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Physico-chemical and Gc-Ms Analysis of *Gossypium hirsutum* (Cotton Seed) Oil

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction and Objectives: The fatty acid profile of cotton seed oil, can be used to assess its nutritional value as ell the industrial applications of the oil as well. The significant amino acids such as Lipase, phytase, and lecithin were confirmed to found in cotton seed oil .Additionally, cotton seed oil is made to contained substantial natural antioxidants and α-tocopherols which give it shelf life longer than other related edible oils. The aim of this study is evaluate the physicochemical properties and characterized the *Gossypium hirsutum* oil using GC-MS analysis.
Materials and Methods: The cotton seed oil was extracted using soxhlet extraction procedure. The physicochemical parameters such as Acid value, Free Fatty acids content, Iodine value, Saponification value, Peroxide value, Viscosity(40°C), Refractive Index, Moisture content, Specific gravity (30°C), and colour index were determined by standard procedures described by AOAC , while the ester value was determined by baltes method. The oil was characterized using gas chromatography mass spectrophotometry (GC-MS) analysis.
Results: The physicochemical parameters showed that the acid value, free fatty acids content, iodine value, Saponification value, ester value, Peroxide value, viscosity, refractive index, moisture

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content, Specific gravity of the oil were 0.94±0.020 (mgKOH/gOil), 0.34±0.016%, 75.70±0.150gI/100g, 210.9±0.023millieqv/g, 209.4±0.027mgKOH/gOil, 8.82±0.010meq/kg, 4.41±0.113cSt, 1.383±0.003, 0.22±0.010%, 0.915±0.001 respectively. While the the oil was found to be Dark red in color. The GC-MS analysis of cotton seed oil shows the presence of twenty five (25) chemical compound ,in which six (6) where found to have biological activity related to Antibacterial activity, Anticancer Drug, Antiseborrhoeic , Anti-inflammatory, Hypocholesterolemic, Cancer Preventive, Insectifuge, Antiarthritic, Antieczemic Hepatoprotective, Antiandrogenic, Nematicide, Antihistaminic, Cytoprotective activity and Anti-inflammatory this includes 4,8-Diaza-2,9-dibenzoyl-5, 6-diphenyl-2,8-decadienedioic acid tridecan-7-ol, Undecanoic acid, octadecanoic acid, 9,12-Octadecadienoic acid(Z,Z)-, (E)-hexadec-7-enal and (Z)-7-Hexadecenoic acid respectively.

Conclusion: Cotton seed oil's physicochemical parameters were found to be within the NAFDAC and Cordex standards, liquids in nature, unsaturated, and slow to oxidation and rancidity and it is suitable for consumption . The GC-MS analysis of cotton seed oil reveals the presence of twenty-five (25) chemical compounds, six (6) of which exhibit biological activity and can be used in industrial, food, additive, and pharmacological formulations.

Keywords: Cotton seed; oil; soxhlet; physicochemical; GC-MS.

1. INTRODUCTION

Cotton seed oil is made from the leftover cotton seed after it has been ginned. Several ethno-medical uses have been discovered for the plant. Cotton seeds are used in traditional medicine to treat a variety of ailments. The world-wide desire to improve the oil potential of locally available cotton cultivars in order to meet the rising demand for edible oils. Modification of the fatty acid profile of cotton seeds to improve their nutritional properties is also becoming more feasible. Cottonseed oil, which is highly reach in palmitic acid and other oxidative stable fatty acids (oleic and stearic acids), may be able to eliminate the need for partial hydrogenation of vegetable oils [2].

The beneficial health role of unsaturated fatty acids cannot be over emphasized, but when it is deep frying for longer period result in to formation of short chain hydroperoxide, aldehydes, and keto derivatives that reduced the flavor of the oil [4]. Cotton seed oil's oxidative stability during frying is due to the presence of a higher %age of saturated fatty acids like palmitic acids etc, which compensates for the instability of unsaturated fatty acids. Partial hydrogenation is therefore used to increase oil stability by converting polyunsaturated fatty acids into monounsaturated and saturated fats while keeping the oil liquid [3]. Partially hydrogenation has its own drawbacks, particularly the production of trans fatty acids, which raise the level of LDL cholesterol while decreasing HDL cholesterol in blood serum [5]. At high temperatures, monounsaturated fatty acid (oleic acid) is rather resilient to oxidative breakdown. Oils with a high oleic acid content have better cooking stability for deep frying and are less susceptible to oxidative deterioration. As a result, it could be raised at the expense of polyunsaturated fatty acids in order to improve quality. Saturated fatty acids do not pose a health danger in and of themselves, but the synthesis of trans fatty acids as a consequence of vegetable oil hydrogenation has considerable cholesterol-raising effects. Cotton seed oil with high quantities of palmitic acid is undesirable due to potential health hazards [6]. On the other hand, increased palmitic acid content is required for oil's oxidative stability when used to create margarine, shortening, and confectionary items.

