure

The method used for determination of free fatty acid (FFA) was that of Cocks and Van Rede (1966), (1.02)g of oil was dissolved in solvent ethanol and diethyl ether (1 : 1) then titrated while swirling against (0.1N) Sodium hydroxide solution using phenolphthalein as indicator and the results recorded.

$$(FFA) = \frac{VxNxM}{10xW}$$

2.1.3.3. Iodine value (IV)

The iodine value (or "iodine adsorption value" or "iodine number" or "iodine index") in chemistry is the mass of iodine in grams that is consumed by 100 grams of a chemical substance. Iodine numbers are often used to determine the amount of instauration in fatty acids. This instauration is in the form of

double bonds, which react with iodine compounds. The higher the iodine number, the more C=C bonds are present in the fat.

$$R - CH = CH - COOH + I_2 \rightarrow R - CHI - CHI - COOH$$

2.1.3.3.1. Procedure

2.1.3.3.1.1. Wij's method

0.2 gram of the oil have been weighted in a completely dry conical flask and dissolved in 10 ml of chloroform, 52 ml of wij's was added and the flask covered and shacked well and put in the dark for half an hour, 10 ml of potassium iodide (10%) was added to the flask and titrated against potassium thiosulfate (0.1M) and the results were recorded.

$$(IN) = \frac{VxMx12.69}{weight\ of\ sample}$$

2.1.4. Extraction by Petroleum ether

2.1.4.1. Objective:

Extraction of oil in colocynthis citrullus seeds using petroleum ether as solvent.

2.1.4.2. Chemicals:

- Petroleum ether

- Ethanol
- Sodium thiosulphate
- Seeds of Bitter

2.1.4.3. Instrumental and apparatus:

- Conical flask
- Filter paper
- Flask shaker
- Funnel

2.1.4.4. Procedure:

500g of colocynthis seeds was cracked into grain mill and placed into (1000ml) beaker and immerged in (1100ml) solution of petroleum ether with continues steering for 24 hours with the beaker covered, after that the solution filtered in a glass vessel and left for one week till all the petroleum ether was evaporated, then the oil abstracted was weighted and the percentage of it were calculated.

2.1.5. Extraction by pressing:

In traditional process volatile oils and essential oils were obtained by simple mechanical pressing and divided into:

1. Coolant pressing
2. Hot pressing

But in this research the sample was pressed in the room temperature.

2.1.5.1. Objective:

Extraction of oil from citrullus colocynithis seeds by pressing.

2.1.5.2. Apparatus:

- Textile sheer (gonzo)
- Small hand squeezer

2.1.5.3. Procedure:

(1000g) of citrullus colocynthis seeds was mashed and put in textile sheer and entered in a small hand squeezer and the pressing process took 4 hours and the results were recorded.

Chapter three

Results and Discussion

3.1. N_hexane extraction

3.1.1. Results:

Table (3.1)

Weight of Container	Weight of Container and oil	Weight of oil
90g	**165g**	**75g**

3.1.2. Calculation:

$$\% = \frac{75}{500}\ x\ 100 = 15\%$$

3.1.3. Conclusion:

In above experiment we found that the ratio of oil by n_hexane extraction is equal to 15%

3.2. Extraction by petroleum ether:

3.2.1. Results:

Table (3.2)

Weight of vessel\g	Weight of oil + vessel\g	Weight of oil\g
90	120	30

3.2.2. Calculations:

$$\% = \frac{30}{500} \times 100 = 6\%$$

3.2.3. Conclusion:

In above experiment we found that the percentage of oil extracted of seeds of citrullus colocynthis is 6%.

3.3. Extraction by pressing:

3.3.1. Results:

No amount of oil was obtained.

3.3.2. Conclusion:

In above experiment we did not have any amount of oil.

3.3.3. Discussion:

- The squeezer is manually (not modern)
- The seeds were small (the squeezer uses for big or medium seeds)

3.4. Free Fatty Acid value (FFA)

3.4.1. Results:

Table (3.3)

Initial value v1	Consumed value v2	Final value v3
0.00	25.00	25.0
0.00	26.00	26.00

3.4.2. Calculations:

V= volume of NaOH

N= Normality of NaOH

M= Molecular weight of fatty acid (282) acetic acid

$$(\text{FFA}) = \frac{25.5 \times 0.1 \times 2.82}{10 \times 1.02} = 70.5$$

Acid value = FFA × 2 = 141 mg/g oil

3.4.3. Conclusion:

In above experiment we found that the amount of Acid value is equal to 141 mg/g oil.

