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Comparative GC-MS Analysis, Antioxidant and cytotoxic activities of *Garcinia kola* Heckel seed and stem-bark n-hexane extract

Seide Akoro*[1], Oyinlade Ogundare[1], Adekunle Oyedola[2]

[1]*Department of Chemical Sciences, College of Basic Science, Lagos State University of Science and Technology, Ikorodu, Lagos State, Nigeria;*

Email:** ***modupe2309@gmail.com

[2]*Department of Chemical Sciences, College of Basic Science, Lagos State Polytechnic, Ikorodu, Lagos State, Nigeria;*

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ABSTRACT

Background & Aim: *Garcinia kola* is one of the well-known tropical medicinal plants known in the traditional management of several diseases especially connected with inflammatory and degenerative diseases. This study was designed to compare the phytochemical contents and cytotoxic activities of crude *G. kola* n-hexane seed and stem-bark extracts by Gas Chromatography-Mass Spectrometry (GC-MS).

Experimental: Fresh seeds and stem-bark of *G. kola* were collected, authenticated and extracted by macerating in n-hexane separately. The compounds in the crude extracts were evaluated using Gas Chromatography-Mass Spectrophotometer (GC-MS). The cytotoxic activity was determined using the brine shrimp lethality assay.

Results: Extraction of the plant materials yielded the crude n-hexane extracts GKSE and GKBE from the *G. kola* seed and stem-bark, respectively. GC-MS analysis detected 54 and 34 compounds in GKSE and GKBE, respectively. The most abundant compounds in both extracts were Lanosta-8,24-dien-3-one and Oxacycloheptadec-8-en-2-one with percentage abundance of 20.72% and 22.71%, respectively. The stem-back extract (LC_{50}=42.76±1.85 µg/mL) showed better cytotoxic activity than the seed extract (LC_{50}=73.15±1.51 µg/mL). GC-MS results for both extracts indicated compounds known for their antioxidant and anticancer properties which may explain some of the various ethno-medical uses of *G. kola* seed and stem-bark.

Recommended applications/industries: This plant could be a source of a new anticancer drug.

1. Introduction

The search for a more potent anticancer agent is unending considering the various medical situations associated with oxidative stress due to ageing and diseased conditions. It is generally believed that medications from natural sources are reported to be safer than synthetic ones (Lourenço *et al.*, 2019). Garcinia is a genus belonging to the diverse tropical family Clusiaceae, which consists of important fruits and medical tree species. An important member of this genus is *Garcinia kola*, commonly known as "bitter kola" (Akoro *et al.*, 2018; Iwu, 1985). Bitter kola is one of the common medicinal plants, used in African ethno medicine as purgative, anti-parasitic, and anti-microbial agents (Iwu, 1985). It is a flowering plant in Nigeria's tropical, moist lowland forests. It is also found in other West African countries like Senegal, Sierra Leone, Liberia, Ghana, Gabon, Ivory Coast, the Democratic Republic of the Congo, Cameroon and the Benin Republic (Iwu, 1985).

In Nigeria, bitter kola is a household medicinal plant, which draws its name from the various tribes: It is known as "orogbo" in Yoruba of south-west Nigeria, and "namijingworo" in Hausa of northern Nigeria, and "ugolo" in Igbo of South-east, Nigeria (Iwu, 1985). The fruits are usually harvested for ceremonial and medicinal uses in these areas where the G. kola trees are found (Iwu, 1985).

The various parts and components of G. kola are useful traditionally as medicine, however, the seed is the most valued and known component of this plant (Akoro *et al.*, 2018; Iwu, 1985). The seed has been used as a ceremonial snack in local communities in Nigeria (Iwu, 1985). The seed has been used as medicine for the treatment of diseases like benign prostatic hyperplasia, multiple sclerosis, and acquired immune deficiency syndrome (AIDS) (Akoro *et al.*, 2018; Kalu *et al.*, 2016; Nwaehujor *et al.*, 2015; Omotoso *et al.*, 2018). The seed has also been reported to have the ability to stop the ebola virus in laboratory trials (Nwaehujor *et al.*, 2015). The kernel has been reported to contain a wide range of medicinally useful phytochemicals like tannins and flavonoids (Nwaehujor *et al.*, 2015). Antioxidant and anti-inflammatory activity of the aqueous, methanol, petroleum ether, ethanol and acetone seed extract was reported (Farombi *et al.*, 2013; Idris *et al.*, 2020).

The cytotoxic effect of G. kola root extracts and fractions was reported by Emmanuel et al. (2021). Kolaviron, a mixture of Garcinia biflavonoids consisting of GB1, GB2 and kola flavanone were isolated from the G. kola seed extract (Iwu, 1985) and later from the stem-bark extracts and reported to have alpha-amylase inhibitory properties (Akoro *et al.*, 2018). Gakolanone, a novel compound was also isolated from the n-hexane extract of G. kola stem-bark (Akoro *et al.*, 2018). The hepatoprotective effect of biflavonoids from G. kola was also reported (Akintonwa and Essien, 1990; Iwu *et al.*, 1987). The biflavonoid mixture has been reported to mitigate lipid peroxidation and hepatotoxicity and reduce carcinogen-induced tissue damage by preventing the initiation and propagation of lipid peroxidative process and scavenging free radicals via enhancement of drug-detoxifying enzyme (Farombi *et al.*, 2000; Farombi, 2000). Mechanisms for the hepatoprotective action of kolaviron: Studies on hepatic enzymes, microsomal lipids and lipid peroxidation in carbon tetrachloride treated rats (Farombi *et al.*, 2000; Farombi, 2000).

