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Chemical Compositions of the Essential Oil Extracted from the Seeds of *Garcina Kola*, and Its Biological Activities

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The essential oil was obtained from the seeds of *Garcina kola* and its compositions were investigated by GC-MS and ICP-MS, respectively. 74 organic compounds and 9 trace elements beneficial to human health were confirmed in this oil. Then, the *in vitro* antioxidant and anticancer activities were evaluated accordingly. The results showed that this essential oil exhibited stronger antioxidant activity against DPPH radicals with the scavenging rate of 94.19% at 0.2 mg/mL, as well as potent inhibition against gastric cancer, lung cancer(A549) and Hela cell lines with the inhibitions of 96.397%±0.929, 98.005%±0.513 and 94.77±2.09 respectively at 8.3 mg/mL. While it exhibited moderate inhibition against the human breast carcinoma cells (MCF-7) with the inhibition of 59.257%±4.544 at 8.3mg/mL. In consideration of *Garcina kola* being consumed in Nigeria for a long time, this essential oil obtained from the *Garcina kola* can be used in the field of food, cosmetic or drugs.

Keywords: Anticancer Activities; Antioxidant; Chemical Compositions; Essential Oil; *Garcina Kola*.

The genus *Garcinia* is the member of the *Clusiaceae* family, and reported to be the medicinal tree species[1]. *Garcina kola* (*G. kola*) is one of the edible seeds that is highly cultivated, culturally valued and been consumed for many years in west and central region of Africa for tradio-medicinal and recreational purposes. It is commonly called "bitter kola" because of the bitter taste of its seeds. The seeds of *G. kola* were highly consumed in Nigeria due to its stimulatory potentials[2]. Extracts from this medicinal plant have been used for the treatment of hoarseness and cough[3], liver diseases and laryngitis[4]. Its seeds also displayed aphrodisiac properties, while its roots and stems can be used as chewing sticks for cleaning human teeth[5]. It can also suppress malaria, gonorrhea, headache and bronchitis[6,7].

G. kola seeds have been reported to be naturally rich in different bioactive ingredients: such as flavonoids, alkaloids, saponins, phenols, tannins and glycosides[8], which also displayed different biological activities: such as

antihepatotoxic[9], hypoglycaemic[10], antibacterial[11], hepatoprotective[12], anti-malarial[13] and anti-cancer[6] activities. Based on the historical records on consumption of *G. kola* in sub-Saharan Africa, it is essential for researchers to focus on its toxicological effects on human beings for safe consumption and extensively studying its biological activities in order to provide more scientific evidences toward traditional consumption of this seeds.

In current work, we obtained the essential oil from the seeds of *G. kola* and determined its chemical compositions. Then, the *in vitro* antioxidant and anticancer activities were also studied.

EXPERIMENT

Material and instruments

Plant Materials

G. kola seeds were purchased from Oko-local market, Ogbomoso, Nigeria, in July, 2019. The seeds were air-dried and powdered. Other chemicals used for chemical extractions and biological evaluations are analytical reagents and commercially available.

Instruments

The trace elements was carried out on the NexION 350X ICP-MS (PerkinElmer, USA); the organic chemical compositions was carried on the GCMS-QP2010 Ultra(SHIMADZU); and the anticancer activity was carried out on the full-wavelength multifunctional microplate reader (Multiskan GO, USA).

Extraction of essential oil

The detailed extraction processes listed below: 43 grams of the powdered *G. kola* seeds was covered using filter paper and added in a Soxhlet extractor with a condenser, which was refluxed and extracted using 500mL n-hexane for 12 hours. After that, the extraction was concentrated under reduced pressure to obtain 0.9 g light yellow oil (Figure 1) with the yield of 1.86%, which was stored in an amber bottle at 4^{o}C.

Determination of trace elements

500 mg of essential oil and 10 mL of 65% nitric acid were added into a 25 mL polytetrafluoroethylene digestion container, which was placed in the Multiwave PRO microwave digestion apparatus. The power ramp rate was regulated as follows: firstly, 0-800W for 10 minutes, while the temperature rose to 120 ^{o}C and the power was maintained for 5 minutes; subsequently, the temperature was adjusted to 150 ^{o}C at 800W for 5 minutes and then maintained for another 5 minutes; finally, the power and the temperature were set 1600W and 180 ^{o}C for 5 minutes, then which was maintained for 20 minutes. After completion, the solution was cooled to room temperature naturally and transferred to a 50 mL volumetric flask, the polytetrafluoroethylene digestion container was washed three times with deionized water and the washing solution was combined and added to the volumetric flask. Then, the solution was diluted with the deionized water to 50mL as the stocking solution.

