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Research Article

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Comprehensive Profiling of Secondary Metabolites and Bio-Activity of Garcinia Kola (Heckel) Leaf

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ABSTRACT

Garcinia kola is a West African medicinal plant traditionally used to manage infections and inflammation. While its seeds have been extensively studied, the leaves remain underexplored despite their potential as sources of antimicrobial agents. This study aims to investigate the phytochemical composition, functional groups, and antimicrobial activity of Garcinia kola leaf extracts using chemical and spectroscopic methods. Qualitative phytochemical screening, Fourier-transform infrared spectroscopy (FTIR), gas chromatography-mass spectrometry (GC-MS), and antimicrobial assays were performed on leaf extracts obtained via cold maceration using ethanol, methanol, aqueous, and n-hexane solvents. Antimicrobial activity was evaluated using the agar well diffusion method and Poison Plate Method against bacterial and fungal clinical isolates. Phytochemical screening revealed tannins as the only secondary metabolite consistently present in all extracts. FTIR analysis indicated key functional groups such as alcohols, aromatic amines, and carbonyls, while GC-MS identified compounds including phytol, neophytadiene, squalene, linoleic acid, and α-tocopherol. Antimicrobial assays showed selective activity only against Pseudomonas aeruginosa, with the n-hexane extract showing the highest inhibition zones (2.1–2.2 mm), while no effect was observed against other tested pathogens. Garcinia kola leaves possess narrow-spectrum antibacterial activity, particularly against Gram-negative bacteria. The presence of bioactive compounds suggests potential therapeutic applications. Further isolation, structural elucidation, and toxicity studies are required to validate these findings and advance the use of Garcinia kola leaf extracts in drug development.

Keywords: Garcinia Kola, Phytochemical Screening, Ftir, Gc-Ms, Antimicrobial Activity, Secondary Metabolites

Introduction

Plants have long served as foundational resources for modern therapeutics, with secondary metabolites such as alkaloids, tannins, flavonoids, and saponins playing crucial roles in drug development and disease management. A global resurgence in herbal medicine, particularly in low-resource settings, is driven by rising antimicrobial resistance, emerging diseases, and the affordability of plant-based remedies. According to the World Health Organization, over 80% of the population in developing countries relies on traditional medicine for primary health care [1].

Garcinia kola Heckel (family Clusiaceae), commonly referred to as bitter kola, is a tropical rainforest tree of remarkable ethnomedicinal value across West and Central Africa. Its seeds are traditionally used to manage liver and gastric disorders, bronchitis, throat infections, and malaria. They are also valued for their aphrodisiac and stimulant properties [2,3]. The plant holds socio-economic importance and is widely traded, but it has

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been listed as vulnerable on the IUCN Red List due to habitat degradation and unsustainable harvesting [4].

Scientific interest in G. kola has primarily centered on its seeds, particularly the biflavonoid complex kolaviron, known for antioxidant, anti-inflammatory, hepatoprotective, and antimicrobial properties [5,6]. Other notable compounds such as garcinianin, garcifurans, and garcinoic acid also contribute to its pharmacological potential but remain underexplored [7,8]. In contrast, the leaves, despite evidence of rich phytoconstituents including flavonoids and tannins, have received comparatively little scientific attention [3,9].

This underrepresentation of leaf-based studies is significant, as leaves are often rich in bioactive compounds and offer a more sustainable option for harvesting. Continued focus on roots and seeds not only narrows pharmacological discovery but also accelerates plant depletion. This poses risks to biodiversity and ethnobotanical heritage [10]. With multidrug-resistant pathogens on the rise, there is a critical need to explore and validate alternative phytotherapeutics derived from underutilized plant parts [11,12].

This study aims to provide a comprehensive phytochemical and bioactivity profile of Garcinia kola leaf extracts. Using GC-MS and FT-IR analyses alongside antimicrobial screening, we assess the potential of the leaf extracts as candidates for novel therapeutic agents. The findings are intended to contribute to ethnopharmacological knowledge and support the sustainable integration of G. kola leaves into herbal medicine and drug discovery frameworks.