Despite the traditional importance, popularity, and known potential of G. kola there is still more to be explored on the chemical components of the different parts of this medicinal plant. The present study was designed to determine and compare the phytocontents of the crude G. kola n-hexane seed and stem-bark extracts using Gas Chromatography-Mass Spectrometry and their cytotoxic activities.

2. Materials and Methods

2.1. Chemicals and materials

Ammonium hydroxide, n-hexane, hydrochloric acid, Dimethyl sulfoxide (DMSO), and other chemicals were of analytical grades and were purchased from Sigma-Aldrich chemical company or Fisher scientific chemical company.

2.2. Collection of samples, preparation and extraction

The plant materials were collected in between July and August, 2021 and authenticated at the University of Lagos Herbarium. The seeds were cut into smaller pieces and then dried in an air-oven maintained at 40^{o}C for about one week until dried and the stem-bark was air-dried in the open laboratory (for about three weeks) until dried. The samples were then grounded to coarse form using a manual grinder. The *G. kola* seed (500 g) and stem-bark (500 g) were macerated by soaking separately in 500 mL and 1000 mL of n-hexane, respectively, for 72 h. The extracts obtained were concentrated using a rotary evaporator at 40°C. The extracts were further dried in an air oven at 40^{o}C and then kept in a sample tubes.

2.3. Qualitative phytochemicals screening

The plant extracts were screened for the presence of secondary metabolites including alkaloids, tannins, Steroids, Flavonoid, Saponin, anthraquinones and reducing sugar using standard methods (Sofowora, 1993).

2.4. GC-MS analysis of extracts

Analysis of the extracts was carried out on GC-MS QP2010SE SHIMADZU (JAPAN) fitted with a MS (Model EI) directly connected with capillary column. Helium carrier gas was used at a constant flow rate of 3.22 mL/min and pressure of 144.4 Kpa. The detector is a secondary electron multiplier with the patented lens

and conversion mode. One µL of the sample was injected. The Initial column temperature was maintained at 60°C for 2 min and increased at 12°C/min to 240°C and hold for 2 min and then increased (12°C/min) to 290 min and hold for another 2 min. The total run time was 21.50 min. The injector and detector temperature was 250°C. Mass spectra were recorded at 70 eV ionization energy. The Identification of the ions was done by the NIST 11 library.

2.5. Cytotoxicity

The cytotoxic activity of the extracts was determined using the brine shrimp lethality test (Akoro *et al.*, 2022; Vanhaecke *et al.*, 1981). The seawater was collected from the Bar Beach (Victoria Island, Nigeria) and then filtered off to get a clear solution. *Artemia salina* leach (brine shrimp eggs) was purchased, hatched in seawater and used as the test organism. The seawater was taken into a transparent glass container. The shrimp eggs were added to one side of the tank and then covered. The brine shrimp was allowed to hatch and matured as nauplii for 24 h. An aerator was passed through the hatching set throughout the time of hatching to give a constant supply of oxygen. The nauplii (10) free from the eggshell were carefully collected from the tank by means of a pipette and added to the various concentrations of plant extracts.

2.5.1. Preparation of the plant extract solutions and the cytotoxicity bioassay

The test plant extracts were prepared by the method of Akoro *et al.* (2022). Each of the test plant samples (32 mg) was taken and dissolved in 200 µL of pure dimethyl sulfoxide (DMSO) and finally, the volume was made to 20 mL with seawater to make a stock solution of concentration 1600 µg/mL from which the concentrations 10, 20, 40, 80, 160 µg/mL were prepared by serial dilution with the seawater. Each of the plant extract solutions (2.5 mL) was added to 2.5 mL of seawater containing 10 nauplii and the experiment was carried out thrice at each concentration. The negative control is a solution of 1% DMSO in seawater containing 10 nauplii. The counting of nauplii was done after 24 h and the number of nauplii that survived in each tube of extract was counted and the percentage mortality was calculated for each concentration. The medial lethal concentration (LC_{50}) was calculated from the plot of Log of the concentration against mean percentage mortality.

2.6. Statistical analysis

Cytotoxic assays were conducted thrice and data were reported as mean ± standard deviation of three values. Results were analyzed using one-way analysis of variance (ANOVA). Student T-test was used to compare means and data were considered statistically significant at $P<0.05$. All graphs were plotted using Excel 2020.

3. Results and discussion

3.1. Extraction and phytochemical Sscreening

The extraction of the plant materials, *G. kola* seed, and stem-bark were carried out by macerating each separately in n-hexane for 72 h to obtain GKSE (yellowish brown oil, 4.9 g, 0.98% w/w) and GKBE (greenish-yellow oil, 6.0 g, 1.2%w/w), respectively. Phytochemical screening of both crude n-hexane extracts for secondary metabolites detected alkaloids, flavonoids, tannins, reducing sugar and steroids in the two extracts while saponins and anthraquinones were detected only in the seed extract (Table 1).

Table 1. Phytochemical contents of *G. kola* seed and stem-bark n-hexane extracts.

Phytochemicals	GKSE extract	GKBE extract
Alkaloids	+	+
Saponins	+	-
Flavonoids	+	+
Tannins	+	+
Anthraquinone	+	-
Reducing sugar	+	+
Steroids	+	+

Keys: (+): detected; (-):not detected; GKSE: *Garcinia kola* n-hexane seed extract; GKBE: *Garcinia kola* n-hexane bark extract.