Cotton seed oil is made from the cotton seed that remains after the cotton has been ginned. The herb has been discovered to have numerous ethno medical benefits. Cotton seeds are used in traditional medicine in a variety of ways, both internally and topically, to treat a variety of ailments. Cotton preparations are widely utilized in the treatment of skin issues and injuries. A drink made from powdered cotton seeds and milk is used to treat headaches. An infusion of seeds is also used to cure dysentery. Cotton seed or leaf extracts are used to treat spots and other skin problems. Cotton is used in Western medicine in the form of dressings, bandages, swabs, and cotton wool. Cotton seeds have been discovered to contain substances that may be advantageous to one's health, with the potential to treat cancer and HIV.

Antimicrobial, antibacterial, hemolytic, and foaming action are among the properties of these

substances [7]. Quantification of such metabolites will aid in the extraction, purification, and identification of bioactive substances for human use. Aromatic compounds, usually phenols or their oxygen-substituted derivatives, are synthesized in an infinite number of ways by plants [8].

Cotton seed oil contains tocopherols, which are natural antioxidants. During the refining process, however, the amount of tocopherols in the oil decreases substantially. When compared to refined cotton seed oil and soybean oil, unrefined cotton seed oil contains more tocopherol and is more resistant to oxidation [9]. Cotton seed oil's fatty acid content is one of its most essential qualities. Cotton seed oil has a polyunsaturated to saturated fatty acid ratio of 2:1. Its fatty acid profile is classified as naturally hydrogenated because it contains 70% unsaturated fatty acids, comprising 18% mono-unsaturated (oleic) and 52 % poly-unsaturated (linoleic) acids, and 26% saturated (mainly palmitic and stearic) acids. These stabilize the oil for frying without requiring further processing or causing trans-fatty acid production [10]. Cotton seed oil's quality, like that of other vegetable oils, is determined by the fatty acid content and unsaponifiable materials stated. Their quantity and oil output vary according on genotype, regional ecological circumstances, and storage conditions [10]. Cotton belongs to the Malvaceae family, and the genus Gossypium contains a variety of commercially important and edible species such as G.hirsutum, G. barbadense, G. arboreum, and G. herbaceum [11]. Cotton cultivars with higher crop yield and productivity have been produced. The most important fiber/food crop in the world is Gossypium hirsutum, which is native to tropical and subtropical climates It is also the fifth most important seed oil crop after soybean, palm, canola, and sunflower, and one of the richest sources of plant (vegetable) protein after soybean. Cotton is one of the most important conventional oilseed crops, with the ability to close the gap between supply and demand for vegetable oils in the United States. Cotton is an important crop that spans around 2.5 % of the world's farmed area and is recognized as a dual-purpose crop, as it is used both for its natural fiber [12] and contributes to over 4% of the world's vegetable oil output.The aim of this research is to evaluate the physicochemical property of cotton seed oil and characterized the oil using GC-MS analysis .

2. MATERIALS AND METHODS

2.1 Collection of Cotton Seeds Sample

Cotton seeds were brought from Dawanau Market in Dawakin Tofa Local Government of Kano State. The seed sample was identified and authenticated at Department of Agricultural Science, Kano University of Science and technology, Wudil with identification NO KUSTAGRIC-CS456. The seeds sample were obtained by removing/ breaking/ external cover mechanically using mortar and pestle. The experiment was carried out at the Department of Food Science and Technology, Kano University of Science and Technology Wudil, Kano.

