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GC-MS Based Phytochemical Profiling and Complementary Antioxidant Activities of Ethanol Extract of *Garcinia kola* Seeds Collected from Kogi State, Nigeria

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ABSTRACT

Garcinia kola Heckel (bitter kola) is widely used in West African traditional medicine and is valued for its diverse phytochemical constituents. This study evaluated the antioxidant activity and chemical composition of an ethanolic seed extract of *G. kola* using complementary in *vitro* antioxidant assays and gas chromatography-mass spectrometry (GC-MS). Qualitative phytochemical screening indicated the presence of flavonoids, phenols, tannins, alkaloids, saponins, and phytates. Antioxidant activity was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging, total antioxidant capacity (TAC), and ferric reducing antioxidant power (FRAP) assays. The extract showed concentration-dependent DPPH scavenging, ranging from 43.24% at 100 µg/mL to 69.38% at 500 µg/mL, with an IC_{50} of 284.09 µg/mL. The TAC and FRAP values were 96.61 µg ascorbic acid equivalents (AAE)/g and 84.89 µg AAE/g at 500 µg/mL, respectively. GC-MS tentatively identified 26 constituents, with n-hexadecanoic acid (27.84%), 2,4-di-tert-butylphenol (10.97%), and 9,12-octadecadienoic acid (approximately 4- 5%) among the more abundant components. These findings indicate measurable antioxidant capacity and chemical diversity, but GC-MS library matching alone does not establish definitive compound identity, biological activity, or causal contribution to the antioxidant response. Further isolation, structural confirmation, quantitative analysis, and mechanistic studies are warranted.

Keywords: *Garcinia kola*, Antioxidant Activity, DPPH, FRAP, Total Antioxidant Capacity, GC-MS, Phytochemicals

INTRODUCTION

Oxidative stress arises when oxidant generation exceeds the capacity of biological antioxidant systems to maintain redox homeostasis. Persistent oxidative imbalance is implicated in the development or progression of several chronic disorders, including cardiovascular, metabolic, inflammatory, and neurodegenerative diseases (Sies, 2020). Consequently, considerable research attention has been directed toward plant-derived antioxidant sources, particularly because many plants contain phenolics, flavonoids, terpenoids, and other secondary metabolites with redox-modulating properties (Shahidi & Ambigaipalan, 2015).

Garcinia kola Heckel, commonly known as bitter kola, is a tropical tree indigenous to West and Central Africa and has a long history of ethnomedicinal use. Its seeds are traditionally consumed and have been investigated for antimicrobial, anti-inflammatory, hepatoprotective, antioxidant, and other biological properties. The phytochemical profile of *G. kola* includes biflavonoids, phenolic compounds, and other secondary metabolites, with kolaviron being one of its best studied biflavonoid-rich fractions (Farombi & Owoeye, 2011; Tauchen *et al.*, 2023).

Despite the established ethnomedicinal importance of *G. kola*, chemical profiles can vary with geographical origin, extraction procedure, plant maturity, and analytical conditions. A combined assessment of antioxidant activity and chemical composition can therefore provide useful preliminary evidence for evaluating the biological potential of a particular plant material. This study investigated the antioxidant activity of an ethanolic extract of *G. kola* seeds using DPPH, TAC, and FRAP assays and characterized its volatile and semi-volatile constituents by GC-MS.

MATERIALS AND METHODS

Plant Material and Extraction

Garcinia kola seeds were purchased from a local market in Lokoja, Kogi State, Nigeria, and authenticated at the Herbarium Unit, University of Benin (Voucher No. UBH-G365). The dried seeds were powdered and extracted with 80% (v/v) ethanol by cold maceration for 72 h. The extract was filtered, concentrated under reduced pressure, and stored at 4 °C until analysis.

Phytochemical Screening

Qualitative phytochemical screening was performed using standard procedures (Sofowora, 2008) to determine the presence of flavonoids, phenols, tannins, alkaloids, saponins, and phytates.

Antioxidant Assays

DPPH radical-scavenging activity was determined according to Brand-Williams *et al*. (1995), with absorbance measured at 517 nm and IC_{50} values calculated from the concentration-response relationship. Total antioxidant capacity was determined using the phosphomolybdenum method and expressed as ascorbic acid equivalents (AAE) per gram of extract (Prieto *et al.*, 1999). Ferric reducing antioxidant power was determined according to the FRAP principle described by Benzie and Strain (1996).