3.2. Gas-Chromatography- Mass Spectrometry (GC-MS) results

Gas-Chromatography- Mass Spectrometry (GC-MS) detected fifty-four compounds in the *G. kola* seed n-hexane extract GKSE (Table 2). Twenty-nine of the compounds have percentage area abundance of 0.5% and above and these constituted 96.76% of all the identified compounds. Seven of the compounds, 2,7,11-Trimethyl-4-phenylthiododeca-2,6,10-triene (RT: 20.117; 5.67%); beta.-Tocopherol (RT:20.236, 10.83%); (2,3-Diphenylcyclopropyl) methyl phenyl

sulfoxide, trans-(RT: 21.966 min; 7.88%); A'-Neogammacer-22(29)-ene (RT: 22.701 min; 10.18%); Supraene (RT: 22.735 min; 10.18); Lanosta-8,24-dien-3-one (RT:23.943 min and 24.904 min; 20.61%) and Tetracosapentaene, 2,6,10,15,19,23-hexamethyl (RT:24.42 min; 9.86%) have percentage area abundance above 5% and they constituted the bulk of the compounds detected (75.21 %) (Table 2, Figure 2). The most abundant compound in the seed extract was Lanosta-8,24-dien-3-one (20.61%).

Table 2. Some of the identified compounds in *G. kola* n-hexane seed extract with area abundance 0.5% and above.

S/N	Retention time	Compound	% Area abundance	Molecular formula	Molecular mass
1	13.571	1-Hexadecanol	0.2	$C_{16}H_{34}O$	242
2	13.665	Pentadecane	0.13	$C_{15}H_{32}$	212
3	14.939	Benzene, 1,1'-(1,2-cyclo butanediyl)bis-, trans-	0.66	$C_{16}H_{16}$	208
4	15.033	Cyclopentane, (2-hexyloctyl)-	0.14	$C_{19}H_{38}$	266
5	15.183	3-Cloropropionic acid, heptadecyl ester	0.25	$C_{20}H_{39}ClO_2$	346
6	15.433	1-Nonadecene	0.64	$C_{19}H_{38}$	266
7	15.515	Nonadecane	0.27	$C_{19}H_{40}$	268
8	15.828	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	0.18	$C_{16}H_{22}O_4$	278
9	16.082	Dodecylcyclohexane	0.54	$C_{18}H_{36}$	252
10	16.61	1,2-Benzenedicarboxylic acid, butyl2-ethylhexyl ester	0.36	$C_{20}H_{30}O_4$	334
11	16.683	1.Heptatriacotanol	0.14	$C_{37}H_{76}O$	536
12	16.814	n-Hexadecanoic acid	1.5	$C_{16}H_{32}O_2$	256
13	16.9	1-Nonadecene	0.35	$C_{19}H_{38}$	266
14	16.971	Nonadecane, 2,3-dimethyl-	0.42	$C_{21}H_{44}$	296
15	17.121	1-Heneicosanol	1.54	$C_{21}H_{44}O$	312
16	17.192	Nonadecane	0.29	$C_{19}H_{40}$	268
17	17.333	2,10-Dodecadien-1-ol,3,7,11-trimethyl-, (Z)-	0,22	$C_{15}H_{28}O$	224
18	17.691	Kaur-16-ene	0.21	$C_{20}H_{32}$	272
19	17.823	Cyclohexane, undecyl	0.32	$C_{17}H_{34}$	238
20	18.25	9,12-Octadecadienoic acid (Z,Z)-	0.27	$C_{18}H_{32}O_2$	280
21	18.319	9-Octadecenoic acid, (E)-	1.03	$C_{18}H_{34}O_2$	282
22	18.466	(R)-(-)-(Z)-14-Methyl-8-hexadecen-1-ol	0.26	$C_{17}H_{34}O$	254
23	18.553	Octadecanoic acid, 2- (2-hydroxyethoxy) ethyl ester	0.59	$C_{22}H_{44}O_4$	372
24	18.767	Trans-Geranylgeraniol	0.47	$C_{20}H_{34}O$	290
25	18.953	1-Heneicosanol	0.63	$C_{21}H_{44}O$	312
26	19.371	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl) cyclohexane	0.25	$C_{33}H_{56}$	452
27	19.864	Dodecylcyclohexane	0.18	$C_{18}H_{36}$	252
28	20.117	2,7,11-Trimethyl-4-phenylthiododeca-2,6,10- triene	5.67	$C_{21}H_{30}S$	314
29	20.236	beta.-Tocopherol	10.83	$C_{28}H_{48}O_2$	416
30	20.929	Octacosanol	0.28	$C_{28}H_{58}O$	410
31	21.175	1-Cyclopentene-1-carbaldehyde,3,4,5-tri(benzyloxy)	2.75	$C_{27}H_{26}O_4$	414
32	21.477	Ethyl trans-4a,cis-4b,trans-8a,cis-10a-perhydro-trans-2,4a,8a-trimethyl-8-oxophenanthrene-2-carbothiolate	0.75	$C_{20}H_{32}O_2S$	336
33	21.71	Hexanoic acid, octadecyl ester	0.66	$C_{24}H_{48}O_2$	368
34	21.783	Dodecylcyclohexane	0.37	$C_{18}H_{36}$	252
35	21.891	Octacosane	0.66	$C_{28}H_{58}$	394
36	21.966	(2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, trans-	2.42	$C_{22}H_{20}OS$	332
37	22.102	(2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, trans-	4.20	$C_{22}H_{20}OS$	332
38	22.237	(2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, trans-	1.26	$C_{22}H_{20}OS$	332
39	22.701	A'-Neogammacer-22(29)-ene	10.18	$C_{30}H_{50}$	410
40	22.735	Supraene	10.28	$C_{30}H_{50}$	410
41	22.931	Naphthalene, 1,2,3,4-tetrahydro-2-phenyl-	0.68	$C_{16}H_{16}$	208
42	23.118	Benzene,1,1'-[1-(2,2- dimethyl-3-butenyl)-1,3-propanediyl]bis-	0.71	$C_{21}H_{26}$	278
43	23.192	2-[5-(2,2-Dimethyl-6-methylene-cyclohexyl)-3-methyl-pent-2-enyl]-1,4-dimethoxy-benzene	0.54	$C_{23}H_{34}O_2$	342
44	23.338	erythro-9,10-Dibromo pentacosane	0.67	$C_{25}H_{50}Br_2$	508
45	23.488	Octacosane	2.07	$C_{28}H_{58}$	394
46	23.641	2-Hydoxymethyl-2,6,8,8-tetramethyltricyclo[5.2.2.0(1.6)]undecane	0.49	$C_{16}H_{28}O$	236
47	23.75	Ursa-9(11),12-dien-3-one	1.12	$C_{30}H_{46}O$	422
48	23.943	Lanosta-8,24-dien-3-one	3.54	$C_{30}H_{48}O$	424
49	24.083	Lup-20(29)-en-28-ol	0.27	$C_{30}H_{50}O$	426
50	24.155	Decanedioic acid, bis (2-ethylhexyl) ester	0.68	$C_{26}H_{50}O_4$	426
51	24.242	erythro-9,10-Dibromopentacosane	0.28	$C_{25}H_{50}Br_2$	508
52	24.431	Tetracosapentaene,2,6,10,15,19,23-hexamethyl	9.86	$C_{30}H_{52}$	412
53	24.592	9,19-Cyclolanostan-3-ol, 24-methylene-,(3-beta)	0.41	$C_{31}H_{52}O$	440
54	24.906	Lanosta-8,24-dien-3-one	17.07	$C_{30}H_{48}O$	424