2.2 Extraction of Oil from *Gossypium Hirsutum* Seeds

The seeds were crushed and placed in paper bags. The sample placed in a Pyrex glass Soxhlet extractor, attached with a water condenser and a Pyrex round bottomed flask (500 mL capacity). Extraction was carried out using a water bath with n-hexane as extraction solvent. The crude fat of cotton seed flour was determined using AOAC-2000 method [13]. Ninety grams (90g) of cotton seed flour were weighed using thimble and covered by purified cotton. Then 200 ml of n-hexane as solvent was added. The sample with the solvent was placed in the soxhlet extractor for the cycle was allowed to repeat many times for about 8 hours. After 8 hours the remaining solvent was evaporated using oven dry method and the extracted fat was cooled in a desiccator and weighed.

After the oil extraction, the solvent was removed under vacuum in a rotary evaporator machine (EYELA, N. N. Series fitted with an Aspirator and a Digital Water Bath SB-651, Japan) at 45°C. The solvent (hexane) and oil were separated using distillation at a temperature of slightly higher than the boiling temperature of hexane, which is recovered again for further extraction with fresh hexane. The oil was stored in the Food Science and Technology laboratory room for physico-chemical properties and GC-MS analysis. All the physicochemical analysis was conducted under laboratory condition. The data were recorded on as %age yield/crude fat(oill contents), acid value and saponification value, Iodine value, refractive index, specific gravity/density, ester value, Peroxide value, free fatty acid, Viscosity and moisture content.

Table 1. Percentage yield of *Gossypium hirsutum* seed oil using soxhlet extraction

Method	% Yield
Soxhlet	19.98±0.005

Value are presented as mean ± SD n=5

2.3 Determination of %age Yield

$$\%\text{age yield} = \frac{\text{Net weight of oil(g)x100}}{\text{Total weight of ground seed}}$$

2.4 Determination of Acid Value (AV)

The acid value was determined using the method described by Bamgboye and Adejumo [14]. Equal volumes (25 ml) of diethylether and ethanol were mixed together and 1 ml of 1% phenolphthalein indicator solution was added and then neutralized with 0.1 M potassium hydroxide solution.

2.4.1 Procedure

Five grams of oil sample was dissolved in the neutralized solvent mixture and titrated with 0.1 M potassium hydroxide solution with constant shaking until a pink color which persists for 15 seconds is obtained.

$$\text{Acid Value (AV)} = \frac{\text{Titrate value(ml) ×56.1}}{\text{Weight of sample used (g)}}$$

Titre value= Blank titre value (B) - Real titre value (R)

2.5 Determination of Saponification Value (SpV)

Determination of saponification value was carried out using the method described by AOAC [14].

2.5.1 Procedure

Two grams of the oil sample was added to a flask with 30 cm^3 of ethanolic potassium hydroxide solution and was then attached to a reflux condenser and heated on a water bath for 1 hour with occasional shaking to ensure the sample was fully dissolved. After the sample cooled, 1cm^3 of phenolphthalein indicator was added and titrated with 0.5M hydrochloric acid until a pink endpoint was reached. A blank determination was also carried out omitting the oil under the same condition and saponification value was calculated using the equation:

$$\text{Saponification Value (SpV)} = \frac{\text{(b-a)x M x 56.1}}{\text{Sample weight (g)}}$$

Titre value= Blank titre value (B) - Real titre value (R)

2.6 Determination of Ester Value

The ester value was determined by subtracting acid value from saponification value [15].

Ester value = Saponification value - Acid value.

2.7 Determination of Refractive Index (RI)

Refractive Index (RI) was determined following method. Melt the sample if it is not already liquid and filter through a filter paper to remove impurities and traces of moisture. Make sure sample is completely dry Circulate stream of water through the instrument. Adjust the temperature of the refractometer to the desired temperature. Ensure that the prisms are clean and dry.

2.7.1 Procedure

Place a few drops of the sample on the prism. Close the prisms and allow standing for 1-2 min. Adjust the instrument and lighting to obtain the most distinct reading possible and determining the refractive index or but yrorefrac to meter number as the case may be [16].

2.8 Determination of Specific Gravity (SG)

Determination of Specific gravity (SG) was conducted using the following method.

2.8.1 Procedure

The pycno meter was filled with the prepared sample in such a manner to prevent entrapment of air bubbles after removing the cap of the side arm. Insert the stopper, immerse in water bath at 300°C and hold for 30 minutes. Carefully wipe off any oil that has come out of the capillary opening. Remove the bottle from the bath, clean and dry it thoroughly. Remove the cap of the side arm and quickly weigh ensuring that the temperature does not fall below 30°C [17].