Parameters of ICP-MS instrument

NexION 350X ICP-MS instrument (PerkinElmer, USA) was used for element analysis. The forward power was set to be 1600W, the plasma gas flow rate was 18L/min, Auxiliary gas flow rate was 1.2L/min, and carrier gas flow rate was 0.97L/min. Meinhard spray chamber was used, dwell time was set as 50ms. The position of torch, carrier gas flow rate, quadrupole ion deflector (QID) voltage and dual-mode correction of detector of ICP-MS were all optimized. ICP-MS tuning solution 5051, 5055 and 5059 (PerkinElmer, USA) was used in smart tune procedure.

The main technique indicators all had met the requirements. For example, intensity of sensitivity indicators of ^{9} Be, ^{115} In and ^{238} U had values above 2000 cps, 40,000 cps and 30,000 cps separately, back-ground intensity atm/z220 was<1 cps, oxide ratio ^{156} CeO $^{+}$/ ^{140} Ce $^{+}$was <2.5%, double-charged ions ratio ^{70} Ce^{2+}/^{140} Ce^{+} was <3.0%, and peak width of Li, Mg, In and U as mass resolution indicators was <0.65–0.8amu.

All detection was performed on kinetics energy discrimination (KED) mode and the flow rate of helium gas was 4.4mL/ min.

GC-MS analysis

In the gas chromatography-mass spectrometer (GC-MS) analysis of the essential oil of *G. kola*, a SHIMADZU GCMS-QP2010 plus system coupled with a SHIMADZU GCMS-QP2010 Ultra mass spectrometer equipped with a capillary column SH-RXi-5Ms (30 m × 0.25 mm i.d, 0.25 ìm film thickness) was used. The oven temperature was held at 60°C (hold 2mins), then programmed to 260°C (hold 10 mins) at a rate of

Fig. 1. The essential oil obtained from the seeds of *G. kola*

3°C/min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The injector temperature was 250°C, and the injection volume was 1 µL in acetone, with a split ratio of 1:30. Mass spectra (MS) were obtained in the electron impact mode (70 eV), and the MS data were acquired in scan mode with a mass range of m/z 40–700. The identification of the components was made based on the retention index (RI) relative to a homologous series of n-alkanes C_8–C_{20} under identical experimental conditions, MS library search (NIST 11s. lib), the Automated Mass Spectral Deconvolution & Identification System (AMDIS_32), and by comparing with MS literature data[14].

Antioxidant activity

Antioxidant activity of the essential oil was evaluated by the DPPH radicals assay in accordance with[15]. The detailed processes listed below: (1) 5 mg DPPH was added into a 25 mL volumetric flask, and MeOH was added to 25 mL. The mixture was shaken fully to give the DPPH stock solution, which was kept away from the light; (2) 1.8mL DPPH stock solution was added into a