GC-MS Analysis

GC-MS analysis was performed using an Agilent 7890A gas chromatograph coupled to a 5975C mass selective detector. Separation was achieved on a DB-5MS column (30 m × 0.25 mm, 0.25 µm). Constituent assignments were based on comparison of mass spectra with the NIST 14 mass spectral library and retention-index information where available. Because library matching alone does not provide definitive structural confirmation, the reported GC-MS assignments are treated as tentative.

Statistical Analysis

Antioxidant assays were performed in triplicate. Results are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, with $p < 0.05$ considered statistically significant, using GraphPad Prism 9.0.

RESULTS AND DISCUSSION

Phytochemical Composition of *Garcinia kola* Seed Extract

Qualitative phytochemical screening indicated the presence of flavonoids, phenols, tannins, alkaloids, saponins, and phytates. The detection of these metabolite classes is consistent with the known chemical diversity of Garcinia species and provides a plausible basis for the antioxidant behaviour observed in the extract (Farombi & Owoeye, 2011; Tauchen *et al.,* 2023).

Table 1: Phytochemical Composition of Garcinia Kola Seed Extract

Phytochemical	Concentration (mg/g, mean ± SD)
Phenols	27.17 ± 0.55
Tannins	82.14 ± 0.39
Flavonoids	19.67 ± 0.56
Alkaloids	3.04 ± 0.48
Saponins	24.18 ± 0.24
Phytates	35.99 ± 0.23

Antioxidant Activities of *Garcinia kola* Seed Extract

The ethanolic extract of *Garcinia kola* seeds exhibited concentration-dependent DPPH radical-scavenging activity, with percentage inhibition increasing from 43.24% at 100 µg/mL to 69.38% at 500 µg/mL. The calculated IC_{50} value was 284.09 µg/mL, indicating appreciable radical-scavenging activity under the experimental conditions. The concentration-dependent increase observed in the present study suggests that the extract contains constituents capable of donating hydrogen atoms or electrons to neutralize DPPH radicals. This finding is consistent with previous reports demonstrating antioxidant activity in *G. kola* seeds (Abubakar *et al*., 2020; Dogara *et al*., 2022).

The DPPH activity obtained in the present study is comparable to that reported by Abubakar *et al*. (2020) in a comparative investigation of aqueous extracts of *G. kola* and *Buchholzia coriacea* seeds. The authors reported 43.62% DPPH radical-scavenging activity for *G. kola* at 100 µg/mL, which is remarkably close to the 43.24% obtained in the present study at the same concentration. *B. coriacea*, another medicinal seed commonly used in West Africa, exhibited 44.66% inhibition at the same concentration. Interestingly, although *B. coriacea* showed slightly higher DPPH scavenging, *G. kola* demonstrated greater inhibition of lipid peroxidation, indicating that antioxidant activity may vary according to the assay employed (Abubakar *et al*., 2020). The close agreement between the present result and the earlier *G. kola* value provides additional evidence for the radical-scavenging potential of the seed.

The IC_{50} of 284.09 µg/mL obtained in this study, however, differs from values reported in some previous investigations of *G. kola*. Dogara *et al*. (2022), in a review of the biological activities of *G. kola*, reported a DPPH IC_{50} of approximately 65.86 µg/mL for an ethanol/aqueous seed extract. In another recent Nigerian study, methanolic *G. kola* extract showed a DPPH IC_{50} of 133.70 ± 0.11 µg/mL, while the corresponding *Cola nitida* extract had an IC_{50} of 226.70 ± 0.21 µg/mL (Apiamu *et al*., 2023). Thus, the IC_{50} obtained in the present study is higher than some previously reported values, suggesting lower DPPH potency under the conditions employed. Nevertheless, such differences should not be interpreted solely as differences in the inherent antioxidant potential of the plant because extraction solvent, extraction duration, geographical origin, phytochemical composition, DPPH concentration, reaction time and other assay conditions can markedly influence IC_{50} values (Dogara *et al*., 2022).

The comparatively higher IC_{50} observed in the present study may therefore reflect differences in the polarity and composition of the ethanolic extract or variation in the concentration of active antioxidant constituents. Previous phytochemical investigations have shown that *G. kola* seeds contain several bioactive phenolic and biflavonoid constituents. In particular, garcinia biflavonoids GB1 and GB2, garcinal and garcinoic acid have been isolated from *G. kola* seeds and implicated in their antioxidant activity (Iwu *et al*., 2009). The presence and relative abundance of these compounds may vary according to environmental and processing factors and could consequently influence the magnitude of antioxidant activity measured in vitro.

