

Physicochemical, Phytochemical and Gas Chromatography- Mass Spectrometric Analyses of *Gmelina Arborea* Root Hexane Extract

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Abstract: This study investigates the physicochemical properties, phytochemical composition, and gas chromatography-mass spectrometric (GC-MS) profile of the hexane extract of *Gmelina arborea* root (GARHE) to evaluate its potential for industrial applications. GC-MS analysis identified five major components. Of these, (9Z,12Z)-octadeca-9,12-dienoic acid (Linoleic acid), a polyunsaturated fatty acid (PUFA), was the most abundant natural compound at 30.90%. Also present were 2,4-Di-tert-butyl-6-nitrophenol (an antioxidant additive) at 2.19% and (9Z)-octadeca-9,17-dienal (an unsaturated aldehyde) at 0.23%. Notably, diisobutyl phthalate (37.47%) and di(2-ethylhexyl) phthalate (29.21%) were identified as significant processing contaminants. The physicochemical properties revealed an acid value of 66.30 mg/g, an iodine value of 61.48 mg/g, and a free fatty acid (FFA) content of 32.07 mg/g. These values suggest a moderate degree of unsaturation and significant hydrolysis, indicating the oil's suitability for applications such as biodiesel production and lubricant formulations rather than direct nutritional uses. Additional parameters, including a peroxidation value of 34.63 mg/g, saponification value of 43.39 mg/g, and esterification value of 22.92 mg/g, further support the extract's industrial relevance due to its oxidative characteristics and high non-saponifiable matter content. Phytochemical screening indicated the absence of alkaloids, flavonoids, phlobatannins, anthraquinones, carotenoids, and glycosides in this hexane extract. However, significant concentrations of terpenoids (133.90 mg/g), saponins (83.00 mg/g), and phenols (18.62 mg/g) were

detected, suggesting potential functional benefits in cosmetic and surfactant applications. This comprehensive analysis provides foundational data supporting the industrial utility of *Gmelina arborea* root hexane extract, particularly in biofuel and lubricant formulations, while highlighting the importance of contamination control during processing.

Keywords: *Gmelina arborea*, phytochemicals, physicochemical properties, gas-chromatography, root oil.

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1.0 Introduction

Gmelina arborea, commonly known as "gamhar" or "beechwood," is a widely grown tree across tropical regions of Asia and Australia, extensively introduced into West Africa, including Nigeria. In Nigeria, it is notably prevalent along roadsides and highways, earning it the nickname "avenue

tree." Primarily valued for its strong and durable timber, used in furniture making, upholstery, carving, and paper production (Wang, 2004), this species, belonging to the Lamiaceae family (though sometimes categorized under Verbenaceae), also holds significant medicinal and economic value (Choudhary et al., 2016; Charu & Arora, 2017; Attanayake et al., 2015). The global demand for natural products in pharmaceuticals, cosmetics, and sustainable industrial materials has spurred renewed interest in ethnobotanically significant plants, making comprehensive chemical profiling crucial for valorizing their potential (Cragg & Newman, 2013).

Traditionally, various parts of *Gmelina arborea* have been utilized in folk medicine, Ayurveda, and other traditional systems for treating ailments such as fever, wounds, ulcers, microbial infections, diabetes, and inflammation (Kumar et al., 2015). Specifically, its roots have demonstrated multiple biological activities, including anti-inflammatory, antimicrobial, and antioxidant effects (Banso & Adeyemo, 2007; Wadasinghe et al., 2022). These therapeutic properties are broadly attributed to their diverse phytochemical composition, which includes both volatile and non-volatile oils extractable through various organic solvents (Marwa et al., 2016). For instance, benzene extraction of its heartwood yields compounds like β -sitosterol and ceryl alcohol (Joshi et al., 1970), while dried root powder contains apigenin and root bark includes compounds like tyrosol (Dighe et al., 2007; Falah et al., 2008). Root-derived aromatic substances, including essential oils, are also commonly applied in cosmetics, perfumery, and pharmaceuticals.

While the plant's diverse applications are acknowledged, the detailed chemical characterization of its root extracts remains less comprehensive, particularly concerning its non-polar constituents. Comparative studies, such as those on hexane root extracts of *Coleus*

forskohlii, have revealed the presence of compounds like α -cedrene, β -cadinene, citronellal, and labdane derivatives, known for antimicrobial, antifungal, and insecticidal properties (Murugesan et al., 2012). This highlights the potential for non-polar extracts to yield industrially relevant compounds. In Nigeria, despite *Gmelina arborea*'s widespread presence and traditional use, its therapeutic applications are often anecdotal or undocumented in local medicinal plant references. This local context underscores the need to bridge the gap between traditional knowledge and modern scientific validation.