Materials and Methods

Collection and Preparation of Plant Material

Fresh leaves of Garcinia kola were collected in June 2024 from Oko Egon, Ilupeju, Ekiti State, Nigeria. Botanical identification and authentication were performed at the Department of Plant Biology, University of Ilorin, where a voucher specimen was deposited in the institutional herbarium for reference. The leaves were washed with distilled water, air-dried at ambient room temperature, and pulverized using a mechanical grinder. The powdered material was stored in clean airtight containers for subsequent analyses.

Extraction Procedure

Cold maceration was used to obtain crude extracts. Exactly 100 g of powdered leaf sample was soaked in 700 mL of each of the following solvents: ethanol, methanol, distilled water, and n-hexane. Each mixture was left to stand for 48 hours with intermittent agitation. Filtration was done using Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator or by low-temperature air-drying based on the solvent used. Extracts were stored at 4 °C in sterile vials until further use [12].

Qualitative Phytochemical Screening

Standard methods were used to detect secondary metabolites including alkaloids, tannins, flavonoids, steroids, saponins, cardiac glycosides, phenolic flavonoids, anthraquinones, and anthocyanins [13,14]. Specific tests included Mayer's and Dragendorff's reagents for alkaloids, ferric chloride for tannins, lead acetate for flavonoids, and Keller-Kiliani and Salkowski tests for glycosides and steroids, respectively. Observations were based on color changes or precipitate formation.

Fractionation and Characterization of Bioactive Compounds

Column chromatography was employed to fractionate the ethanol and methanol extracts using a 1:1 solvent mixture of ethanol and methanol as the mobile phase. Thin-layer chromatography (TLC) was used to monitor the separation of compounds.

Fourier-transform infrared (FTIR) spectroscopy was used to identify functional groups using a Thermo-Nicolet Nexus 670 FTIR spectrometer within the range of 4000 to 400 cm^{-1}. Functional group interpretation was carried out following established methods [15].

Gas chromatography-mass spectrometry (GC-MS) was used to determine the chemical composition of the extracts using a Shimadzu GCMS-QP2010 Plus system. Retention times and fragmentation patterns were compared with entries in the NIST library for compound identification [12].

Antimicrobial Assay

Antimicrobial activity was tested against nine clinical isolates obtained from the Department of Microbiology, University of Ilorin. These included six bacterial strains (Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, and Salmonella typhi) and three fungal strains (Fusarium oxysporum, Candida albicans, and Trichophyton rubrum).

Bacterial suspensions were adjusted to 0.5 McFarland standard. The agar well diffusion method was used on Mueller-Hinton Agar (MHA) for antibacterial screening, while antifungal testing was done on Potato Dextrose Agar (PDA) using the Poison Plate Method. Plates were incubated at 37 °C for 24 hours (bacteria) and at room temperature for 5 days (fungi). Zones of inhibition were measured in millimeters [1,16].

Results

Phytochemical Screening of Garcinia kola Leaf Extracts

Qualitative phytochemical analysis revealed the consistent presence of tannins across all solvent extracts aqueous, ethanol, methanol, and n-hexane (Table 1). Other phytoconstituents such as alkaloids, flavonoids, phenolic flavonoids, steroids, cardiac glycosides, anthraquinones, and anthocyanins were not detected in any of the extracts. The exclusive detection of tannins indicates their abundance and solubility across polar and non-polar solvents.

Tannins are polyphenolic compounds known for their antimicrobial, antioxidant, and anti-inflammatory properties, which support the ethnomedicinal relevance of Garcinia kola [2,3]. The absence of other metabolites could be due to their low concentrations or limitations in detection sensitivity during qualitative screening, as previously observed in related studies [17,18].

