



CHEMICAL CONSTITUENTS OF SOLVENT EXTRACTED BITTER KOLA NUT OIL AND ITS BENEFICIAL HEALTH PROPERTIES

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ABSTRACT

This study investigated chemical composition and biological activity of bitter kola oil. Solvent extraction was done using Soxhlet extraction method and the chemical composition of the oil was investigated using standard procedures. The oil yield of bitter kola nut oil was 2.480%. The color was deep brown. The chemical composition of the oil showed 58 different chemical compounds of which 40 are reported to have biological activity. 23.1% of the chemical composition of bitter kola oil obtained was made up of fatty acids. The fatty acid compositions of the oil showed saturated and unsaturated components that ranged from 0.59 – 10.10% and 0.72 – 4.13%, respectively. Some of the saturated components of bitter kola nut oil include Palmitic; Lauric; Myristic; Pentadecanoic and Stearic fatty acids while the unsaturated components were Oleic; Linoleic and Hydnocarpic fatty acids. In conclusion, this study showed that bitter kola oil comprises of many biologically active compounds which could be used could beuse in food formulation, pharmaceutical and chemical industries.

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1.0 Introduction

Nuts and seed are nutrient dense foods with complex matrices rich in unsaturated fatty acids (60 – 98%), high quality protein, fiber, minerals (iron, potassium, magnesium, calcium, phosphorus and selenium), and other bioactive compounds such as tocopherols, phytosterols, and phenolic compounds (Nzekwe *et al.*, 2015). Prominent nuts and seeds are groundnut, almonds, cashew nuts, cotton seeds, palm kernels, pecans, sesame seeds, soybean seed, sunflower seeds and walnuts. They have been widely utilized in various food formulation and development due to their nutritional and health benefits (Gonzalez-Perez and Arellano, 2009). Vegetable oils extracted from nut and seeds are important ingredients in the food, agro-allied and chemical industries. Prominent vegetable oils are palm oil, soybean oil, canola oil, and sunflower oil which contribute more than 70% of the global vegetable oils consumption (Kojima *et al.*, 2016). The world major exporting countries in vegetable oils are Indonesia, Malaysia, and Argentina while leading importers being India, China and EU (Kojima *et al.*, 2016). Global demand for vegetable oils is greatly affected by the exponential increase in the world's population, rising awareness of the health benefits of the unsaturated fatty acids present in the vegetable oils and increased industrial usage (Kojima *et al.*, 2016). Recent advances in technology increases global utilization of vegetable oils therefore put pressure on conventional nut and seeds. Therefore, there is dire need to explore non-conventional nuts and seeds. One of the non-conventional nuts and seeds is bitter kola (*Garcinia kola*). Bitter kola is a species of dicotyledonous flowering plant in the *Malpighiales* plantae order and *Clusiaceae* family (Manourova *et al.*, 2019). It is a perennial crop growing in the forest, distributed throughout West and Central Africa where its favorable climatic conditions

are met (Iwu, 1993). In Nigeria, bitter kola is primarily cultivated in Southern states such as Ekiti, Osun, Oyo, Ogun, Ondo, Edo and Delta. Bitter kola stem and leaves have been employed over the centuries to treat different ailments but the most valued products of bitter kola are from its nuts, which possess anti-microbial, anti-diabetic and anti-inflammatory properties (Okoye *et al.*, 2014). Eleyinmi *et al.* (2006) documented that bitter kola nut comprises of crude protein (39.52 g/kg), lipid extract (43.25 g/kg), ash (11.42 g/kg), crude fiber (114.02 g/kg), and a carbon: nitrogen ratio of 57.88. Despite enormous benefits of bitter kola vegetable oil, it is still classified as an underutilized nut. Therefore, there is need to investigate its chemical and biological properties as this will provide scientific data that will promote possible utilization of bitter kola vegetable oil for both domestic and industrial usage.