Given the limited scientific data on the precise chemical makeup of *Gmelina arborea* roots, especially through advanced analytical techniques like Gas Chromatography–Mass Spectrometry (GC-MS), this study aims to explore the non-polar bioactive components of its hexane root extract. The selection of hexane as the extraction solvent is driven by its high affinity for non-polar compounds, such as fatty acids, steroids, and certain terpenoids, which are often overlooked in more polar extracts but hold significant industrial and pharmacological interest (Harborne, 2003). Specifically, the objectives are to characterize its physicochemical properties, identify and quantify its phytochemical constituents, and determine its bioactive compounds through GC-MS analysis. By achieving these objectives, this study will provide foundational data to validate the extract's traditional medicinal claims and, more importantly, assess its suitability for modern industrial applications, particularly in sectors requiring sustainable raw materials for biofuel, lubricant, and cosmetic formulations. The insights gained will form a critical bridge between the ethnobotanical utility and the scientifically-backed potential of *Gmelina arborea* root.

2.0 Materials and methods

2.1 Plant collection

Fresh root samples of *G. arborea* were harvested from the trees growing at the back of

Department of Pure and Applied Chemistry, within the Campus of the Ladoké Akintola University of Technology, Ogbomoso, Oyo State, Nigeria, in June, 2023. Hassan Ganiyu Foluso of the Crop, Soil, and Pest Management department of the Microbiology section at the Federal University of Technology, Akure, Ondo State, Nigeria, verified the plant's identity after a voucher specimen with the number 0333 was placed.

2.2 Preparation of plant material

The harvested root samples were cut into smaller pieces and air-dried by spreading on the laboratory bench. The air-dried sample was pulverized in a mechanical grinder and packed in air-tight container for storage at 4 °C in a refrigerator.

2.3 Extraction of plant material

Exactly 2000g of the dried powdered root sample of *Gmelina arborea* was extracted with 7.3 litres of hexane solvent in a 10 L Pyrex extraction jar at room temperature for 96 hours. The extract measuring 5.7 litres was filtered using Whatman No. 1 filter paper and then evaporated to dryness on a rotary evaporator. The extracts weighing 8g, which amounts to 0.4% yield, are a yellow oil that later solidified.

2.4 Preliminary Phytochemical Analysis

The preliminary qualitative and quantitative phytochemical screening for the presence of tannins, saponins, alkaloids, cardiac glycosides, flavonoids, and others was carried out on the hexane extract of the root of *Gmelina arborea*. by the method of (Harbone, 2003).

2.5 Physicochemical properties Analysis

The physicochemical parameters such as acid, FFA, iodine, saponification, peroxidation and esterification values were determined using standard methods prescribed by (AOAC, 1994).

2.6 Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analyses

Constituents of GARHE oils were determined by gas chromatography-mass spectrometry

(GC-MS) using an Agilent 7890N gas chromatography hyphenated with an Agilent system mass detector Triple Quad 5975A in EI mode at 70 eV (m/z range 40 - 600 amu) with an ion source temperature of 250°C and an Agilent ChemStation data system. GC column was equipped with

an HP-5MS column (30 m $\times$ 320 μ m $\times$ 0.25 μ m), a split-split less injector heated at 200°C and a flame ionization detector (FID) at 230°C. Oven temperature was programmed as follows: Initial

temperature 80°C held for 2 min, increased 5°C/min to final temperature of 240°C held for 6 min. Helium was the carrier gas at flow rate of 2 mL/min. Injection volume was 2.0 μ L (split ratio 1:20). The components were identified by comparison of their mass spectra with NIST 2014 library data of the GC-MS system as well as by comparison of their retention indices (RI) with the relevant literature data (Adams, 2007).

The relative amount of each individual component of the extracted oil was expressed as the

percentage of the peak area relative to the total peak area. RI value of each component was determined relative to the retention times of a homologous n-alkane series with linear interpolation on the HP- 5MS column.