3.4.4. Discussion:

The low Acid value indicates on lack of free fatty acids and that means the oil is not rancidified and it's good for use.

3.5. Iodine value

Consumer volume of potassium thiosulfate = 17 ml

3.5.1. Calculations

Iodine value $= \frac{17*0.1*12.69}{2}$

$= 107.865$ g/100g

3.5.2. Conclusion:

There was Found to be that the iodine value is equal = 107.865 g/100g.

3.6. Peroxide value:

3.6.1. Results:

Table (3.4)

Initial value V_1	**Consumed value V2**	**Final value V_3**
0.00	1.00	1.00
0.00	1.10	1.10

3.6.2. Calculations:

b= reading of blank (ml)

a= reading of oil sample (ml)

S= original weight of oil sample (ml)

$\frac{1.05\times0.01\times1000}{1.01}$ =10.4 mg/g oil

3.6.3. Conclusion:

There was found to be that the peroxide value is equal =

10.4 mg/g oil

3.6.4 General Discussion:

The results obtained from oil extraction process of citrullus colocynthis seeds through methods:

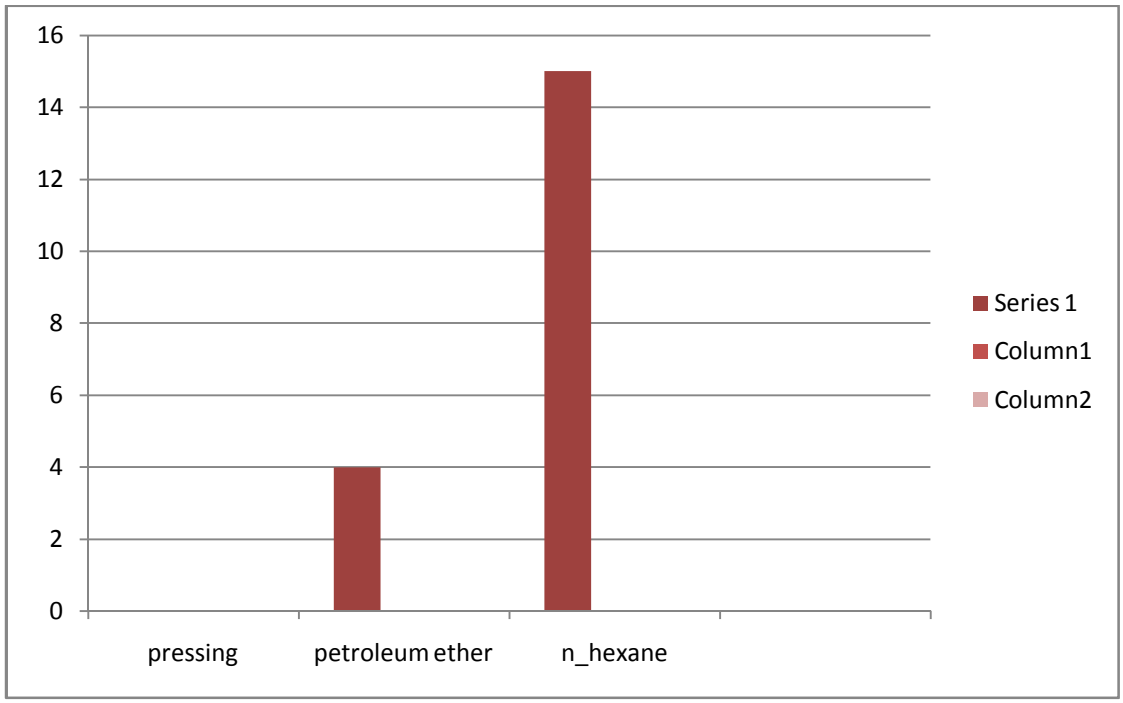
1. n_hexane
2. petroleum ether
3. pressing

Is showed in the table below:

Table (3.5)

Type of abstraction	n_hexane	Petroleum ether	Pressing
Percentage	15%	6%	0%

Figure (3.1) Graf describing the ratio of extractions by solvent



3.7. Conclusion:

Found that the ratio of volatile oil in citrullus colocynthis plant from different sources of Sudan like followed:

1. Contrast in ratio of oil in seeds by using (n_hexane 15% _ petroleum ether 4% _ pressing 0%)
2. Top ratio was obtained by using n_hexane.

3.8. Recommendations:

1. Conduct study to compare the extraction ratio from seeds by using solvent and steam distillation.
2. Determined the organic and inorganic components of the seeds to rest take advantage of it.
3. Need to conduct researches to identify the other contents of citrullus colocynthis.

Chapter four

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