The FRAP assay provided additional evidence of antioxidant activity in the present study. The FRAP response increased progressively from 59.08 to 84.89 µg AAE/g across the tested concentrations, demonstrating increasing ferric-reducing capacity with increasing extract concentration. This observation is consistent with the concentration-dependent reducing activity previously reported for *G. kola*. Joshua *et al*. (2017) observed concentration-dependent FRAP activity in ethanolic extracts of both *G. kola* and *Cola nitida*, with *G. kola* showing greater reducing activity than *C. nitida*. Similarly, Apiamu *et al*. (2023) reported stronger antioxidant activity for methanolic *G. kola* extract than for *C. nitida*, with DPPH and FRAP assays demonstrating the greater antioxidant effectiveness of *G. kola*. These findings support the present observation that *G. kola* possesses appreciable electron-donating and ferric-reducing capacity.

A particularly relevant comparison can also be made with *Buchholzia coriacea*, another medicinal seed occurring in the West African region. Abubakar *et al*. (2020) reported measurable FRAP activity in both *G. kola* and *B. coriacea*, although the relative activity varied according to concentration. At 100 µg/mL, the percentage FRAP activity of *G. kola* was 38.35%, compared with 46.65% for *B. coriacea*. At lower concentrations, differences between the two seeds were also observed. These findings indicate that

closely related or similarly used medicinal seeds can exhibit different antioxidant responses depending on the assay and concentration employed (Abubakar *et al*., 2020). Therefore, the present FRAP results further establish the reducing capacity of *G. kola* but should not be directly ranked against published numerical values unless identical assay conditions and reporting units are used.

The total antioxidant capacity (TAC) of the ethanolic *G. kola* seed extract was 96.61 µg AAE/g. Although TAC provides a useful estimate of the overall antioxidant capacity of the extract, direct numerical comparison with studies reporting TAC in different standards or units should be undertaken cautiously. Nevertheless, the measurable TAC observed in the present study complements the DPPH and FRAP findings and indicates that the extract possesses multiple antioxidant properties. The combined increase in DPPH radical scavenging and FRAP response suggests that the extract is capable of both radical neutralization and electron donation.

The antioxidant activity observed in the present study may be associated with the phenolic compounds and biflavonoids present in *G. kola*. Iwu *et al*. (2009) isolated GB1, GB2, garcinal and garcinoic acid from *G. kola* seed fractions and reported that these constituents contributed to the antioxidant potential of the plant. In addition, Apiamu *et al*. (2023) reported significantly higher phenolic, flavonoid and tannin contents in *G. kola* than in *C. nitida*, together with stronger DPPH and FRAP responses. Such findings support the possibility that phenolic and related secondary metabolites contribute substantially to the antioxidant activity observed in the present ethanolic extract.

Overall, the present findings demonstrate that ethanolic *G. kola* seed extract possesses appreciable antioxidant activity, as evidenced by concentration-dependent DPPH radical scavenging, increasing ferric-reducing capacity and measurable total antioxidant capacity. The DPPH result at 100 µg/mL was particularly comparable with the value previously reported for aqueous *G. kola* extract by Abubakar *et al*. (2020). The results are also consistent with studies comparing *G. kola* with other West African medicinal and edible seeds, particularly *B. coriacea* and *C. nitida*, which have demonstrated antioxidant properties but varying degrees of activity (Abubakar *et al*., 2020; Joshua *et al*., 2017; Apiamu *et al*., 2023). The observed differences among studies emphasize the influence of extraction solvent, phytochemical composition and experimental conditions on antioxidant measurements. Further characterization of the extract, including determination of total phenolic and flavonoid contents and identification or quantification of individual antioxidant constituents, would help establish the chemical basis of the antioxidant activity observed in *G. kola* seeds.

Table 2: Antioxidant Activity of *Garcinia kola* Seed Extract

Assay	Result
DPPH IC_{50}	284.09 µg/mL
TAC	96.61 µg AAE/g
FRAP at 500 µg/mL	84.89 µg AAE/g

The use of complementary antioxidant assays is important because DPPH, FRAP, and TAC do not measure exactly the same chemical property. The DPPH assay primarily reflects radical-scavenging capacity, whereas FRAP reflects reducing power and the phosphomolybdenum assay provides an estimate of total reducing capacity. Accordingly, the agreement among the assays strengthens the evidence that the extract contains redox-active constituents, although in vitro antioxidant activity should not be equated with therapeutic efficacy in humans (Gülçin, 2020).