In general, the classes of compounds identified were alkanoic acids, alkanols, ester, alkanal, alkanone, alkanes, alkenes, haloalkane, thiosulphoxide, and ethers.

Carboxylic acids containing thioether and sulphoxide like 2,7,11-Trimethyl-4-phenylthio dodeca-2,6,10-triene ($C_{31}H_{30}S$; 5.67%); Ethyltrans-4a,cis-4b,trans-8a,cis-10a-perhydro-trans-2,4a,8a-trimethyl-8-oxophenanthrene-2- ($C_{20}H_{32}O_2S$; 0.75%); (2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, trans- ($C_{22}H_{20}OS$; 7.887%) constitute 14.3 % of the compounds identified in *G. kola* seed extract.

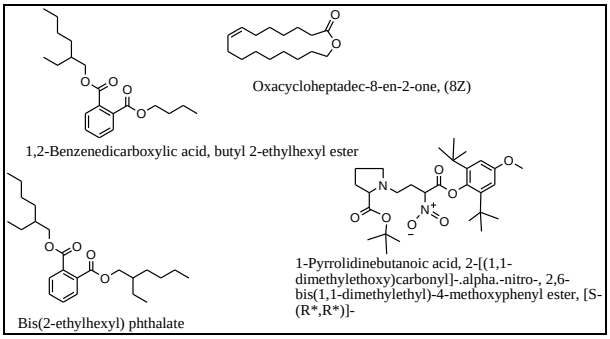

Figure 2. Chemical structures of some abundant compounds and Oxacycloheptadec-8-en-2-one, (8Z) in the *G. kola* n-hexane stem-bark extract.

Other compounds that make 4.153 % of the remaining components of the seed extract were 2-Hydroxymethyl-2,6,8,8-tetramethyltricyclo[5.2.2.0(1.6)]undecane; Octacosanol; 1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetram; (R)-(-)-(Z)-14-Methyl-8-hexadecen-1-ol; 9,12-Octadecadienoic acid (Z,Z)-; Cyclohexane, undecyl-; Kaur-16-ene; 2,10-Dodecadien-1-ol, 3,7,11-trimethyl-, (Z); Nonadecane, 2,3-dimethyl-; 1-Heptatriacotanol; 1,2-Benzenedicarboxylic acid, butyl 2-ethyl hex; 1,2-Benzenedicarboxylic acid, bis (2-methylpro; 3-Chloropropionic acid, heptadecyl ester; Cyclopentane, (2-hexyloctyl)-; Pentadecane and 1-Hexadecanol (Table 2).

In the seed extract chromatogram, multiple peaks were obtained for (2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide; 1-Heneicosanol; 1-Nonadecene; Dodecyclcyclohexane; erythro-9,10-Dibromopentacosane; Lanosta-8,24-dien-3-one; Nonadecane and Octacosane. The observation of multiple peaks may be due to the complexity of the extract and the high retention time of some of the compounds which causes impairment of column efficiency (Ogunlesi *et al.*, 2010).

Mphahlele (2019) reported the presence of 2,7,11-Trimethyl-4-phenylthiododeca-2,6,10- triene in turmeric (*Curcuma longa*), a natural product which is known for its antioxidant and anticancer properties. Beta-tocopherol is a natural form of vitamin E that reduces damage to the body cells because of its well-known and reported free radical scavenging properties (Mathur *et al.*, 2015). Trans- (2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide and related compounds that contain sulphur, thioethers and carboxylic acids containing sulphoxide have been reported to be effective in the healing of bruised skin due to the accumulation of free radical bruise healing and anti-ageing skin effects (Ogunlesi *et al.*, 2010).