2. Materials and Methods

2.1 Source of materials

Mature bitter kola nut was obtained from Teaching and Research farm, Obafemi Awolowo University, Ile-Ife, Nigeria. All chemicals needed for this work were of analytical (Sigma Chemical (St. Louis, MO, USA and Fisher Scientific, Oakville, ON, Canada) grades.

2.2 Sample preparation

About 5 kg of matured bitter kola nut was sorted and washed with potable water to remove foreign particles attached to the nuts. The cleaned bitter kola nut was decorticated, dried in a hot-air oven (AFOS Mini Kiln, Hull, England) and milled with a laboratory milling machine (Retsch GmbH and Co., Haan, Germany). The milled bitter kola nut was then subjected into solvent extraction process using n-hexane (analytical grade) as extraction solvents. Milled bitter kola nut (1.00 kg) was measured and weighed into the extraction thimble of known weight. The loaded thimble was then placed in a Soxhlet apparatus. The extraction solvent (5 L) was measured into the round bottom flask for each extraction process. Then, the temperature of the heating mantle was set to 50 °C. As the temperature of the solvent increased, as a result of heat convection, solvent began to vaporize and vapor formed then passed through the thimble and began to condense due to the cooling effect of the water flowing in and out of the Soxhlet extractor setup. This continuous process of heating and cooling was carried out until considerable amount of bitter kola oil was extracted after 8 h. At the end of the experiment, the thimble was removed from the Soxhlet extractor set-up and the rest of the solvent in the apparatus was recovered. Subsequently, a simple distillation process was carried out on the miscella in order to separate bitter kola oil from the solvent (Figure 1). Then, the extracted oil was labeled accordingly.

2.3 Determination of moisture content

The moisture content was determined using the oven drying method. 10 g of decorticated bitter kola nut sample were placed inside a moisture can. The sample was then dried in a Uniscope Laboratory Oven set at the temperature of 105 °C for 3 h till no change in mass was observed. After drying, the sample was placed inside a dessicator and allowed to cool to room temperature. Then, the sample was weighed and the values were recorded. The moisture content of the bitter kola nuts was then expressed as percentage moisture content (wet basis) using Equation 1:

$$MC_{(w.b)} = \frac{M_b - M_c}{M_b - M_a} \times 100\% \quad (1)$$

Where: M.C (w.b) = moisture content (wet basis); M_a = weight of moisture can; M_b = weight of moisture can plus sample weight before drying and; M_c = weight of moisture can plus sample weight after drying.