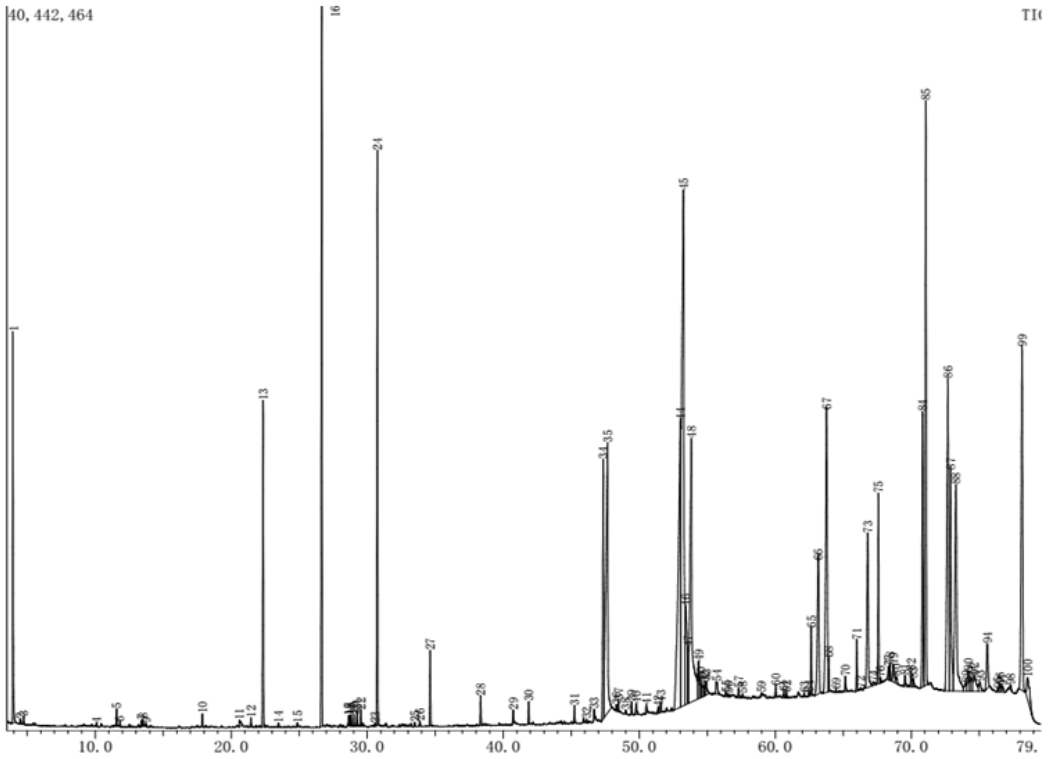
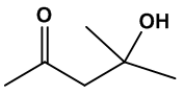
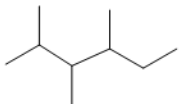
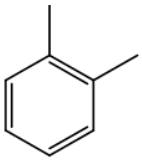
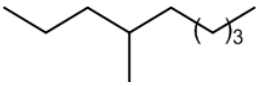
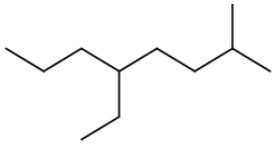
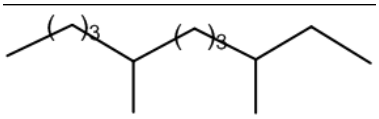
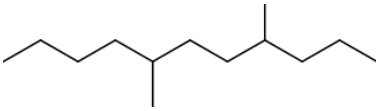
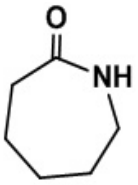
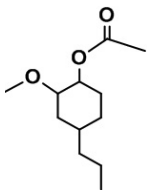
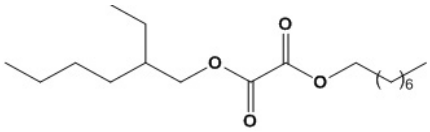
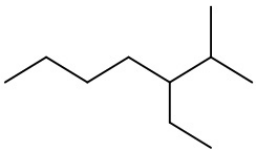
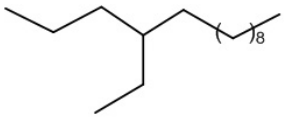
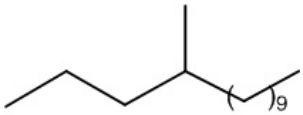
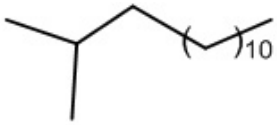
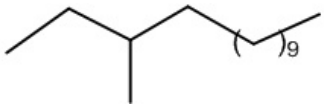
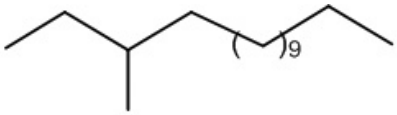
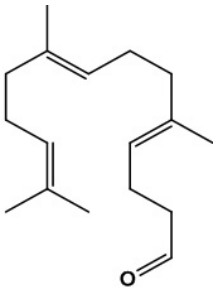
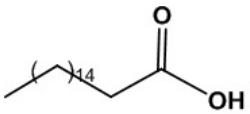
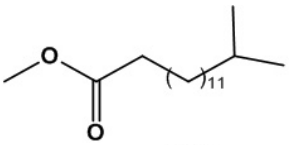
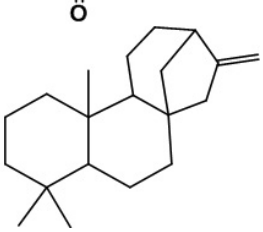
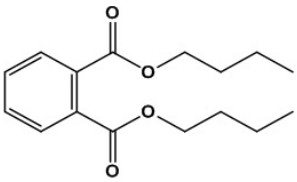
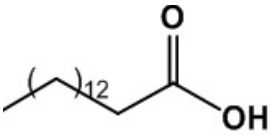
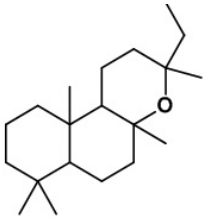
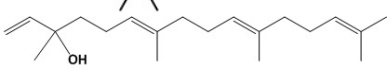