GC-MS Profiling

GC-MS analysis tentatively identified 26 constituents. The more abundant assigned components included n-hexadecanoic acid (27.84%), 2,4-di-tert-butylphenol (10.97%), and 9,12-octadecadienoic acid (approximately 4-5%), together with phytol and several long-chain hydrocarbons and fatty-acid derivatives.

Table 3: GC-MS Profile of the Ethanolic Seed Extract of *Garcinia kola*

Peak No.	Peak Area*	Library ID	NIST Quality
1	4,488	3-Hexanol	83
2	4,019	Oxiran, 2-methyl-3-propyl, trans	47
3	39,973	Dodacane	91
4	70,634	2,4-Di-tert-butylphenol	95
5	102,600	Heptadecane	80
6	89,840	Hexadecane	94
7	115,545	Octadecane	87
8	102,600	Heptadecane	96
9	68,330	Methadone, (1-hydroxycyclohexyl)…	91
10	109,279	2-Bromododecane	87
11	102,600	Heptadecane	86
12	138,502	Neophytadiene	97
13	74,580	1-Undecene, 4-methyl…	42
14	235,614	Octacosane	86
15	136,513	7,9-Di-tert-butyl-1-oxaspiro(4,5)…	95
16	269,761	9-Hexadecenoic acid, eicosyl ester…	72
17	117,418	n-Hexadecanoic acid (palmitic acid)	99
18	155,852	Phytol	76
19	155,888	Heneicosane	86

Peak No.	Peak Area*	Library ID	NIST Quality
20	140,139	9,12-Octadecadienoic acid (Z,Z) (linoleic acid)	99
21	140,137	9,12-Octadecadienoic acid (Z,Z) (linoleic acid)	96
22	144,272	Octadecanoic acid (stearic acid)	95
23	142,238	Eicosane	91
24	40,006	Tetracosane	74
25	233,419	Phthalic acid, di(2-propylpentyl)…	72
26	115,547	Octadecane	90

Library quality values are reported as supplied by the original manuscript. The GC-MS profile indicates substantial chemical complexity. n-Hexadecanoic acid was the most abundant tentatively assigned constituent, followed by 2,4-di-tert-butylphenol and 9,12-octadecadienoic acid. These compounds have been reported in different contexts to possess biological activities; however, the present experiment does not demonstrate that any single compound is responsible for the antioxidant activity. In particular, 2,4-di-tert-butylphenol requires cautious interpretation because a library match does not distinguish with certainty between a naturally occurring constituent and a possible environmental, processing, or analytical contaminant. Confirmation with authentic standards, retention indices, and complementary spectroscopic data would strengthen the chemical identification.

The findings are broadly consistent with previous reports describing antioxidant and other biological properties of Garcinia-derived phytochemicals (Farombi & Owoeye, 2011; Tauchen *et al.*, 2023). The present study nevertheless provides only preliminary evidence, and further work should establish the contribution of individual constituents using purified compounds, quantitative analysis, mechanistic assays, and appropriate biological models.

CONCLUSION

The ethanolic seed extract of Garcinia kola exhibited measurable in vitro antioxidant activity across DPPH, TAC, and FRAP assays and contained a chemically diverse profile of constituents tentatively identified by GC-MS. The concentration-dependent DPPH response and the reducing capacity measured by TAC and FRAP support the presence of redox-active components in the extract. The predominance of n-hexadecanoic acid and detection of other potentially bioactive constituents provide a useful basis for further phytochemical investigation. However, the present findings do not establish therapeutic efficacy or assign the antioxidant activity to individual GC-MS constituents. Future studies should focus on isolation and structural confirmation, quantitative profiling, mechanistic antioxidant assays, and appropriate safety and in vivo evaluation.

AUTHOR CONTRIBUTIONS

Conceptualization, K.O.A.; methodology, S.F.O.; formal analysis, E.A.T and B.A.J.; investigation, K.O.A. and R.J.A.; writing- original draft, K.O.A. and R.J.A.; writing- review and editing, K.O.A.; supervision. All authors read and approved the final manuscript.

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DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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