3.0 Results and Discussion

The hexane extract of *Gmelina arborea* root (GARHE) yielded a yellow oil, which solidified upon cooling, representing a 0.4% (w/w) yield from the pulverized root material. This solidified extract was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) for chemical profiling, and a qualitative phytochemical analysis, along with quantitative physicochemical parameter determination, was also performed.

GC-MS Analysis of *Gmelina arborea* Root Hexane Extract (GARHE)

The GC-MS chromatogram of GARHE (Figure 1) revealed five distinct peaks. Table 1 summarizes the retention times (tR), relative

peak areas (%), identified compounds, IUPAC and common names, base peaks (m/z), molecular ion peaks (m/z), and molecular weights (g/mol) for each component.

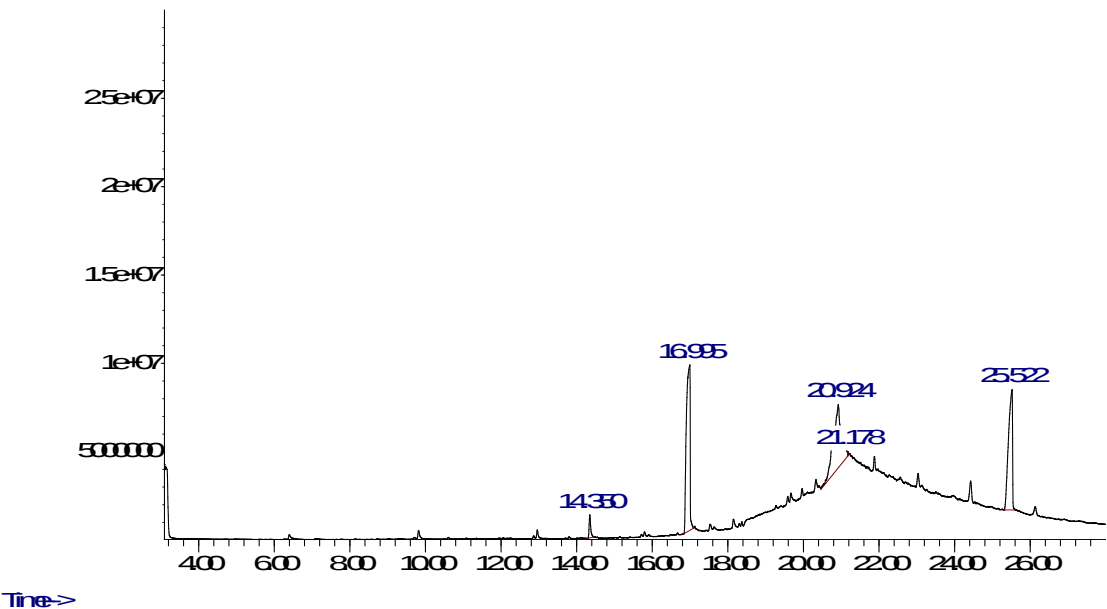


Fig 1: Gas chromatogram of GARHE oil.

Table 1: Retention time, peak area (%), constituents identified, molecular weight, molecular ion, and structures of compounds present in the oil of *Gmelina arborea* Root Hexane Extract (GARHE)

Peak #	RT (min)	Area (%)	IUPAC Name	Common Name	Base Peak (m/z)	Molecular Ion (m/z)	Molecular Weight (g/mol)	Structure
1	14.348	2.19	2,4-Di-tert-butyl-6-nitrophenol	DBNP (Antioxidant additive)	191	261	261	(Structure)
2	16.997	37.47	Bis(2-methylpropyl) benzene-1,2-dicarboxylate	Diisobutyl phthalate (Plasticizer)	149	278	278	(Structure)
3	20.922	30.90	(9Z,12Z)-Octadeca-9,12-dienoic acid	Linoleic acid (Fatty acid)	67	280	280	(Structure)
4	21.180	0.23	(9Z)-Octadeca-9,17-dienal	Linolelaidic aldehyde	81	264	264	(Structure)
5	25.523	29.21	Bis(2-ethylhexyl) benzene-1,2-dicarboxylate	Di(2-ethylhexyl) phthalate (DEHP, Plasticizer)	149	390	390	(Structure)

