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

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https://dx.doi.org/10.13005/bpj/2163 \\
(Received: 08 June 2020; accepted: 20 April 2021)

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 \textit{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.*
antihepatotoxic⁹, hypoglycaemic¹⁰, antibacterial¹¹, hepatoprotective¹², anti-malarial¹³ and anti-cancer⁶ 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(SHAMADZU); 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°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 °C and the power was maintained for 5 minutes; subsequently, the temperature was adjusted to 150 °C at 800W for 5 minutes and then maintained for another 5 minutes; finally, the power and the temperature were set 1600W and 180 °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 of⁹ Be, ¹¹⁵ In and ²³⁸ U had values above 2000 cps, 40,000 cps and 30,000 cps separately, back-ground intensity atm/z²²₂₀ was<1 cps, oxide ratio ¹⁵⁶ CeO⁺/¹⁴⁰ Ce⁺ was <2.5%, double-charged ions ratio ⁷⁰ Ce²⁺/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 im 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
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 C₈–C₂₀ 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.

Antioxidant activity
Antioxidant activity of the essential oil was evaluated by the DPPH radicals assay in accordance with. 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) 1.8 mL DPPH stock solution was added into a
**Table 1.** The organic chemical compositions of the obtained essential oil

| No. | Names                           | Structures | RT/mins |
|-----|---------------------------------|------------|---------|
| 1   | Acetonyl dimethyl carbinol      | ![Structure](image1) | 3.94    |
| 2   | 2,3,4-trimethyl hexane          | ![Structure](image2) | 4.49    |
| 3   | 1,2-dimethylbenzene             | ![Structure](image3) | 4.73    |
| 4   | 4-methyldecane                  | ![Structure](image4) | 10.10   |
| 5   | 5-ethyl-2-methyloctane          | ![Structure](image5) | 11.57   |
| 6   | 3,7-dimethylundecane            | ![Structure](image6) | 11.80   |
| 7   | n-undecane                      | ![Structure](image7) | 13.40   |
| 8   | 4,7-dimethylundecane            | ![Structure](image8) | 13.51   |
| 9   | n-dodecane                      | ![Structure](image9) | 17.88   |
| 10  | Azepin-2-one                    | ![Structure](image10) | 20.60   |
11 n-hexadecane

12 Acet Eugenol

13 n-hexadecane

14 2-ethylhexylnonylester

15 3-ethyl-2-methylheptane

16 4-ethyltetradecane

17 4-methyltetradecane

18 2-methyltetradecane

19 3-methyltetradecane

20 hexadecane

21 3-methylpentadecane
| No. | Compound                                | Percentage |
|-----|-----------------------------------------|------------|
| 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

32 n-eicosanoic acid

33 Methyl-11,14-eicosadienoate

34 Methyl-9-octadecenoate

35 9,12-octadecadienoic acid

36 11-octadecenoic acid

37 2-methyltetrasocane

38 Ethyl-9-octadecenoate

39 3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraen-1-ol

40 2,2-dimethyloctanol

41 Heptadecylacetate
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
| No. | Chemical Name                                      | Molecular Weight |
|-----|---------------------------------------------------|------------------|
| 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)carbothioylecarbamate      | 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
61  2,13-octadecadien-1-ol
62  5,12-dihydroxy-ergost-25-ene-3,6-dione
63  trans-Geranylgeraniol
64  9-octadecenamide
65  Thunbergol
66  3,7-dimethyl-2,6-octadienylbutanoate
67  2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-oxirane
68  Cholestan-3,5,6-triol
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 $\left(1 - \frac{A}{A_0}\right) \times 100$. 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).
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 (5000 cells/well) with 100 µL DMEM supplemented with 10% fetal bovine serum, and cultured at 37°C in a humidified CO₂ incubator (95% air, 5% CO₂) 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.

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**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 °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 \(\{1-(\text{OD}_{450 \text{treated}}/ \text{OD}_{450 \text{negative control}})\} \times 100\).

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 -NH₂ 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 1691 cm⁻¹ was confirmed as =C=O, -COOH and -CHO; the stronger stretching band at 1465 cm⁻¹ was attributed to the absorbance of C=C; the weak peak at 1377 cm⁻¹ was attributed to P=O; the weak peak at 1220 cm⁻¹ was attributed to the C=S; while the weak 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±0.43 mg/kg) and Zn (2.30 ± 008 mg/kg)\(^7\); Cu (38.4 mg/kg) and Co (102 mg/kg)\(^8\); Fe (17.75 ± 0.30 mg/100 g), Zn (2.30 ± 0.01 mg/100 g), Cu (0.78 ± 0.20 mg/100 g) and Co (0.55 ± 0.20 mg/100 g)\(^9\); Zn (3.5 mg/100 g) and Cu (1.5 mg/100 g)\(^10\). 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 confirmed 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, etc21. 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 stock 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

This work was financially supported by the National Natural Science Foundation of China (21875252).

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