Table 1: Qualitative Phytochemical Constituents of Garcinia kola Leaf Extracts

✓ = Present, – = Absent

Phytochemical	Aqueous	Ethanol	Methanol	n-Hexane
Tannins	✓	✓	✓	✓
Alkaloids (Mayer's)	–	–	–	–
Alkaloids (Dragendorff's)	–	–	–	–
Flavonoids	–	–	–	–
Phenolics flavonoids	–	–	–	–
Cardiac glycosides	–	–	–	–
Steroids	–	–	–	–
Anthraquinones	–	–	–	–
Anthocyanins	–	–	–	–

FTIR Spectroscopy Analysis

FTIR spectra revealed broad and sharp peaks representing diverse functional groups (Table 2). Alcohol (–OH) stretching vibrations between 3744–3769 cm^{-1} were evident in all extracts, confirming the presence of phenolics and polyols. Peaks around 2991–3013 cm^{-1} and 1180–1191 cm^{-1} were indicative of carboxylic acids, aldehydes, and isothiocyanates, known for antimicrobial and antioxidant properties. Aromatic amines and sulfonyl chlorides were prominent in ethanol, methanol, and n-hexane extracts, while the isocyanate peak at 2350 cm^{-1} appeared only in ethanol. Acid halides, which may contribute to antimicrobial activity, were found consistently across all solvents at 1804–1840 cm^{-1} [15].

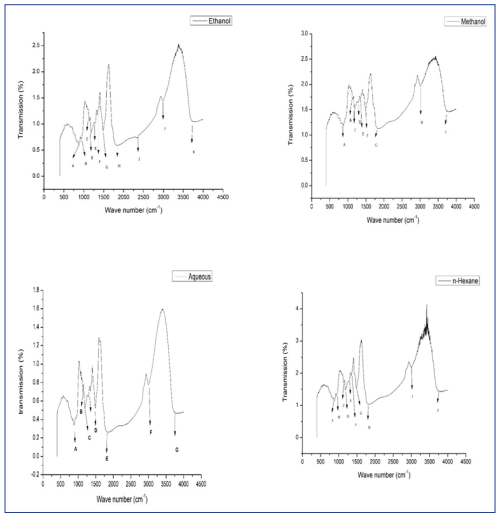

Figure 1: FTIR spectra of Garcinia kola leaf extracts showing major functional groups across solvents: Aqueous, Ethanol, Methanol and n-Hexane

Table 2: Major FTIR Absorption Bands Across Extracts of Garcinia kola Leaf

Wavenumber (cm^{-1})	Functional Group	Aqueous	Ethanol	Methanol	n-Hexane
3744-3769	Alcohol (–OH stretch)	✓	✓	✓	✓
2991-3013	Amine salt / Carboxylic acid	✓	✓	✓	✓
1180-1191	Aldehyde/ Isothiocyanate	✓	✓	✓	–
1293-1349	Aromatic amine	–	✓	✓	✓
1475-1490	Methylene/ Sulfonyl chloride	✓	✓	✓	✓
1135-1168	Aliphatic ether/ Methylene	✓	✓	✓	✓
868-960	Alkanes/ Alkaline groups / Vinylidene	✓	✓	✓	✓
1804-1840	Acid halide / Nitro compound	✓	✓	✓	✓
2350	Isocyanate	–	✓	–	–

GC-MS Analysis

GC-MS profiling identified several bioactive compounds across the extracts (Table 3). Methanol extract had the most diverse composition, with phytol, neophytadiene, and squalene compounds with well-documented antioxidant and antimicrobial activities [12]. The n-hexane extract contained fatty acids such as palmitic acid and linoleic acid, as well as α-tocopherol (Vitamin E), known for cardioprotective and anti-inflammatory effects.

The aqueous extract had fewer compounds but showed unique bicyclic derivatives. These results reflect the chemical richness of G. kola leaves, validating their potential as sources of therapeutic agents.