The GC-MS analysis identified three primary constituents of plant origin: 2,4-Di-tert-butyl-6-nitrophenol (DBNP), (9Z,12Z)-Octadeca-9,12-dienoic acid (Linoleic acid), and (9Z)-Octadeca-9,17-dienal (Linolelaidic aldehyde). DBNP, an antioxidant additive, was detected at 2.19%. Its presence in plant oils is relatively rare, making its identification in GARHE noteworthy, potentially contributing to the extract's stability or indicating its environmental ubiquity as an industrial additive. Linoleic acid, a polyunsaturated fatty acid (PUFA), represented a significant portion of the extract at 30.90%. This concentration is comparable to that found in common vegetable oils such as soybean oil (25.90%), sesame oil (40.90%), and pumpkin seed oil (46.40%) (Fránčáková et al., 2015), highlighting its potential as a source of this essential fatty acid. (9Z)-Octadeca-9,17-dienal, an unsaturated aldehyde, was present at a minor concentration of 0.23%. This compound is a known product of lipid oxidation (Cahyana et al., 2015), and its low concentration in the extract aligns with expectations for such a degradation product.

It is important to note the significant presence of two phthalate esters: Bis(2-methylpropyl) benzene-1,2-dicarboxylate (Diisobutyl phthalate, 37.47%) and Bis(2-ethylhexyl) benzene-1,2-dicarboxylate (Di(2-ethylhexyl) phthalate, DEHP, 29.21%). These compounds are well-known plasticizers and industrial contaminants. Their high concentrations in the extract strongly suggest their introduction during the extraction or processing steps, likely through contact with polymer materials such as rubber tubing used in the solvent distillation or filtration setup. While their presence is indicative of contamination rather than natural plant constituents, their prevalence underscores the need for scrupulous material selection in phytochemical studies.

Physicochemical Properties of GARHE

Table 2 presents the determined physicochemical parameters of GARHE,

providing insights into its overall quality, stability, and potential industrial applicability.

Table 2: Physicochemical parameters of *Gmelina arborea* Root Hexane Extract (GARHE)

Parameter	Amount (mg/g)
Acid Value	66.30 ± 0.36
FFA Value	32.07 ± 1.47
Iodine Value	61.48 ± 0.99
Peroxidation Value	34.63 ± 1.00
Saponification Value	43.39 ± 0.33
Esterification Value	22.92 ± 0.68

The acid value of 66.30 mg/g and a corresponding free fatty acid (FFA) value of 32.07 mg/g are notably high. This suggests significant hydrolysis of the glycerides within the oil, leading to an elevated concentration of free fatty acids. Such high values typically indicate oil degradation (e.g., from microbial action, enzyme activity, or improper storage) rather than a desirable trait for direct edible consumption. However, this characteristic makes the oil particularly suitable for industrial applications like biodiesel production, where high FFA content can be directly converted through esterification or transesterification processes, and soap manufacturing, as free fatty acids readily react with alkali (Warra, 2019).

The iodine value of 61.48 mg/g indicates a moderate degree of unsaturation. This value suggests that GARHE contains a significant proportion of unsaturated fatty acids, consistent with the GC-MS finding of linoleic acid (a polyunsaturated fatty acid), but also a considerable amount of saturated or monounsaturated fatty acids. While a higher iodine value typically correlates with higher oxidative instability, this moderate value points to a balance, making it comparable to oils like olive oil or peanut oil (Warra, 2015). For industrial uses, this moderate unsaturation can offer a good balance of fluidity and oxidative stability, for instance, in lubricant formulations.

The saponification value of 43.39 mg/g is relatively low compared to typical vegetable oils, which often have values above 180 mg/g. This low value suggests a high proportion of non-saponifiable matter (compounds that do not form soaps, such as waxes, sterols, and long-chain hydrocarbons) or a high molecular weight of the fatty acids present. This property, combined with the high FFA, further supports the oil's potential in applications such as **fuel additives** and the manufacture of specialized lubricants, where the presence of non-saponifiable components can impart desirable physical properties like improved viscosity or film strength.

The peroxidation value of 34.63 mg/g indicates a moderate level of primary oxidation products (hydroperoxides) in the oil. This value confirms some degree of oxidative instability, which is consistent with the presence of unsaturated fatty acids like linoleic acid. While high peroxide values are undesirable for edible oils due to rancidity, this characteristic can be beneficial in certain industrial contexts, particularly for applications where controlled oxidation or specific functional properties are desired, such as in drying oils or certain lubricant bases.

Finally, the esterification value of 22.92 mg/g represents the amount of fatty acids present in esterified form (i.e., as triglycerides or other esters). Compared to the high FFA value, this indicates that a significant portion of the fatty acids are free. The moderate esterification value, along with high FFA, further supports the oil's potential for industrial transformations like biodiesel synthesis (via transesterification or esterification of free fatty acids) and soap making.