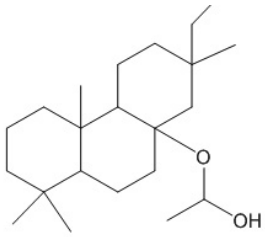
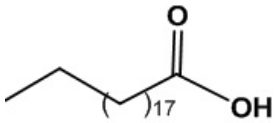
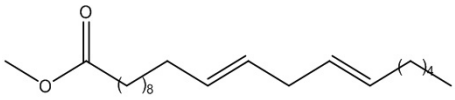
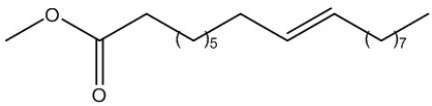
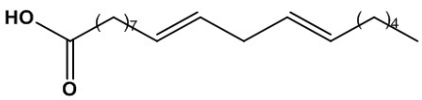
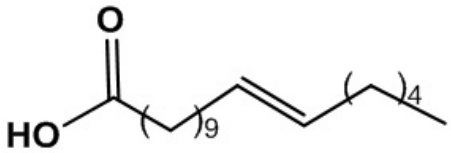
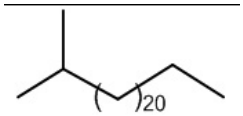
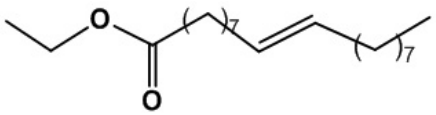
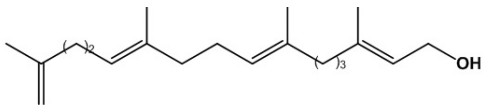
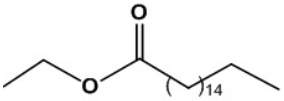
Fig. 2. The GC-MS chromatogram of the essential oil of *G. kola*

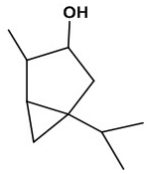
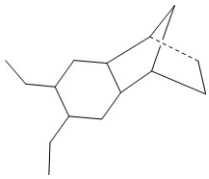
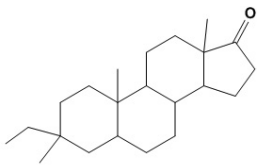
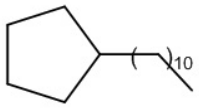
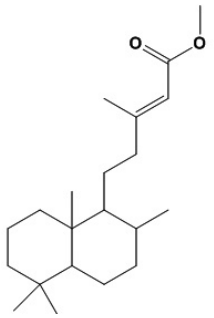
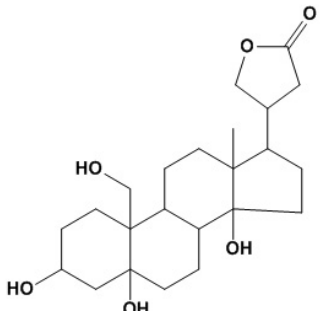
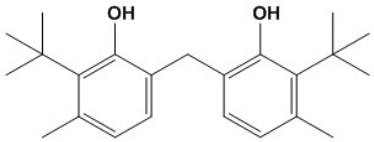
Table 1. The organic chemical compositions of the obtained essential oil

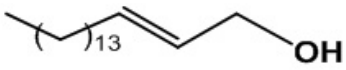
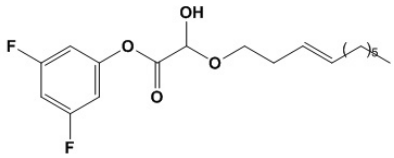
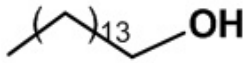
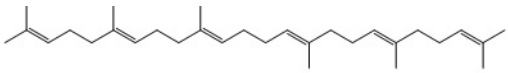
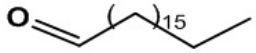
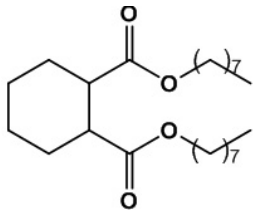
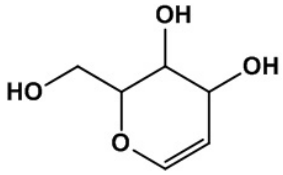
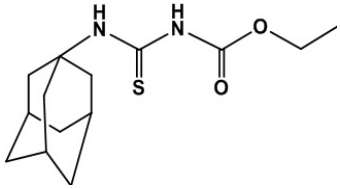
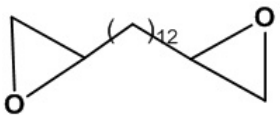
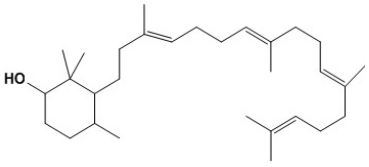
No.	Names	Structures	RT/mins
1	Acetonyl dimethyl carbinol		3.94
2	2,3,4-trimethyl hexane		4.49
3	1,2-dimethylbenzene		4.73
4	4-methyldecane		10.10
5	5-ethyl-2-methyloctane		11.57
6	3,7-dimethylundecane		11.80
7	n-undecane		13.40
8	4,7-dimethylundecane		13.51
9	n-dodecane		17.88
10	Azepin-2-one		20.60