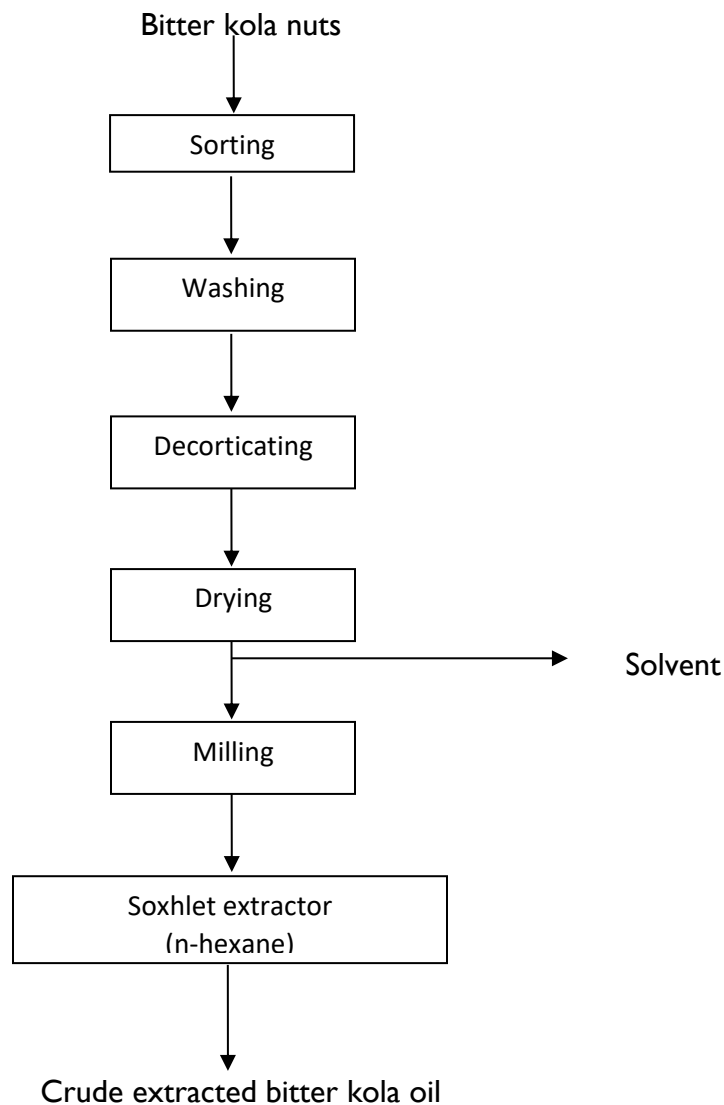


Figure 1: A flow chart showing solvent extraction of bitter kola oil

2.4 Determination of oil yield

Solvent extracted bitter kola oil was transferred to a beaker, and then it was heated in a water bath for 2 h, in order to completely evaporate the solvent from it. At the end of the heating process, the volume of the recovered bitter kola oil was measured and expressed as percentage oil yield, using equation 2:

$$OY\% = \frac{W_0}{W_s} \times 100 \quad (2)$$

Where: OY = Oil Yield, %; W_s = Weight of milled bitter kola nut used, g W_0 = Weight of extracted bitter kola oil, g

2.5 Color

The color of the extracted bitter kola oil was determined by physical observation.

2.6 Determination of chemical composition of solvent extracted bitter kola oil

The chemical components of bitter kola oil such as alkanes, alkenes, alcohols and aromatic hydrocarbons were evaluated using AOAC (2002) methods. First, the oil sample was pre-treated with a process of methylation, following which it was subjected to GC-MS (Gas Chromatography-Mass Spectrometry (Model GL – 15A, Shimadzu Corporation, Kyoto, Japan) analysis. The column temperature of the GC-MS was programmed between 60 °C and 300 °C.

2.7 Fatty acid profile

The fatty acid profile of solvent extracted bitter kola oil was determined by using gas chromatography as a separation method. Fatty acids (FAs) were determined by means of indirect method, which involves their conversion to corresponding methyl esters (fatty acid methyl esters; FAMES) due to their reaction with trimethylsulfonium hydroxide solution. Individual fatty acids were then quantified by means of two distinct methods. Firstly, quantification was based on the received peak area, following which the results were normalized without correction factor. Secondly, quantification was based on the external calibration curve (AOCS, 2009).

2.8 Statistical Analysis

Values obtained after all analyses were taken as data, which were then analyzed inferentially with the aid of statistical tools such as mean, analysis of variance (ANOVA), and Tukey test ($p < 0.05$).

3. Results and Discussion

The initial moisture content of the bitter kola nut was 49.67% (w.b) (Table I). The initial moisture content value obtained is lower than 60.48 and 71.9% reported by Odebunmi *et al.* (2009) and Onyekwelu *et al.* (2015), respectively but higher than 15.20% documented by Adeyeye *et al.* (2017). This wide range of moisture content obtained from various studies could be due to different levels of maturity of bitter kola nut samples, loss of moisture during transportation and storage and different farming practices. The high moisture content value (49.67%, w.b) obtained shows that the nut is susceptible to moisture-dependent deterioration. Also, it shows that bitter kola nut has poor keeping quality unless it is subjected to drying.