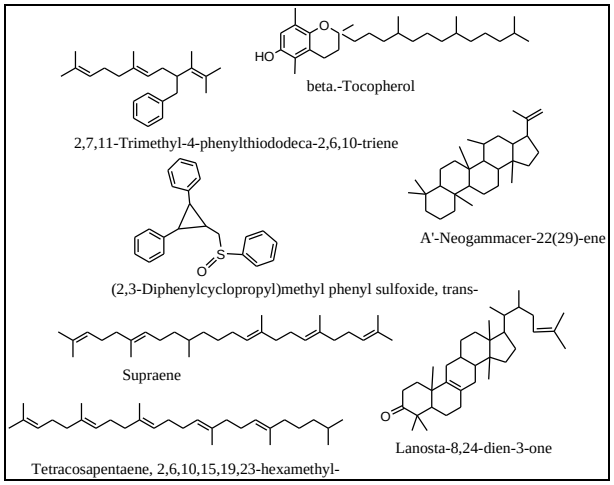

Figure 1. Chemical structures of some abundant compounds and Lanosta-8,24-dien-3-one in the *G. kola* n-hexane seed extract.

A'-Neogammacer-22(29)-ene (10.18%), also known as hop-22(29)-ene (NCBI, 2021a) is a triterpene consisting of a C=C double bond at the 22 (29)-position. Related compounds to (hop-22(29)-ene) such as hop-17(21)-ene, and neohop-13(18)-ene have been reported to show anti-tumour activities in an earlier study (Konoshima *et al.*, 1996). Supraene (10.28%) also known as trans-squalene (NCB1, 2022b) has been reported in nutritional, medicinal, and pharmaceutical aspects to have chemopreventive and chemotherapeutic properties which inhibit the growth of tumours on the skin, lung, breast, and colon (Güneş *et al.*, 2013; NCB1, 2022b; Reddy and Couvreur, 2009; Wołosik *et al.*, 2013). Supraene also stimulates the immune system in the treatment of ailments like HIV, leukaemia, papilloma, and herpes, among others (Güneş *et al.*, 2013; NCB1, 2022b; Reddy and Couvreur, 2009; Wołosik *et al.*, 2013). Several derivatives of Lanosta-

8,24-dien-3-one have been reported to have antioxidant and anticancer properties (Manourová *et al.*, 2019; Srivastava *et al.*, 2015).

In the *G. kola* stem-bark crude extract (GKBE), GC-MS detected thirty-four compounds (Table 3). These compounds consist of acids and long-chain fatty acids, long-chain fatty acid esters, alkanes, alkenes, alkanones, long-chain fatty alcohols, ethers, thioesters, and haloalkane (Table 3). Thirty-three of the compounds which make up 99.65% of the compounds identified have a percentage area abundance above 0.5% (Table 3).

Table 3. Compounds identified from the *G. kola* n-hexane stem-bark extract by GC-MS.

S/N	Retention time	compound	% Area abundance	Molecular formula	Molecular mass
1	15.820	1,2-Benzenedicarboxylic acid, bis (2-methylproyl) ester	1.56	$C_{16}H_{22}O_4$	278
2	16.078	Cyclohexane, undecyl-	0.66	$C_{17}H_{34}$	238
3	16.594	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	6.13	$C_{20}H_{30}O_4$	334
4	16.784	n-Hexadecanoic acid	1.11	$C_{16}H_{32}O_2$	256
5	17.017	Ethyl13-methyl-tetradecanoate	0.91	$C_{17}H_{34}O_2$	270
6	17.112	1-Nonadecene	1.25	$C_{19}H_{38}$	266
7	17.686	1-Naphthalenepropan ol,.alpha.-ethenyl decahydro-.alpha. ,5,5,8a-tetramethyl-2-methylene-,	2.02	$C_{20}H_{34}O$	290
8	17.817	Dodecylcyclohexane	1.13	$C_{18}H_{36}$	252
9	18.291	Palmitoleic acid	1.35	$C_{16}H_{30}O_2$	254
10	18.461	Oxacycloheptadec-8-en-2-one, (8Z)	25.59	$C_{16}H_{28}O_2$	252
11	18.542	Ethyl Oleate	1.02	$C_{20}H_{38}O_2$	310
12	18.941	n-Tetracosanol-1	1.34	$C_{24}H_{50}O$	354
13	19.029	Nonadecane	0.36	$C_{19}H_{40}$	268
14	19.361	1-Naphthalenepropan ol,.alpha.-ethenyl decahydro-2-hydroxy-alpha.,2,5,5,8a-pentamethyl-2, [1R-[1.alpha.(R*),2.beta.,4a.beta.,8a.alpha.]]	1.32	$C_{20}H_{36}O_2$	308
15	19.771	Cyclohexane,1-(cyclohexylmethyl)-4-methyl-,cis	0.55	$C_{14}H_{26}$	194
16	19.855	Dodecylcyclohexane	0.83	$C_{18}H_{36}$	252
17	20.917	Octacosanol	2.22	$C_{28}H_{58}O$	410
18	20.992	2-(2-ethyl hexanamido) ethyl2-(2-ethyl hexanamidoethyl) hexanoate	0.95	$C_{26}H_{50}N_2O_4$	454
19	21.168	Benzene, (3-nitropropyl)-	2.79	$C_9H_{11}NO_2$	165
20	21.701	Cyclohexanepropanol, .alpha.-methyl-	2.04	$C_{10}H_{20}O$	156
21	21.769	Dodecylcyclohexane	1.58	$C_{18}H_{36}$	252
22	21.987	Bis(2-ethylhexyl) phthalate	10.88	$C_{24}H_{38}O_4$	390
23	22.091	(2,3-Diphenyl cyclopropyl) methylphenylsulfoxide, trans-	3.28	$C_{22}H_{20}OS$	332
24	22.164	Erucic acid	1.14	$C_{22}H_{42}O_2$	338
25	22.233	(2,3-Diphenyl cyclopropyl) methylphenylsulfoxide, trans-	0.82	$C_{22}H_{20}OS$	332
26	22.638	erythro-9,10-Dibromo pentacosane	1.06	$C_{25}H_{50}Br_2$	508
27	23.19	2-[5-(2,2-Dimethyl-6-methylene-cyclohexyl)-3-methyl-pent-2-enyl]-1,4-dimethoxy-benzene	1.01	$C_{23}H_{34}O_2$	342
28	23.758	15-Tetracosenoic acid, methyl ester, (Z)-	2.43	$C_{25}H_{48}O_2$	380
29	24.142	Decanedioic acid, bis (2-ethylhexyl) ester	3.48	$C_{26}H_{50}O_4$	426
30	24.229	1-Nonadecene	0.99	$C_{19}H_{38}$	266
31	24.415	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-	4.35	$C_{10}H_{18}O$	154
32	24.618	1-Pyrrolidinebutanoic acid, 2-[(1,1-dimethyl ethoxy) carbonyl] -.alpha.-nitro-,2,6-bis(1,1-dimethylethyl)-4-methoxy phenylester, [S-(R*,R*)]-	6.5	$C_{28}H_{44}N_2O_7$	520
33	24.827	1-Pyrrolidinebutanoic acid, 2-[(1,1-dimethylethoxy) carbonyl]-.alpha.-nitro-,2,6-bis(1,1-dimethylethyl)-4-methoxy phenylester, [S-(R*,R*)]-	5.23	$C_{28}H_{44}N_2O_7$	520
34	24.875	2-Pentanone, 4-cyclohexylidene-3,3-diethyl-	2.13	$C_{15}H_{26}O$	222