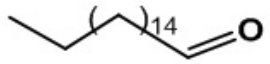
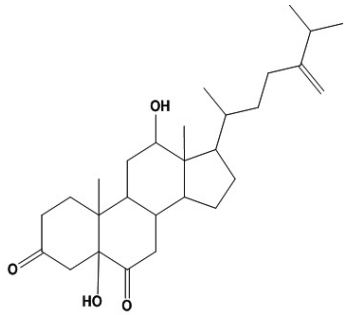
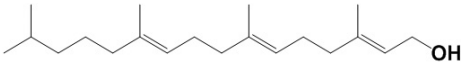
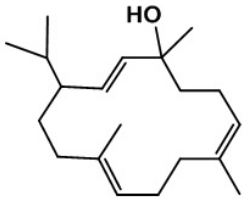
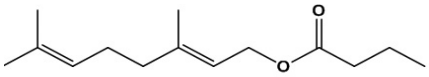
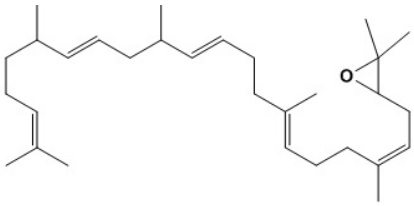
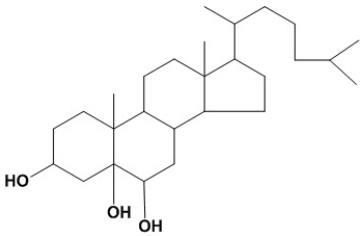
11	n-hexadecane		21.46
12	Aceteugenol		24.87
13	n-hexadecane		26.66
14	2-ethylhexylnonylester		28.63
15	3-ethyl-2-methylheptane		28.69
16	4-ethyltetradecane		28.80
17	4-methyltetradecane		29.03
18	2-methyltetradecane		29.24
19	3-methyltetradecane		29.54
20	hexadecane		30.57
21	3-methylpentadecane		33.50

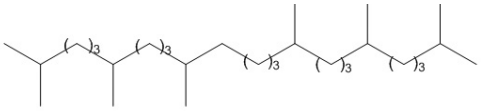
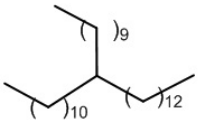
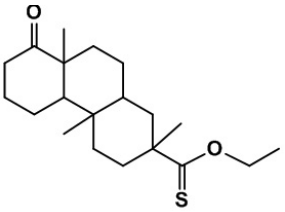
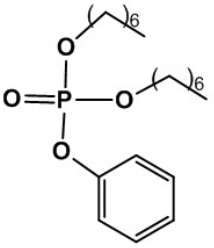
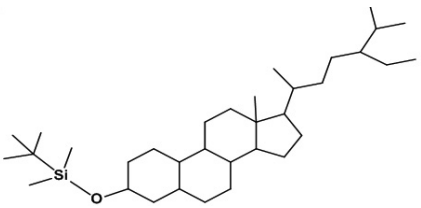
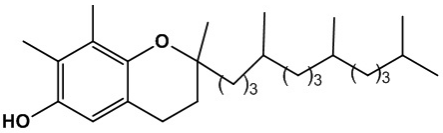
22	5,9,13-trimethyl-4,8,12-tetradecatrienal		33.87
23	Stearic acid		40.74
24	n-eicosane		41.88
25	Methyl-14-methylpentadecanoate		46.13
26	Ent-kaurene		46.69
27	Phthalic acid		47.35
28	Palmitic acid		47.67
29	1H-naphtho[2,1-b]pyran		49.00
30	3,7,11,15-tetramethyl-1,6,10, 14-hexadecatetraen-3-ol		49.43

31	Phenanthrenol		49.77
32	n-eicosanoic acid		50.53
33	Methyl-11,14-eicosadienoate		51.39
34	Methyl-9-octadecenoate		51.59
35	9,12-octadecadienoic acid		53.03
36	11-octadecenoic acid		53.25
37	2-methyltetracosane		53.42
38	Ethyl-9-octadecenoate		53.61
39	3,7,11,15-tetramethyl-2,6,10, 14-hexadecatetraen-1-ol		54.37
40	2,2-dimethyloctanol		54.73
41	Heptadecylacetate		54.83