Specific Gravity at 30 degree C / 30 degree

$$C = \frac{A - B}{C - D}$$

A = weight in gm of specific gravity bottle with oil at 30°C
B = weight in gm of specific gravity bottle at 30°C
C = weight in gm of specific gravity bottle with water at 30°C

2.9 Determination of Peroxide Value

Peroxide value was determined by [18].

2.9.1 Procedure

Exactly two grams (2g) of the oil sample was weighed in ground neck flask and 10ml of chloroform was added to dissolve the butter. This was followed by addition 15ml acetic acid and 1ml 50% of KI solution. The mixture was shaked and keep it in the dark for 5minutes. After which, 25ml of water was added and titrated with 0.002M sodium thiosulphate.

2.10 Determination of Free Fatty Acid (FFA)

2.10.1 Procedure

Determination of the FFA in Oil sample was carryout by potentiometric titration in Ethanol/Diethyl ether (1:1, V/V) as solvent with NaOH in Isopropyl alcohol. 29500 ml absolute Ethanol and 500ml Diethyl ether were mixed in a bottle. Five grams (5gm) of sample was weighed into a 150 ml beaker and dissolved in 70 ml of the solvent. The mixture was heated gently to increase the solubility of the oil. After a complete dissolution the sample was titrated with 0.1N NaOH. using phenolphthalein as indicator until the pink colour was formed for approximately 30 seconds.

$$\%FFA = \frac{\text{Conc. Of consumption titrant as first equiv.} - B \times T \times M \times F1}{\text{Weight of the sample (g)} \times \text{conversion factor}}$$

2.11 Determination of Iodine Value by Wij's Method

2.11.1 Principal

Iodine value give the degree of unsaturated fatty acid of oil or fat and It is the relative measure of the unsaturated bonds present in the oil or fat. Iodine value expressed in grams of iodine absorbed by 100g of oil or fat. Unsaturated compounds absorbed iodine (in suitable form) and saturated compounds. The amount of iodine absorbed in compounds is the measure of unsaturated of the oil.

2.11.2 Procedure

Iodine value was determined according to titre metric method of Pearson, [19]. The oil sample (2g) weighed into a dry 500ml conical flask and 10ml of carbon tetrachloride was added to the oil. Exactly 20ml of Wij's solution was added and allowed to stand in the dark for 30minutes, 15ml of (10%) potassium iodine and 100ml of distilled water was added and then titrated with 0.1N sodium Thiosulphate solution using starch as indicator before the end point. a blank was also prepared alongside the oil sample. The iodine value was calculated using the formula below:

$$\text{Iodine value (Wij's)} = \frac{V2 - V1 \times 1.269}{\text{Weight of sample}}$$

Where, V1=volume of sodium thiosulphate required for sample, V2 =volume of sodium thiosulphate required for blank.

2.12 Determination of Moisture Content

Moisture content of cotton seed was determined according to Association of Official Analytical Chemistry [13] (AOAC, 2000) using the official method 925.09 by oven drying method.

2.12.1 Procedure

A crucible was cleaned and dried in an oven at 105°C for 1 hour and placed in desiccators to protect moisture absorption. Weight of crucible (W1) was determined. 5 gm sample of cotton seed flour was weighed in the dry crucible (W2) dried at 105°C for 3 hours and after cooling the sample in desiccator to room temperature it was weighed again (W3). The moisture content of cotton seed flour was calculated using the formula below:

$$\%Mo = \frac{W2 - W3}{W2 - W1} \times 100$$

%MO = %ages of moisture content
W2= weight of the crucible plus weight of fresh sample

W1= weight of the empty crucible
W3= weight of the crucible plus weight of the sample after oven dried.

2.13 Determination of oil Viscosity

The standard determination of kinematic viscosity generally employs a glass u-tube viscometer with a capillary tube build into one leg. This procedure was described in ASTM D445 and ISO 3104.