Four of the compounds -1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester ($C_{20}H_{30}O_4$; 6.13%); Bis(2-ethylhexyl) phthalate ($C_{24}H_{38}O_4$;10.88%); Oxacycloheptadec-8-en-2-one, (8Z) ($C_{16}H_{28}O_2$; 25.59%); 1-Pyrrolidinebutanoic acid, 2-[(1,1-dimethyl ethoxy) carbonyl] -.alpha.-nitro-, 2,6-bis(1,1-dimethylethyl)-4-methoxy phenylester, [S-(R*, R*)]- ($C_{28}H_{44}N_2O_7$; 11.73%)- have percentage abundance above 5% and they constitute 67.218 % of the total compounds detected. The most abundant of the compounds eluted was Oxacycloheptadec-8-en-2-one, (8Z) (25.59%) which is also known as Ambrettolide (Reddy and Couvreur, 2009). Ambrettolide is a flavour and fragrance agent commonly used as a perfume base (Reddy and Couvreur, 2009).

As in the seed extract, some of the compounds identified in the stem-bark extract -1-Nonadecene; 1-Naphthalenepropanol, .alpha.-ethenyldecahydro-

alpha.,5,5,8a-tetramethyl-2-methylene-; Dode cylcyclohexane; (2,3-Diphenyl cyclopropyl) methylphenylsulfoxide, trans- and –Pyrrolidine butanoic acid, 2-[(1,1-dimethylethoxy) carbonyl] -.alpha.-nitro-, 2,6-bis(1,1-dimethylethyl)-4-methoxy phenylester, [S-(R*,R*)]- also eluted at multiple retention time as indicated by multiple peaks due to the complexity of the extract and high retention time of some of the compounds as earlier explained (Ogunlesi *et al.*, 2010).

Comparing the crude *G. kola* seed and stem-bark extracts, the compounds - Octacosanol, (2,3-Diphenylcyclopropyl) methyl phenylsulfoxide, trans-, Nonadecane, n-Hexadecanoic acid (Palmitic acid), -Cyclohexane, undecyl-, 1,2-Benzenedicarboxylic acid butyl 2-ethylhexyl ester, 1-Nonadecene, Dodecylcyclohexane, 2-[5-(2,2-Dimethyl-6-methylene-cyclohexyl)-3-methyl-pent-2-enyl]-1,4-dimethoxy-benzene and Decanedioic acid, bis (2-ethylhexyl) ester - are detected in both extracts. However, Octacosanol, (2,3-Diphenylcyclopropyl) methyl phenylsulfoxide, trans-, Nonadecane, and n-Hexadecanoic acid (Palmitic acid) are more abundant in the seed extract than the stem-bark extract while -Cyclohexane, undecyl-, 1,2-Benzenedicarboxylic acid butyl 2-ethylhexyl ester, 1-Nonadecene, Dodecylcyclohexane, 2-[5-(2,2-Dimethyl-6-methylene-cyclohexyl)-3-methyl-pent-2-enyl]-1,4-dimethoxy-benzene and Decanedioic acid, bis (2-ethylhexyl) ester are more abundant in the stem-bark extract. Octacosanol is similar to vitamin E, and has been reported to exert biological effects like antioxidant, antitumor, anti-inflammatory and anti-fatigue effects among others (Srivastava *et al.*, 2015). 2,3-Diphenylcyclopropyl methyl phenylsulfoxide has the potential to heal wounds (Ogunlesi *et al.*, 2010). Palmitic acid is found in most plants and its use in skin-care products and its anti-inflammatory properties have been reported (Carta *et al.*, 2017).