42	Isothujol		54.92
43	1,4-methanonaphthalene		55.71
44	Androstan-17-one		56.38
45	Cyclopentyldecane		56.57
46	5-(decahydro-5,5,8a-trimethyl)-2-methylene-1-naphthalenyl)-3-methyl-2-methylpentenoate		57.60
47	Strophanthidol		58.99
48	p-Cresol		60.64

49	(2Z)-2-octadeceno-1-ol		60.82
50	Decyl-3,5-difluorophenylester		62.14
51	n-hexadecan-1-ol		62.50
52	Squalene		63.16
53	Octadecanal		63.77
54	Di-n-octylphthalate		63.93
55	Glucal		64.48
56	Ethyl(1-adamantylamino) carbothioylcarbamate		66.28
57	Diepoxyhexadecane		67.12
58	n-heneicosane		67.59
59	2,2,5-trimethyl-3-(3,8,12,16-tetramethyl heptadeca-3,7,11,15-tetraenyl)cyclohexanol		67.72

60	Heptadecanal		68.33
61	2,13-octadecadien-1-ol		68.44
62	5,12-dihydroxy-ergost-25-ene-3, 6-dione		68.68
63	trans-Geranylgeraniol		68.91
64	9-octadecenamide		69.54
65	Thunbergol		70.14
66	3,7-dimethyl-2, 6-octadienylbutanoate		70.83
67	2,2-dimethyl-3-(3,7,12,16, 20-pentamethyl-3,7,11,15, 19-heneicosapentaenyl)- oxirane		74.02
68	Cholestane-3,5,6-triol		74.42

69	Spinacene		74.61
70	11-decyltetracosane		75.60
71	Ethyl trans -4a,cis-4a, trans-8a,cis-10a-perhydro-trans-2, 4a,8a-trimethyl-8-oxophenanthrene-2-carbothiolate		76.54
72	Di-n-octylphenylphosphate		76.71
73	Stigmasterol tbdms		77.30
74	Gamma-tocopherol		78.56

10mL volumetric flask and diluted with 8.2mL MeOH. After being fully mixed, the solution was kept away from the light as the working solution; (3)5 mL of the DPPH working solution was taken out and the absorbance A_0 was measured at 519 nm, which was repeated for three times; (4) 1.0 mg essential oil was added in 5mL fresh DPPH working solutions and shaken to mix well, which was also measured the absorbance as A at 519 nm. The DPPH scavenging ability was calculated at the ratio{$[(A_0\text{-}A)\ /A_0]\times100$}. Each experiment was repeated for 5 times.

Preliminary *in vitro* anticancer evaluation

The anticancer activity of this essential oil was evaluated against gastric cancer, the human breast carcinoma cells (MCF-7), lung cancer(A549) and Hela cell lines using the counting kit-8 (CCK-8) method [16]. The evaluation process was described elsewhere with some modifications. Briefly, the oil was dissolved in DMSO at a concentration of 16.6mg/mL, then diluted successively with DMSO for seven different concentrations (16.6 mg/mL, 8.3 mg/mL, 4.15 mg/mL, 2.075 mg/mL, 1.0375 mg/mL, 0.51875 mg/mL and 0.209375

mg/mL respectively) as stock solutions for below experiment.

The Procedure for Anticancer Evaluation

The target cancer cell lines were seeded in 96-well plates (5000cells/well) with 100 µL DMEM supplemented with 10% fetal bovine serum, and cultured at 37 °C in a humidified CO_2 incubator (95% air, 5% CO_2) for 24h. While the cell lines grew to 90% in logarithmic growth, the culture medium was removed from each well, and 100 µL fresh DEME was added to each well. Then, 10 µL different concentrations of essential oil solutions were added into each well (every concentration was repeated for 5 times) and the plates were incubated for another 48h at 37 °C. Subsequently, 10 µL CCK8 was added to each well,