2.13.1Procedure

A certain amount of cotton seed crude oil sample was poured into a beaker then transferred to the viscometer. The viscometer have been cleansed with a non toxic solvent and dried. The viscometer, containing the crude, was inserted, into the water bath at the temperature of 40°C. The pump was used to raise the level of the crude to the starting mark on the left hand limp of the viscometer; another finger used to close the other limp to avoid the flow of oil due to air. The finger is removed to allow his flow of oil down the capillary at that point, the time at which the oil flow down is taken and recorded. The viscosity then is obtained by multiplying the constant of the viscometer by the time obtained from the equation below [20].

$$V = Ct + B/t \text{ [cSt]}$$

where C = the instrument calibration constant,

B = the instrument type constant depending on the capillary diameter,
t = efflux time in seconds.

2.14 Colour Detection of the Oil

The color of the cotton seed oil was analyzed by physical observation.

2.15 Gas Chromatography – Mass Spectroscopy (Gc-Ms) Analysis of Cotton Seed Oil

Gas chromatography-mass spectrometry (GC-MS) was performed with GCSM (QP2010plus Shimadzu, Japan). The analysis was conducted by gas chromatography with flame ionization detection (GC/FID) and mass spectrometric detection (GC/MS). In the first instance, gas chromatograph (model HP-5890 Series II) equipped with a split-splitless injector, an HP-5 capillary column (25 mm x 0.32 mm, film thickness 0.52µm) and a flame ionization detector was employed. Hydrogen was used as the carrier gas (1 mL/min). The injector was heated at 250^{o}C, the detector maintained at 300^{o}C, while the column temperature will be linearly programmed from 80-280^{o}C (10o/min and held at 80oC (1min), 200^{o}C (4min) and 280^{o}C (5min). The GC-MS analysis was performed, using an HP G 1800C Series II GCD analytical system, equipped with an HP-5MS column (30m x 0.25mm x 0.25µm). Helium was used as a carrier gas (0.9mL/min). The transfer line was heated at 260^{o}C. The EI mass spectra (70eV) was acquired in the scan mode in the mix range 40-600. In each case 1µL of sample solution in methanol (10µL/mL) was injected in the split mode (1:30). Identification of constituents were carryout by matching their mass spectra and retention indices with those obtained from authentic samples and/or NSIT/Wiley spectra libraries, using different types of search (PBM/NIST/AMDIS) and available literature data [21]. The %age compositions were obtained from electronic integration measurements using flame ionization detection.

2.16 Statistical Analysis

The data was statistically analyzed at P-value ($p<0.05$) significantly accepted and a comparison between the groups was performed using one-way analysis of variance (ANOVA) by Graphpad instat3 software (2000) version 3.05 by Graphpad Inc. The data are given as the mean ± standard deviation.

3. RESULTS AND DISCUSSION

3.1 Percentage Yield of *Gossypium hirsutum* (Cotton Seed) Oil

The percentage yield of cotton seed oil extracted with n-hexane was found to be (19.980.005%). (Table 1). This might be deemed acceptable yield levels, similar findings revealed that the oil content of cotton seeds from various types of edible oil were ranged from 15.85 to 19.49 %. According to Nangbes et al [22], the oil content of several cotton genotypes ranged from 15.84 to 21.35 %. Different geographical locations and genetic variability may be linked to differences in oil yield. Cotton cultivars with high oil, protein, and low moisture and carbohydrate content make them a viable source of edible oil. The oil content of *Gossypium hirsutum* seed is equivalent to well-known seed oils such as linseed (33.33) and soybean (18.35), etc.

3.2 Physicochemical Properties of Gossypium hirsitum (Cotton seed) Oil

The physicochemical analysis showed that the average value of Saponification value, iodine Value, peroxide Value, Viscosity (40°C), Specific gravity, Refrective index (30°C) , free fatty acids content, The acid value, and Moisture content of the seed oil of *Gossypium hirsutum* were found to be (210.9±0.023 millieqv/g), (75.70±0.150), (8.82±0.010millieqv/g oil), (4.41±0.113), (0.915±0.001 at 30°C), (1.383±0.003), (0.34±0.016%). (0.642±0.020mg OH/gOil) and (0.22±0.010%) respectively. While the colour of the oil was observed to be dark red (table 2).

The physicochemical examination of oil is primarily performed for both culinary and industrial applications. According to Table 1, when compared to other traditional oil seed crops, the protein content of cotton seed oil in this study was similar to that of safflower (20–22%), sunflower (16.5–19.6%), and cotton seed (19.40%), as described in the literature [22]. Cotton seed oil has a moisture level of 0.220.010 %. The moisture content of seeds is determined by their maturity and quality. The ability of seeds to store moisture is determined by their moisture content. [22].