Table I: Physico-chemical properties of bitter kola oil

Properties	HEXSO
Initial moisture content	49.67% (w.b)
Oil yield (%)	2.480
Physical observation (Colour)	Deep brown

HEXSO = N-hexane solvent extracted bitter kola oil

The percentage oil yield value of bitter kola nut obtained was 2.48% (Table I). The oil yield value obtained is higher than 2.09% documented by Olatunde *et al.* (2021) but lower than 8.5% reported by Ordu *et al.* (2018). These disparities in values of oil yield are attributed to different levels of maturity of bitter kola nut samples and different handling and storage practices. The oil yield obtained was lower when compared with oil yield of conventional nuts and seeds such as

groundnut (40 – 50%), palm kernel (39.53%), sesame (44.80%), rapeseed (40.00%), sunflower (25.50%) and canola (40.00%) (Pahl, 2008; Dim, 2013; Chauchan et al., 2016; Sitepu et al., 2021).

Color of bitter kola nut oil by physical observation is deep brown (Table 1). Similar observation was documented by Adeyeye et al. (2017). However, Ordu et al. (2018) reported reddish-brown while Olatunde et al. (2021) reported light yellow. These disparities in color of bitter kola oil may be attributed to different levels of maturity of bitter kola nut samples, loss of moisture during transportation and storage, different types of growing soil, different farming practices and different methods of extraction. The color of bitter kola oil is affected by constituents of the nut such as tannins, saponins, terpenoids and flavonoids (Adeyeye et al. 2017). Color is an important parameter used as an indication of adulteration of oils (Lira et al., 2020).

The chemical composition of bitter kola nut oil is presented in Table 2. Fifty-eight (58) different chemical compounds are obtained over 68 peaks, using Gas Chromatography- Mass Spectrometry analysis method (GC-MS).

Table 2: Chemical composition of bitter kola nut oil

Peak No	Compound	RT	%Peak Area
1	.beta.-Myrcene	5.734	0.22
2	Heptane, 3-(chloromethyl)-	5.919	0.08
3	Heptane, 3-(chloromethyl)-	5.970	0.17
4	Benzoic acid	8.123	0.85
5	1-Dodecene	8.242	0.17
6	Dodecane	8.377	0.85
7	Geraniol	8.875	0.12
8	1-Tridecene	10.559	0.62
9	Tetradecane	10.686	2.69
10	Phenol,3,5-bis(1,1-dimethylethyl)	11.689	3.89
11	11-(2-Cyclopenten-1-yl)undecanoic acid, (+)- (Hydnocarpic acid)	11.883	0.72
12	Dodecanoic acid (Lauric acid)	12.289	0.59
13	1-Hexadecanol	12.641	1.08
14	Hexadecane	12.756	3.96
15	2-Bromo-4,6-di-tert-butylphenol	12.807	0.71
16	1-Octadecanesulphonyl chloride	13.554	0.16
17	Tetradecanoic acid (Myristic acid)	14.166	1.24
18	1-Nonadecene	14.507	1.04
19	Heneicosane	14.607	4.01
20	Pentadecanoic acid (Pentadecylic acid)	15.011	0.60
21	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9- diene-2,8-dione	15.344	0.17
22	1-Octadecanesulphonyl chloride	15.429	0.44
23	Malonic acid, 2-chloropropyl pentadecyl ester	15.461	0.31
24	Hexadecanoic acid, methyl ester	15.527	0.51
25	Dibutyl phthalate	15.667	4.06