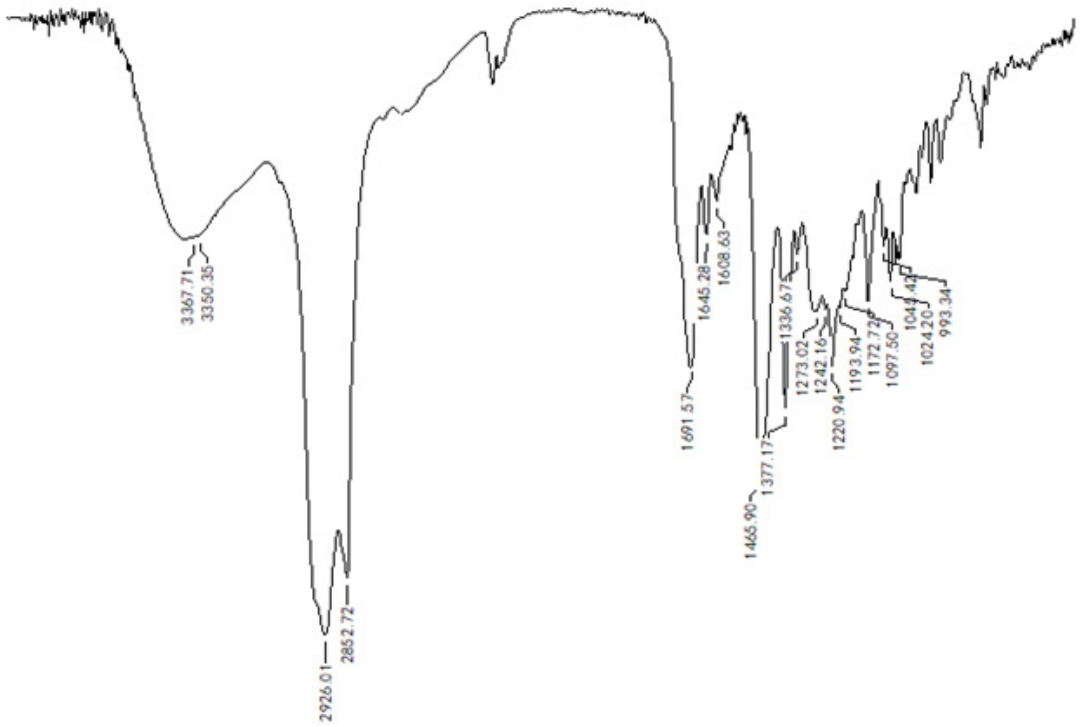


Fig. 3. FT-IR spectra of bitter kola oil in the frequency range 4000-500cm^{-1}

Table 2. Regression equation, correlation coefficient (R) and detection limit and the contents of trace elements in this essential oil

Elements	Internal standard elements	Regression equations	R	Detection limit (ng/mL)	Content of each element in oil (µg/g)
Se	Ge	Y=0.0045X+0.0050	0.9927	5.783	0.104
Sr	Rh	Y=0.0120X+0.0023	0.9959	0.1689	1.302
Cu	Ge	Y=2.3178X+415.7236	0.9945	35.53	3.497
Zn	Ge	Y=0.2807X+1.6809	0.9986	0.4763	7.598
Co	Sc	Y=3.0012X+0.1006	0.9989	0.02179	0.023
Ni	Sc	Y=0.8618X+7.3903	0.9988	0.4008	0.147
Mn	Sc	Y=0.4714X+0.2261	0.9994	0.3177	12.329
Fe	Sc	Y=1.0349X+46.4863	0.9988	7.313	18.642
V	Sc	Y=1.0952X+0.0440	0.9992	0.07894	0.023

Table 3. The scavenging ability of essential oil at different DPPH concentrations

$V_{DPPH\ stocking\ solution}$ (mL)	V_{MeOH} (mL)	Total V(mL)	Essential oil (mg)	Scavenging rate (%)
0.5	4.5	5	4	79.55
0.75	4.25	5	4	85.07
0.9	4.1	5	4	85.67
1	4	5	4	82.59

Table 4. The scavenging ability of different amounts of essential oil at 5mL DPPH stocking solutions

No.	Essential oil amounts (mg)	DPPH stocking solutions (mL)	Scavenging rate (%)
1	0.1	55	51.47
2	0.25	5	88.43
3	0.5	5	94.91
4	1	5	95.25
5	2	5	91.80
6	4	5	87.82
7	5	5	84.80
8	10	5	63.13

and the plates were cultured at 37 ^{o}C for another 4 hours. The optical density was measured at a wavelength of 450 nm on an ELISA microplate reader. DMEM and DMSO solution (*V*/*V*: 10/1) was used as a negative control. The results were expressed as the inhibition calculated at the ratio {[1-(OD_{450} treated/ OD_{450} negative control)] ×100}.

RESULTS AND DISCUSSION

Organic chemical compositions of the essential oil

The organic chemical compositions of this essential oil were confirmed by GC-MS (Figure 2), and 74 compounds were confirmed in this oil. The result was summarized in table 1.