The amount of potassium hydroxide (KOH) required to saponify one gram of fat or oil as described by (AOCS Method Cd 3–25 and AOCS Method Cd 3c–91) is known as saponification value. The analyzed saponification value was 210.90.023 mgKOH/g of oil. Cotton seed oil saponification values are comparable to those reported in the literature for cotton seed oils and numerous other standard seed oils [23]. The result was in conformity with NAFDAC and CODEX's standard guidelines, as well as some additional literature. Cotton seed oil has high saponification properties, which means the oil have normal triglycerides and can be used in soap making [24]. Saponification is only relevant if the oil is being used for industrial reasons; it has no nutritional value. However, because each fat has a fixed fatty acid content within biological constraints, the saponification value can be used to characterize and identify the fat [25].

The amount of potassium hydroxide in milligrams required to neutralize one gram of chemical material is known as acid value. The number of carboxylic acid groups in a fatty acid or a combination of compounds is measured by the acid number. According to Othman and Ngassapa [25], the acid value of cotton seed oil was found to be (0.6420.020mg KOH/g), which is within the standard limits. According to the WHO, the acid value of edible oil should not exceed 4 mg/g, as indicated in various literatures. However, the results of this study were lower than those published in some study [22]. Low acidity indicates that the oil is of good quality [26]. Low acidity in oil suggests that it will remain stable for a long time and will guard against rancidity and peroxidation. This could be due to the availability of natural antioxidants and other phytochemicals such as flavonoids. The acid value of an oil is used to determine its edibility and eligibility for usage in the paint and soap industries. High acid value in oil showed that the oil may not be suitable for use in cooking (edibility), but however, can be used for production of paints, liquid soap and shampoos [26].

Table 2.Physicochemical Properties of *Gossypium hirsitum* (Cotton seed) Oil

Properties	Values	Standard
Saponification Value(mgKOH/g oil)	210.9±0.02	185-265
Iodine Value(gI/ 100g)	75.70±0.150	Below 90-90
Ester value	135.2±0.127	-
Peroxide Value(meq/Kg)	8.82±0.010	10
Viscosity (cSt)(40°C)	4.41± 0.113	-
Specific Gravity(30°C)	0.915±0.001	0.0-1.0
Refractive Index(30°C)	1.383±0.003	-
Free Fatty acids (%)	0.34±0.016	0.3%max
Acid Value(mg KOH/g oil)	0.642±0.020	0.6
Moisture content	0.22±0.010	0.2
Dark red	-	-

All values are the average of three (3) replicates presented as mean±Standard deviation

Cotton seed oil's refractive index (RI) value was discovered to be 1.3830.003, which is consistent with Nagaraj's refractive index findings [27]. Cotton oil has a RI of 1.470 at 32°C, according to the WHO. Rossell and Pritchard [22] reported that the refractive index ranged from 1.4590 to 1.468 at 30°C. The density and refractive indices of the oils studied in this study were very similar to those of several other oil seed crops. The refractive index of the oil was increased when the double bond in the fatty acid composition increased [28]. The amount of enzymatic or chemical hydrolytic products in oil is represented by free fatty acids (FFA). Cotton seed oil had a free fatty acid value of 0.340.016 %, which was lower than the results given by Anhwange et al., [29]. High levels of free fatty acids in vegetable oils are undesirable because they impair the oil's palatability and shelf life [30]. At 30°C, the specific gravity of cotton seed oil was determined to be (0.9150.001 g/cm3). This figure is comparable to that reported in [22]. Cotton seed oil has a viscosity of 4.41 0.113, which is close to the finding of [31].

The iodine value (also known as the iodine adsorption value, iodine number, or iodine index, is the amount of iodine consumed by 100 grams of a chemical compound. Iodine values are frequently used to determine the percentage of unsaturation in fats, oils, and waxes. Cotton seed oil has an Iodine value of 75.700.150, which is consistent with the findings of Anigo et al.,[31]. The refractive index was found to be 1.3830.003, which was less than the value as reported ealier [32].