26	n-Hexadecanoic acid (Palmitic acid)	15.955	10.10
27	m-Cymene,5-tert-butyl	16.033	1.63
28	n-Nonadecanol-1	16.201	1.94
29	Eicosane	16.290	3.45
30	1,6,10,14-Hexadecatetraen-3-ol,3,7,11,15-tetramethyl-(E,E)	16.417	0.72
31	Kaur-16-ene	16.781	0.22
32	n-Heptadecanol-1	16.875	0.50
33	9-Octadecenoic acid, methyl ester, (E)-	16.999	0.23
34	Octadecane,1-chloro-	17.233	0.64
35	9,12-Octadecadienoic acid (Z,Z) (Linoleic acid)	17.408	3.92
36	9-Octadecenoic acid, (E)- (Oleic acid)	17.490	4.13
37	Octadecanoic acid (Stearic acid)	17.698	1.80
38	trans-Geranylgeraniol	17.875	0.87
39	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	17.951	0.38
40	9-Hexadecenoic acid, octadecyl ester, (Z)-	18.068	1.10
41	Heneicosane	18.167	1.84
42	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-thenyl-10,14-dimethylene-pentadec-4-enyl) cyclohexane	18.490	0.75
43	17-Pentatriacontene	19.376	0.63
44	9-Octadecenamide, (Z)	19.455	1.44
45	[1,1'-Biphenyl]-2,3'-diol,3,4',5,6'-tetrakis (1,1-dimethylethyl)	19.540	0.81
46	Behenic alcohol	20.029	0.27
47	Eicosane	20.108	1.01
48	Phosphoric acid, tris (2-ethylhexyl) ester	20.587	1.33
49	Trichloroacetic acid, undecyl ester	20.791	1.09
50	Eicosane	20.994	0.77
51	Bis (2-ethylhexyl) phthalate	21.121	5.09
52	1H-Pyrazole-5-carboxamide,3-cyclopropyl-N-(3,5-dimethyltricyclo [3.3.1.1(3,7)] dec-1-yl)-1-methyl-	21.542	0.22
53	2-methylhexacosane	21.807	0.68
54	Oleoyl chloride	21.252	1.48
55	Behenic alcohol	21.433	1.04
56	Eicosane	22.575	0.85
57	Methoxy-THC	22.620	0.42
58	9-Octadecenamide, (Z)-	22.906	0.37
59	Decanedioic acid, bis (2-ethylhexyl) ester	23.177	0.75
60	Eicosane	23.313	0.29
61	Squalene	23.433	7.11
62	1-Heptacosanol	23.955	4.10
63	Octadecane,3-ethyl-5-(2-ethylbutyl)-	24.056	1.78
64	trans-Geranylgeraniol	24.165	0.51

65	1-(2,5-Bis-methoxy-phenyl)-3,7,11-trimethyl-dodeca-2,6,10-triene	24.240	0.66
66	Oct-5-en-2-ol, 8-(1,4,4a,5,6,7,8,8a-octahydro-2,5,5,8a-tetramethylnaphth-1-yl)-6-methyl-	24.587	1.71
67	Purine-2,6-dione, 1,3-dimethyl-7-(2-oxo-2-phenylethyl)-8-(piperidin-1-yl)-3,7-dihydro	24.697	1.35
68	Ethyl trans-4a, cis-4b,trans-8a,cis-10a-perhydro-trans-2,4a,8a-trimethyl-8-oxophenanthrene-2-carbothiolate	24.864	0.78