Table 3: Selected compounds identified from GC-MS of Garcinia kola extracts

Compound Name	Extract Source	Retention Time (min)	Known Bioactivity
Phytol	Methanol	28.78	Antioxidant, antimicrobial
Squalene	Methanol	29.42	Anticancer, anti-inflammatory
Neophytadiene	Methanol	27.22	Antioxidant, antimicrobial
Hexadeconoic acid	Ethanol	21.93	Antimicrobial, antioxidant
9,12-Octadecadienoic acid	Ethanol	24.64	Anti-inflammatory, cardioprotective
n-Hexadecanoic acid	n-Hexane	21.94	Antibacterial, anti-inflammatory
Vitamin E (α-Tocopherol)	n-Hexane	31.57	Antioxidant
Bicyclo[3.1.1]heptane derivative	Aqueous	6.89	Antimicrobial (tentative)
Tetracosanoic acid	Ethanol	33.01	Antibacterial, surfactant properties

Antimicrobial Activity

The antimicrobial assays demonstrated selective antibacterial activity. Only Pseudomonas aeruginosa was susceptible to the extracts, with n-hexane extract producing the highest inhibition zones (2.1–2.2 mm). No inhibition was observed for E. coli, S. aureus, K. pneumoniae, P. vulgaris, S. typhi, or for fungal strains (Candida albicans, Fusarium oxysporum, Trichophyton rubrum) (Table 4).

This narrow-spectrum activity suggests that specific non-polar bioactives may selectively target Gram-negative bacteria. Similar observations were reported by Akinmoladun et al. and Okwu & Iroabuchi, who attributed selective inhibition to lipid-soluble phytoconstituents like triterpenes and fatty acids [20,19].

Table 4: Antimicrobial Activity of Garcinia kola Leaf Extracts (Zone of Inhibition in mm)

Test organism	Aqueous	Ethanol	Methanol	n-Hexane
		Bacteria		
Pseudomonas aeruginosa	1.4, 1.2, 1.3	1.2, 1.0, 1.3	0.0, 0.0, 0.0	2.1, 2.2, 1.8
Staphylococcus aureus	0.0, 0.0, 0.0	0.0, 0.0, 0.0	0.0, 0.0, 0.0	0.0, 0.0, 0.0
Escherichia coli	0.0, 0.0, 0.0	0.0, 0.0, 0.0	0.0, 0.0, 0.0	0.0, 0.0, 0.0
Klebsiella pneumoniae	0.0, 0.0, 0.0	0.0, 0.0, 0.0	0.0, 0.0, 0.0	0.0, 0.0, 0.0
Proteus vulgaris	0.0, 0.0, 0.0	0.0, 0.0, 0.0	0.0, 0.0, 0.0	0.0, 0.0, 0.0
Salmonella typhi	0.0, 0.0, 0.0	0.0, 0.0, 0.0	0.0, 0.0, 0.0	0.0, 0.0, 0.0
		Fungi		
Fusarium oxysporum	0.0, 0.0, 0.0	0.0, 0.0, 0.0	0.0, 0.0, 0.0	0.0, 0.0, 0.0
Candida albicans	0.0, 0.0, 0.0	0.0, 0.0, 0.0	0.0, 0.0, 0.0	0.0, 0.0, 0.0
Trichophyton rubrum	0.0, 0.0, 0.0	0.0, 0.0, 0.0	0.0, 0.0, 0.0	0.0, 0.0, 0.0

Discussion

This study aimed to assess the phytochemical composition, functional groups, and antimicrobial activity of Garcinia kola leaf extracts using qualitative screening, FTIR, GC-MS, and microbiological assays. The consistent detection of tannins across all solvents suggests their high abundance and universal solubility in both polar and non-polar media. Tannins are polyphenolic compounds widely known for their antimicrobial, antioxidant, and anti-inflammatory effects through mechanisms such as protein precipitation, inhibition of microbial adhesion, and disruption of enzymatic activities [3,14]. Their dominance in G. kola leaves aligns with previous findings by Ugwuowo et al., who reported high tannin content in leaf methanolic and ethanolic extracts [9].

The absence of other major phytoconstituent groups in this study (e.g., alkaloids, flavonoids, and steroids) may result from low concentrations below qualitative detection limits or from extraction inefficiencies due to solvent compatibility. Akinmoladun et al. noted that different solvent types significantly influence the extractable phytochemical profile in Garcinia species [19].