The cotton seed oil detected was dark crimson in appearance. The presence of coloring pigments such as carotenoids and chlorophyll, which must be removed during oil bleaching, contributes to the intensity of the color of cotton seed oil. Oils with the least amount of color intensity are thought to be more desirable from a commercial standpoint [33]. Oils with low color values are preferable for food and household use [34]. Cotton seed oil is also notable for the inclusion of gossypol, a poisonous polyphenolic component. This pigment gives unrefined cotton seed oil a dark red color. During neutralization, most of the gossypol is eliminated. By subtracting the acid value from the saponification value, the ester value was calculated. The ester value examined was (135.20.127), which is comparable to some research finding [35].

3.3 GAS CHROMATOGRAPHY-MASS SPECTROSCOPY (GC-MS) ANALYSIS OF GOSSYPIUM HIRSITUM (COTTON SEED) OIL

The GC-MS analysis of cotton seed oil shows the presence of twenty five (25) chemical compounds ,in which six (6) where found to have biological activity related to Antibacterial activity, Anticancer Drug, Antiseborrhoeic , Anti-inflammatory, Hypocholesterolemic, Cancer Preventive, Insectifuge, Antiarthritic, Antieczemic Hepatoprotective, Antiandrogenic, Nematicide, Antihistaminic, Cytoprotective activity and Anti-inflammatory this includes 4,8-Diaza-2,9-dibenzoyl-5, 6-diphenyl-2,8-decadienedioic acid tridecan-7-ol, Undecanoic acid, octadecanoic acid, 9,12-Octadecadienoic acid(Z,Z)-, (E)-hexadec-7-enal and (Z)-7-Hexadecenoic acid respectively. While the remaining 19 compounds yet no activity was reported (Figure 1)(Table 3).The presence of this various chemical compounds indicated that cotton seed oil is very important source of chemical compounds needed in various industries like chemical industries, food industries, pharmaceutical industries etc. and also provide more light on production of cheapest traditional medicines.

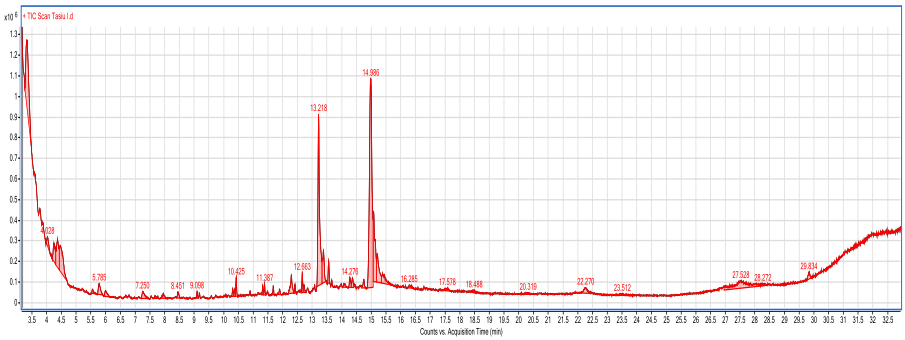
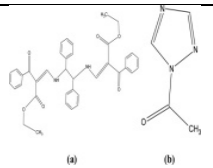
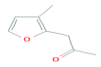
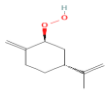
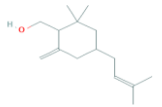
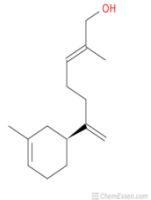
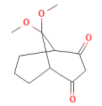
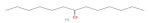
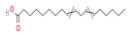


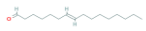
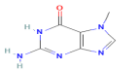
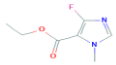
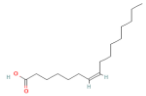
Fig. 1. Chromatogram of GC-MS of Gossypium hirsitum cotton seed oil

Table 3. Gas Chromatography-Mass Spectroscopy (Gc-Ms) Analysis of Gossypium Hirsitum (Cotton Seed) Oil

Peak No	Retention Time	Area	Height	Molecular weight (g/mol)	IUPAC Name	Molecular Formula	Structural Formula	Nature and Medical Important
1	4.028	181893.93	44020.94	616	4,8-Diaza-2,9-dibenzoyl-5,6-diphenyl-2,8-decadienedioic acid ester	$C_{38}H_{36}N_2O_6$		It has Antibacterial activity [36]
2	4.246	434527.18	88485.81	134.22	Benzene,2-ethyl-1,4-dimethyl	$C_{10}H_{14}$		No activity reported
3	4.372	76310.4	136139.19	224.38	5,10-Pentadecadien-1-ol, (Z,Z)	$C_{15}H_{28}O$		No activity reported
4	5.562	72318.2	19972.445 3285.69	138.16	3-Methyl-2-(2-oxopropyl)furan	$C_8H_{10}O_2$		No activity reported
5	5.997	151948.54	28581.97	142	(1E)-1-ethylideneindene	$C_{11}H_{10}$		No activity reported
6	7.25	295422.35	33680.03	222.37	2S,4R-p-Mentha-1(7),8-diene-2-hydroperoxide	$C_{15}H_{26}O$		No activity reported