RT: Retention time

The compound with the highest concentration is n-Hexadecanoic acid (Palmitic acid) (10.10%), a saturated fatty acid with reported anti-inflammatory, anti-oxidant and anti-microbial biological activities (Abubakar and Majinda, 2016). Other identified compounds with relatively high concentrations were: Squalene (7.11%), Eicosane (6.37%), Heneicosane (5.85%), Bis(2-ethylhexyl)phthalate (5.09%), 9-Octadecenoic acid (4.13%), 1-Heptacosanol (4.10%), Dibutyl phthalate (4.06%), Hexadecane (3.96%), 9-12-Octadecadienoic acid(Z,Z) (Linoleic acid) (3.92%), Phenol, 3,5-bis(1,1-dimethylethyl) (3.89%), and Tetradecane (2.69%); while the compound with the least concentration is Geraniol (0.12%), a fatty alcohol with reported anti-microbial, anti-diabetic, anti-inflammatory, anti-oxidant, cardio-protective, anti-depressant and insecticidal biological activities (Lira *et al.*, 2020). Total percentage of fatty acids obtained is 23.10%. This includes saturated fatty acids (14.33%): Palmitic acid, C16:0 (10.10%); Lauric acid, C12:0 (0.59%); Myristic acid, C14:0 (1.24%); Pentadecanoic acid, C15:0 (0.60%) and Stearic acid, C18:0 (1.80%). Palmitic acid (10.10%) is the prominent saturated fatty acid, a fatty acid with anti-microbial, anti-inflammatory and anti-oxidant properties used in food production, soap production, skincare, cosmetics and other industries (Abubakar and Majinda, 2016) while Lauric acid (0.59%), a fatty acid with anti-microbial and anti-viral activities (Lira *et al.*, 2020), has the least concentration among the saturated fatty acids although Adeyeye *et al.* (2017) documented higher concentration of total saturated fatty acids (25.74%), with lower percentage of Palmitic acid (5.50%) and higher percentage of Lauric acid (2.57%). Total percentage of unsaturated fatty acids is 8.77% which includes: Hydnocarpic acid, C16:1 (0.72%), Linoleic acid, C18:2 (3.92%) and Oleic acid, C18:1. The unsaturated fatty acid with the highest concentration is Oleic acid (4.13%), a monounsaturated fatty acid with antimicrobial activity, cholesterol lowering capacity and reported health benefits (Lira *et al.*, 2020), while the least concentration of unsaturated fatty acids is Hydnocarpic acid (0.72%); another monounsaturated fatty acid with anti-oxidant properties which is traditionally used to treat leprosy (Lira *et al.*, 2020). Although, Adeyeye *et al.* (2017) documented a higher concentration of total unsaturated fatty acids (74.26%), with higher percentage of Oleic acid (24.23%). Also, total percentages of mono-unsaturated and poly-unsaturated fatty acids were 4.85 and 3.92%, respectively. The ratios of poly-unsaturated fatty acid to total saturated fatty acid (P/S) and oleic to linoleic are 0.27 and 1.05, respectively. Although, Adeyeye *et al.* (2017) reported higher P/S ratio (1.91) and lower oleic/linoleic ratio of 0.57; which indicates that bitter kola oil they obtained was not as stable and suitable for frying compared to the oil obtained in this study. These differences in fatty acid profile could be as a

result of different levels of maturity of bitter kola nut samples, loss of moisture during transportation and storage, different types of growing soil and different farming practices.

The biological activities of chemical compound of bitter kola oil are presented in Table 3. Forty (40) chemical compounds found in bitter kola oil are reported to have biological activities such as anti-oxidant, anti-inflammatory, anti-microbial, anti-fungal, anti-diabetic, anti-cancer, anti-tuberculosis, anti-depressant, cardio-protective, anti-septic, anti-viral, anti-aging, anti-tuberculosis, anti-epileptic, anti-cytotoxic, psychoactive and insecticidal activity. The anti-microbial effect of bitter kola oil may be attributed to the long aliphatic chains and fatty acid ester in it, which have been reported to build up in the cell membrane and mitochondria of a cell, thus resulting in disruption of the cell integrity and the death of the cell (Belakhdar *et al.*, 2015).

Table 3: Biological activities of compounds in bitter kola nut oil

Peak No	Compound	%Peak area	Class of compound	Biological activity
1	.beta.-Myrcene	0.22	Acyclic alkene	Anti-oxidant, anti-aging, anti-inflammatory
4	Benzoic acid	0.85	Aromatic hydrocarbon	Anti-microbial
5	1-Dodecene	0.17	Acyclic alkene	Anti-bacterial
6	Dodecane	0.85	Acyclic alkane	Anti-oxidant
7	Geraniol	0.12	Terpene alcohol	Anti-microbial, anti-diabetic, anti-inflammatory, anti-oxidant, cardio-protective, anti-depressant, insecticidal
8	1-Tridecene	0.62	Acyclic alkene	Pheromone and allomone properties
9	Tetradecane	2.69	Acyclic alkane	Anti-microbial, anti-tuberculosis
10	Phenol,3,5-bis(1,1-dimethylethyl)	3.89	Aromatic hydrocarbon	Anti-oxidant
11	11-(2-Cyclopenten-1-yl)undecanoic acid, (+)-(Hydnocarpic acid)	0.72	Fatty acid	Anti-oxidant
12	Dodecanoic acid (Lauric acid)	0.59	Fatty acid	Anti-microbial
13	1-Hexadecanol	1.08	Fatty alcohol	Anti-microbial and anti-oxidant
14	Hexadecane	3.96	Acyclic alkane	Anti-microbial and anti-oxidant
17	Tetradecanoic acid (Myristic acid)	1.24	Fatty acid	Larvicidal and repellent activity
18	1-Nonadecene	1.04	Acyclic hydrocarbon	Anti-fungal
19 and 41	Heneicosane	5.85	Alkane	Anti-microbial