3.3. Cytotoxicity Assay

Cytotoxicity assay on both extracts was carried out using the brine shrimp lethality test as previously described (Akoro et al, 2022; Vanhaecke *et al.*, 1981) and the graph of mean percentage mortality for both extracts indicated dose-dependent mortality (Figure 3).

Higher percentage mortality was recorded in the GKBE extract than in GKSE as the concentration of extract increased. LC_{50} for GKSE and GKBE was 73.15±1.51 µg/mL and 42.76±1.85 µg/mL, respectively, suggesting a higher cytotoxic activity in the GKBE extract than the GKSE extract (Karan and Aydin, 2018; Zhang *et al.*, 2007). Sasikumar and Ghosh (2019) reported in a work that LC_{50} lower than 100 µg/mL is effective against tumour cells. According to a report, the American National Cancer Institute (NCI) stated that crude extract with IC_{50} less than 30 µg/mL indicates cytotoxicity (Charles-Okhe *et al.*, 2022).

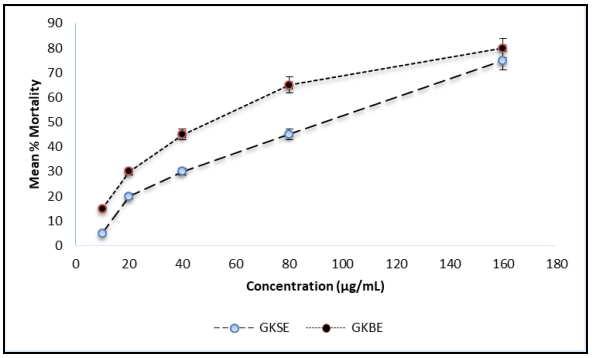

Figure 3. Comparative mean percentage mortality of GKSE and GKBE at different concentrations

4. Conclusion

The seed and the stem-bark extracts contain compounds which have been reported to possess anticancer properties and some of their derivatives are anticancer agents. Some of these compounds including 2,7,11-Trimethyl-4-phenylthiododeca-2,6,10- triene, β-tocopherol, 2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, trans-A'-Neogammacer-22(29)-ene, Lanosta-8,24-dien-3-one, supraene, and n-Hexadecanoic acid (Palmitic acid) may be responsible for the cytotoxic activities recorded in these plant extracts. These results indicated the rich phytochemical contents of the *G. kola* seed and stem-bark n-hexane extracts making this plant a possible source of new anticancer drugs.

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6. References

Akintonwa, A. and Essien, A. R. 1990. Protective effects of Garcinia kola seed extract against

paracetamol-induced hepatotoxicity in rats. *Journal of Ethnopharmacology*, 29 (2): 207-11

Akoro, S.M., Aiyelaagbe, O.O., Onocha, P.A. and Gloer, J.B. 2018. Gakolanone: a new benzophenone derivative from Garcinia kola Heckel stem-bark. *Natural product research*, 34 (2): 241-250.

Akoro, S.M., Omotayo, M. A., Ogundare, O.C., Akpovwovwo, S.A. and Bello, G.P. 2022. Phytochemical Analysis, Antioxidant and Cytotoxic Activity of Lannea egregia Engl. & K. Krause Stem Bark Extracts. *Pharmaceutical Sciences and Research*, 9(3):149 - 156

Carta, G., Murru, E., Banni, S and Manca, C. 2017. Palmitic Acid: Physiological Role, Metabolism and Nutritional Implications. *Frontier in Physiology*, 8: 902.

Charles-Okhe, O., Odeniyi, M.A., Fakeye, T.O., Ogbole, O.O., Akinleye, T.E., and Adeniji, A. J. 2022. Cytotoxic activity of crude extracts and fractions of African peach (nauclea latifolia smith) stem bark on two cancer cell lines. *Phytomedicine Plus*, 2(1): 100212.

Emmanuel, E.E., Paul, S.T., Ime, R.E. and Mohammad, I.C. 2021. Isolation and characterization of 5-hydroxymethylfurfural, antiglycation, antihyperglycaemic, antioxidant, and cytotoxic effects of Garcinia kola Heckel roots extract and fractions. *South African Journal of Botany*, 140: 62-67.

Farombi, E.O., Tahnteng, J.G., Agboola, O.A., Nwankwo, J.O. and Emerole, G.O. 2000. Chemoprevention of 2-acetylaminofluorene induced hepatotoxicity and lipid peroxidation in rats by kolaviron- a Garcinia kola seed extract. *Food and Chemical Toxicology*, 38: 535–541.

Farombi, E.O. 2000. Mechanisms for the hepatoprotective action of kolaviron: Studies on hepatic enzymes, microsomal lipids and lipid peroxidation in carbon tetrachloride treated rats. *Pharmacological Research* 42: 75–80.

Farombi, E.O., Adedara, I.A., Ajayi, B.O., Ayepola, O.R. and Egbeme, E.E. 2013. Kolaviron, a natural antioxidant and anti-inflammatory phytochemical prevents dextran sulphate sodium-induced colitis in rats. *Basic* and *Clinical Pharmacology* and *Toxicology*. 113 (1): 49-55.

Güneş, F.E., Üniversitesi, M., Fakültesi, S. B.,Bölümü,B. D., and Türkiye, I. 2013. Medical use of squalene as a natural antioxidant derleme/review. *Journal of Marmara University Institute of Health Sciences*, 3: 221–229.