IR analysis of the obtained oil

The obtained *G. kola* oil was also carried out the FT-IR analysis. The FT-IR spectra displayed in figure 3. It appeared a brs medium absorption peak at 3367 cm^{-1}, this is the characteristic absorption of the -OH, -NH and -NH_2 groups; it appeared strongest absorptions at 2852-2962 cm^{-1}, which owe to the =C-H, and -C-H stretching band, because this oil contains the saturated alkane, unsaturated alkane, saturated fatty acids, unsaturated fatty acids, and benzene; the strong stretching band at 1691cm^{-1} was confirmed as -C=O, -COOH and -CHO; the stronger stretching band at 1465cm^{-1} was attributed to the absorbance of C=C; the weak peak at 1377cm^{-1} was attributed to P=O; the weak peak at 1220 cm^{-1} was attributed to the C=S; while the week peak at 1220 cm^{-1} was attributed to the C-Si and C-Si-O. The peak signals of FT-IR are consistent well with the functional groups of compositions listed in table 1.

Trace elements in this essential oil

The trace elements in the seeds of *G. kola* have also been reported accordingly: Fe (6.10±0.43mg/kg) and Zn (2.30 ± 008 mg/kg)[17]; Cu (38.4mg/kg) and Co (102 mg/kg)[18]; Fe (17.75 ± 0.30 mg/100g), Zn (2.30 ± 0.01 mg/100g), Cu (0.78 ± 0.20 mg/100g) and Co (0.55 ± 0.20 mg/100g)[19]; Zn (3.5 mg/100g) and Cu (1.3 mg/100g)[20]. However, in this study, we obtained the essential oil from the seeds of *G. kola* and determined the trace elements using ICP-MS. Firstly, series of standard working solutions were successively measured to build the standard curve, and the regression equations were obtained accordingly. The regression equation, correlation coefficient and linear range of each element are shown in **table 2**. The trace elements

in this oil were tested accordingly and the contents were calculated according to the regression equations and the results also showed in **table 2**. From **table 2**, we can know that this essential oil is rich in nine essential microelements beneficial to human health (it is reported that 18 kinds of essential microelements have been conformed as necessary to human health and life, namely, iron, copper, zinc, cobalt, manganese, chromium, selenium, iodine, nickel, fluorine, molybdenum, vanadium, tin, silicon, strontium, boron, rubidium, arsenic, etc[21]. This study indicated that this essential oil is suitable for human consumption without causing any health problems.

***In vitro* biological evaluation**

Antioxidant effect of this essential oil

The radical scavenging ability of this essential oil was evaluated in the conventional system of DPPH radicals. Firstly, we added 4.0 mg oil to 5 mL different concentrations of DPPH solutions respectively and studied the scavenging ability to optimize the best experimental conditions. The results showed in table 3, which showed that the optimized DPPH concentration is 0.036mg/mL and the highest scavenging ability is 85.67%.

Then, we also added different amounts of essential oil to 5mL DPPH stocking solution respectively to optimize the optimal concentration of essential oil with the highest scavenging ability. The results (table 4) showed that the essential oil exhibited the scavenging ability in a concentration-dependent manner from 0.1 mg to 1.0 mg and exhibited the highest scavenging ability with 95.25% at 1.0 mg/5mL, while the scavenging ability reduced with the contents increasing of the essential oil. Then, we tested the scavenging ability of this essential oil at the optimized conditions: 5 mL of 0.036mg/mL DPPH solution, the essential oil 1.0 mg. The experiment was repeated for five times and the scavenging abilities are: 94.87%, 92.81%, 93.44%, 94.17% and 95.66% respectively; the average scavenging ability is 94.19%.

Preliminary *in vitro* anticancer evaluation

The preliminary *in vitro* anticancer result of this essential oil against gastric cancer, the human breast carcinoma cells (MCF-7), lung cancer(A549) and Hela cell lines showed that this oil exhibited potent anticancer activity against gastric cancer, lung cancer(A549) and Hela cell lines at the concentration of 8.3mg/mL with the inhibitions of 96.397%±0.929, 98.005%±0.513 and 94.77±2.09 respectively, while it exhibited moderate inhibition against the human breast carcinoma cells (MCF-7) with the inhibition of 59.257±4.544. The result implies that this oil can be also used as the anticancer agent.

CONCLUSIONS

In this work, we obtained the essential oil from *G. kola* seeds, and determined its chemical compositions using GC-MS and ICP-MS. The GC-MS results provide much information about the kinds of compositions for further study of this oil. The isolation of this oil will be carried out soonest. Then, we also evaluated the *in vitro* antioxidant and anticancer activities of this oil. The results showed that this oil exhibited potent antioxidant and anticancer activities against four cancer cell lines.

The seeds of *G. kola* have been consumed by human beings in Nigeria for a long time, which means that the seeds were non-toxic to the human beings. Therefore, this oil is also non-toxic and could be the functional edible oil and probably used in the fields of the food, cosmetic or drugs to replace synthetic antioxidant and anticancer drugs. The further and deeply research will be studied later.

ACKNOWLEDGMENTS

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