20	Pentadecanoic acid (Pentadecylic acid)	0.60	Fatty acid	Anti-microbial
24	Hexadecanoic acid, methyl ester	0.51	Fatty acid ester	Anti-microbial
25	Dibutyl phthalate	4.06	Phthalate ester	Anti-microbial
26	n-Hexadecanoic acid (palmitic acid)	10.10	Fatty acid	Anti-inflammatory, anti-oxidant, anti-microbial
28	n-Nonadecanol-1	1.94	Fatty alcohol	Anti-bacterial
29, 47, 50,56 and 60	Eicosane	6.37	Acyclic alkane	Anti-microbial
32	n-Heptadecanol-1	0.50	Fatty alcohol	Anti-bacterial
33	9-Octadecenoic acid, methyl ester, (E)-	0.23	Fatty acid methyl ester	Anti-microbial
35	9,12-Octadecadienoic acid (Z,Z) (Linoleic acid)	3.92	Fatty acid	Anti-oxidant activity
36	9-Octadecenoic acid, (E)- (Oleic acid)	4.13	Fatty acid	Anti-microbial
37	Octadecanoic acid (stearic acid)	1.80	Fatty acid	Anti-oxidant activity
59	Decanedioic acid, bis (2- ethylhexyl)ester	0.75	Fatty acid ester	Anti-microbial
61	Squalene	7.11	Aromatic hydrocarbon	Anti-oxidant and anti-cardiovascular disease
62	1-Heptacosanol	4.10	Fatty alcohol	Anti-microbial, anti-oxidant
63	Octadecane,3-ethyl-5-(2- ethylbutyl)-	1.78	Acyclic alkane	Anti-microbial

The biologically active compounds with relatively high concentration are n-Hexadecanoic acid (Palmitic acid) (10.10%), which has reported anti-inflammatory, anti-oxidant and anti-microbial properties (Abubakar and Majinda, 2016); Eicosane (6.37), Heneicosane (5.85%), Dibutyl phthalate (4.06%) and Hexadecane (3.96%) which have reported anti-microbial activity (Usha *et al.*, 2015; Ahsan *et al.*, 2017; Kim, 2020). Although, there is dearth of information on the biological activity of Kaur-16-ene, its derivatives have been reported to have anti-inflammatory activity (Chavan *et al.*, 2010). Hexadecanoic acid methyl ester, 9-Octadecenoic acid methyl ester and 9,12-Octadecadienoic acid were also documented by Fapohunda *et al.* (2017) although, they occurred in higher concentrations of 4.08, 12.42 and 5.04% respectively. These differences in chemical composition of bitter kola nut oil could be as a result of different levels of maturity of bitter kola nut samples, loss of moisture during transportation and storage, different types of growing soil and different farming practices.

4. Conclusion

This study shows the chemical composition and biological activity of bitter kola nut oil. Chemical compounds such as n-Hexadecanoic acid (Palmitic acid), Eicosane Heneicosane, Dibutyl phthalate and Hexadecane which occur in relatively high concentration have notable anti-inflammatory, anti-oxidant and anti-microbial properties. Also, other compounds such as 1-Dodecene, n-Nonadecanol and n-Heptadecanol-1 have anti-bacterial activity. Behenic alcohol has anti-viral activity, Squalene has anti-cancer activity and Geraniol, which has the lowest concentration, has anti-depressant, cardio-protective and anti-diabetic activity. Based on the favorable reported biological activities of the components of bitter kola nut oil, the oil can find its application in food formulation, pharmaceutical and chemical industries subject to further clarification.

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