Idris, A. E., Seke Etet, P. F., Saeed, A. A., Farahna, M., Satti, G. M. H., AlShammari, S. Z., and Hamza, M. A. 2020. Evaluation of metabolic, antioxidant and anti-inflammatory effects of Garcinia kola on diabetic rats. *Saudi Journal of Biological Sciences*, 27(12): 3641–3646.

Iwu M. M. 1985. Antihepatoxic constituents of Garcinia kola seeds. Experientia, 41 (5): 699–700.

Iwu, M., Igboko, O. A., Onwuchekwa, U. and Okunji, C. O. 1987. Evaluation of the antihepatotoxicity of the bioflavonoids of Garcinia kola seeds. *Journal of Ethnopharmacology*, 21: 127–142.

Kalu, W.O., Okafor, P. N., Ijeh, I.I., and Eleazu, C. 2016. Effect of kolaviron, a biflavanoid complex from Garcinia kola on some biochemical parameters in experimentally induced benign prostatic hyperplasic rats. *Biomedicine and Pharmacotherapy*, 83: 1436–1443.

Karan, T., and Aydin, A. 2018. Anticancer potential and cytotoxic effect of some freshwater cyanobacteria. *Tropical Journal of Pharmaceutical Research*, 17 (11): 2183-2188

Konoshima T, Takasaki M, Tokuda H, Masuda K, Arai Y, Shiojima K, Ageta H. 1996. Anti-tumor-promoting activities of triterpenoids from ferns. I. Biological & pharmaceutical bulletin, 19: 962-965

Lourenço, S. C., Moldão-Martins, M. and Alves, V. D. 2019. Antioxidants of Natural Plant Origins: From Sources to Food Industry Applications. *Molecules* (Basel, Switzerland), 24(22): 4132.

Manourová, A; Leuner, O., Tchoundjeu, Z., Patrick Van Damme, P.V., Verner, V., Pribyl, O and Bohdan Lojka, B. 2019. Medicinal Potential, Utilization and Domestication Status of Bitter Kola (Garcinia kola Heckel) in West and Central Africa. Forests, 10 (2): 124-129.

Mathur, P., Ding, Z., Saldeen, T., and Mehta, J. L. 2015. Tocopherols in the Prevention and Treatment of Atherosclerosis and Related Cardiovascular Disease. *Clinical cardiology*, 38 (9): 570–576.

Mphahlele, M.P. 2019. The sustained delivery system of curcuminoids-rich plant Curcuma longa, l for topical application. (Master's Dissertation). Johannesburg: University of Johannesburg. Available on the Internet at: http://hdl.handle.net/102000/0002.

National Center for Biotechnology Information. (2022a). PubChem Compound Summary for CID

92155, Diploptene. Available on the Internet at: https://pubchem.ncbi.nlm.nih.go v/compound/ Diploptene.

National Center for Biotechnology Information (2022b). PubChem Compound Summary for CID 638072, Squalene. Available on the Internet at: https: //pubchem. ncbi. nlm. nih.gov/ compound/Squalene.

Nwaehujor, C.O., Udegbunam, R.I., Ode, J.O., Udegbunam, S.O. 2015. Analgesic anti-inflammatory antipyretic activities of Garcinia hydroxybiflavanonol (GB1) from Garcinia kola. *Journal of the Korean Society for Applied Biological Chemistry*, 58: 91–96.

Ogunlesi, M., Okiei, W. and Osibote, A. E . 2010. Analysis of the essential oil from the leaves of Sesamum radiatum, a potential medication for male infertility factor, by gas chromatography-mass spectrometry. *African Journal of Biotechnology*, 9 (7): 1060-1067.

Omotoso, G.O., Ukwubile, I.I., Arietarhire, L., Sulaimon, F. and Gbadamosi, I.T. 2018. Kolaviron protects the brain in cuprizone-induced model of experimental multiple sclerosis via enhancement of intrinsic antioxidant mechanisms: Possible therapeutic applications? *Pathophysiology*, 25: 299–306.

Reddy and P. Couvreur, P. 2009. Squalene: a natural triterpene for use in disease management and therapy. *Advanced Drug Delivery Reviews*, 61, (15): 1412–1426.

Sasikumar, K., and Ghosh, A. R. 2019. Brine shrimp cytotoxicity potential of alpha-mangostin and dulxanthone D from Garcinia mangostana. *Bangladesh Journal of Pharmacology*, 14: 123-124.

Sofowora, A. 1993. Screening plants for bioactive agents. In: Medicinal Plants and Traditional Medicinal in Africa, second ed., Spectrum Books Ltd., Sunshine House, Ibadan, Nigeria, pp. 134–156.

Srivastava, R., Mukerjee, A., and Verma, A. 2015. GC-MS Analysis of Phytocomponents in, Pet Ether Fraction of Wrightia tinctoria Seed. *Pharmacognosy Journal*, 7(4): 249-253.

Vanhaecke, P., Persoone, G., Claus, C., & Sorgeloos, P. 1981. Proposal for a short-term toxicity test with *Artemia nauplii*. *Ecotoxicology and environmental safety*, 5(3), 382–387.

Wołosik, K., Knaś, M., Zalewska, A., Niczyporuk, M., and Przystupa, A. W. 2013. The importance and perspective of plant-based squalene in cosmetology. *Journal of Cosmetic Science*, 64(1): 59–66.

Zhang, M., Aguilera, D., Das, C., Vasquez, H., Zage, P., Gopalakrishnan, V. and Wolff, J. 2007. Measuring cytotoxicity: a new perspective on LC50. *Anticancer Research*, 27(1A): 35–38.