Peak No	Retention Time	Area	Height	Molecular weight (g/mol)	IUPAC Name	Molecular Formula	Structural Formula	Nature and Medical Important
7	7.667	65523.02	13198.97	168.23	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)	$C_{10}H_{16}O_2$		No activity reported
8	7.953	105320.31	24225.4	222.37	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl	$C_{15}H_{26}O$		No activity reported
9	8.451	98551.23	296502.05	338.7	Tetracosane	$C_{24}H_{50}$		No activity reported
10	9,098	65658	32505.81	220.35046	(2E)-2-methyl-6-(3-methylcyclohex-3-en-1-yl)hepta-2,6-dien-1-ol	$C_{15}H_{24}O$		No activity reported
11	10,305	86474.77	37503.31	266.5	nonadec-1-ene	$C_{19}H_{38}$		No activity reported
12	11.387	927729.09	53276.21	294.6	(E)-henicos-10-ene	$C_{21}H_{42}$		No activity reported
13	11.678	77718.47	42435.41	268.5	Nonadecane	$C_{19}H_{40}$		No activity reported

Peak No	Retention Time	Area	Height	Molecular weight (g/mol)	IUPAC Name	Molecular Formula	Structural Formula	Nature and Medical Important
14	11.89	98204.11	25762.11	212.24	9,9-dimethoxybicyclo[3.3.1]nonane-2,4-dione	$C_{11}H_{16}O_4$		No activity reported
15	12.296	374520.9	89013.66	200.36	tridecan-7-ol	$C_{13}H_{28}O$		Its use as Anticancer Drug[37]
16	13.218	3391493.88	831914.39	228.41	pentadecan-7-ol	$C_{15}H_{32}O$		No activity reported
17	13.384	664405.41	157120.99	186.2912	Undecanoic acid	$C_{11}H_{22}O_2$		**antifungal agent and** Antiseborrhoeic [38]
18	13.555	248757.23	108813.99	284.5	octadecanoic acid	$C_{18}H_{36}O_2$		anti-inflammatory lipid [39]
19	14.093	72366.06	16333.58	172.26	methoxymethoxycyclooctane	$C_{10}H_{20}O_2$		No activity reported
20	15.083	1464719.73	337713.12	280.4	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$		Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge(cide), antihistaminic, antieczemic, antiacne, 5-α reductase inhibitor,

Peak No	Retention Time	Area	Height	Molecular weight (g/mol)	IUPAC Name	Molecular Formula	Structural Formula	Nature and Medical Important
								antiandrogenic, antiarthritic, anti coronary, antimicrobial [40]
21	20.319	73665.97	7836.08	214.39	tetradecan-1-o	$C_{14}H_{30}O$		No activity reported
22	22.27	265710.12	27270.4	238.41	(E)-hexadec-7-enal	$C_{16}H_{30}O$		Cytoprotective activity[41]
23	23.512	119139.76	97488.9	165.15	2-amino-7-methyl-1H-purin-6-one	$C_6H_7N_5O$		No activity reported
24	28.272	81460.26	10158.79	172.16	4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester)	$C_7H_9FN_2O_2$		No activity reported
25	29.834	146114.54	33146.47	254.41	(Z)-7-Hexadecenoic acid	$C_{16}H_{30}O_2$		Anti-inflammatory[40]

4. CONCLUSION

The physicochemical parameters of cotton seed oil were found within the NAFDAC and Cordex standard, hence its non drying oil of low saturation, slow to oxidation and rancidity, and can remain liquid for a long time and is suitable for consumption. The GC-MS analysis of cotton seed oil shows the presence of twenty five (25) chemical compounds, in which six (6) where found to have biological activity that can be employed for industrial, foods, additive and other pharmaceutical formulation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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