Phytochemical Analysis of GARHE

The preliminary quantitative phytochemical screening of GARHE oil is presented in Table 3.

Table 3: Phytochemical constituents of *Gmelina arborea* Root Hexane Extract (GARHE) oil

S/N	Parameter	Amount (mg/g)
1	Terpenoids	133.90 ± 4.8
2	Saponins	83.00 ± 0.30
3	Tannins	0.73 ± 0.03
4	Steroids	1.31 ± 0.01
5	Phenols	18.62 ± 0.2

The phytochemical screening of GARHE revealed the presence of five major classes of compounds: terpenoids, saponins, tannins, steroids, and phenols. Notably, alkaloids, flavonoids, phlobatannins, anthraquinones, carotenoids, and glycosides were not detected in this hexane extract. The absence of these more polar compounds is expected given the non-polar nature of hexane as an extraction solvent. While the absence of certain typically pharmacologically active compounds like flavonoids and alkaloids might limit its immediate application as a broad-spectrum pharmacological agent, the presence of others indicates different types of biological or industrial potential.

The terpenoid concentration of 133.90 mg/g is notably high, falling within the range observed in plant waxes and essential oils (50-200 mg/g). Terpenoids are a diverse group of organic compounds derived from isoprene units, known for their characteristic aromas and often exhibiting antimicrobial, anti-inflammatory, and antioxidant properties (Christen et al., 2010). This high content suggests potential applications for GARHE in industries requiring natural flavoring, fragrance, aromatherapy, cosmetics (e.g., perfumery, skin care), or even as a natural insecticidal agent.

The saponin concentration of 83.00 mg/g is considerable and aligns with levels found in other plant-based extracts (1-100 mg/g). Saponins are amphipathic glycosides known for their foam-forming and emulsifying properties, as well as potential anti-

inflammatory and antioxidant activities. Their high concentration suggests the extract could be valuable as a natural surfactant or emulsifying agent in soap making and cosmetic formulations, providing a natural alternative to synthetic detergents.

Phenols were detected at 18.62 mg/g, a concentration consistent with plant-based oils (1-50 mg/g). Phenolic compounds are well-known for their antioxidant properties due to their ability to scavenge free radicals. While the overall antioxidant capacity of this extract might be modest due to the lower concentration compared to highly phenolic extracts, this value suggests it could contribute to mild antioxidant benefits, potentially making the oil useful as a natural preservative in food, cosmetics, or pharmaceuticals to extend shelf life.

The concentrations of tannins (0.73 mg/g) and steroids (1.31 mg/g) were relatively low. Tannins, typically found in the range of 0.1-10 mg/g in plant oils, are known for their astringent and antioxidant properties. The low concentration here suggests minimal contribution to astringency or strong antioxidant activity. Steroids, present at a low level, are important biological molecules that can have various effects, though their low concentration here indicates a limited role in the overall industrial application compared to other components.

4.0 Conclusion

The hexane extract of *Gmelina arborea* root (GARHE) yielded a solidified oil rich in bioactive constituents, notably linoleic acid and terpenoids, alongside industrially relevant compounds such as 2,4-di-tert-butyl-6-nitrophenol. Although two identified phthalate esters are likely contaminants from processing equipment, the overall chemical profile of the extract suggests significant potential for industrial applications. The physicochemical parameters, including high acid and free fatty acid values, moderate iodine and saponification indices, and notable peroxide content, indicate

that the oil may not be ideal for nutritional purposes but holds promise for use in biodiesel production, lubricants, soap, and cosmetic formulations. Phytochemical screening confirmed the presence of beneficial secondary metabolites, further enhancing its value in non-pharmacological applications. Future work should focus on refining the extraction process to eliminate contamination and exploring the bioactivity and toxicological profiles of the extract to support its safe industrial utilization.

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Declaration:**Ethical Approval**

Not Applicable

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Authors' Contribution

Ismaila J. Olawale performed plant collection, extraction, phytochemical and

physicochemical analyses, and drafted the manuscript. Sunday O. Oladoye supervised methodology, guided data interpretation, and revised the work critically. Fatai Afolabi designed the study, coordinated GC-MS analyses, interpreted data, finalized the manuscript, and served as corresponding author.