The FTIR spectra revealed the presence of diverse functional groups, such as alcohols (O–H), carbonyls (C=O), alkanes (C–H), isocyanates, and sulfonyl chlorides. These functional groups are commonly associated with bioactive molecules possessing antimicrobial, antioxidant, or anti-inflammatory activities [15]. The O–H stretching vibrations between 3744–3769 cm^{-1} signify phenolic compounds, which are also supported by the presence of fatty acids and phenolics in GC-MS data. Notably, isocyanate and acid halide peaks, which are uncommon in many medicinal plant extracts, indicate the possible presence of nitrogen- or halogen-containing bioactive compounds, warranting further study.

GC-MS analysis revealed an array of pharmacologically active compounds, including phytol, squalene, neophytadiene, linoleic

acid, palmitic acid, and α-tocopherol. Phytol, a diterpene alcohol derived from chlorophyll degradation, is reported to possess antibacterial, anti-inflammatory, and antioxidant properties [18]. Squalene, a triterpene hydrocarbon, has demonstrated anticancer and immune-enhancing activities in earlier pharmacological evaluations [12]. Linoleic and palmitic acids are fatty acids with membrane-disrupting potential against bacterial pathogens, while neophytadiene contributes anti-inflammatory and anti-microbial properties [5,7]. These compounds, particularly when abundant in non-polar solvents like n-hexane, explain the observed selective antibacterial activity.

The antimicrobial assay revealed that G. kola leaf extracts exhibited selective antibacterial activity against Pseudomonas aeruginosa, a multidrug-resistant Gram-negative pathogen known for its intrinsic resistance mechanisms and efflux pumps [6]. The highest zone of inhibition (2.1–2.2 mm) was observed in the n-hexane extract, indicating that non-polar compounds, likely diterpenoids and long-chain fatty acids, are responsible for the bioactivity. This finding supports earlier observations by Okwu and Iroabuchi, who reported that G. kola extracts had targeted antibacterial activity, particularly when non-polar solvents were used [20].

The lack of antimicrobial activity against other bacterial and fungal pathogens, including E. coli, S. aureus, K. pneumoniae, and Candida albicans, suggests that the leaf extracts do not possess broad-spectrum antimicrobial activity in their crude forms. This may be attributed to insufficient concentrations of active constituents or to selective bioactivity of specific compounds. Related studies by Prasad et al. and Erukainure et al. documented similar narrow-spectrum activity in plant extracts rich in tannins and fatty acids, suggesting a need for fractionation and compound isolation to optimize efficacy [18,2].

Importantly, the absence of antifungal activity could stem from resistance mechanisms in fungal membranes or insufficient exposure to specific antifungal agents such as alkaloids and flavonoids, which were not detected in this study. Several antifungal plant extracts require phenolic or steroidal constituents to show potency, as emphasized by Altemimi et al. [12].

Overall, the results suggest that G. kola leaves, despite being underexplored compared to the seeds, harbor promising bioactive agents. However, their crude extracts exhibit limited antimicrobial scope. Future research should focus on purification, isolation of active principles, cytotoxicity profiling, and synergistic assays with existing antibiotics to determine pharmaceutical viability.

Conclusion

This study provides a comprehensive evaluation of the phytochemical constituents and antimicrobial activity of Garcinia kola leaf extracts. Tannins were consistently detected across all solvent extracts, suggesting their pharmacological relevance. FTIR and GC-MS analyses confirmed the presence of functional groups and bioactive compounds with known antimicrobial and antioxidant properties, including phytol, squalene, and α-tocopherol. Antimicrobial testing revealed selective inhibition of Pseudomonas aeruginosa, particularly by the n-hexane extract, indicating the potential of non-polar constituents against Gram-negative bacteria. However, the absence of activity against Gram-positive bacteria and fungi suggests limited broad-spectrum efficacy. Further studies are recommended to isolate active compounds, evaluate toxicity, and investigate mechanisms of action and synergistic potential with existing antibiotics.

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