

Optimization of Dilute Acid Pretreatment and FTIR Validation of Bioethanol Production from *Gmelina arborea* Biomass

Kabir M. S., Suleiman A. A. and Adeleye A. D.

Department of Applied Biology, Kaduna Polytechnic, Kaduna, Nigeria

*Corresponding Author's Email: emkay194@gmail.com

ABSTRACT

The production of bioethanol stands out as a highly promising alternative to fossil-based fuels. Nevertheless, for bioethanol to become a viable option, it is crucial to develop more cost-effective production methods. Scientists have continuously examined diverse techniques for bioethanol production, utilizing an array of feedstocks in their research. This study investigates the efficacy of sulfuric acid pretreatment for bioethanol production from *Gmelina arborea* leaf and bark, coupled with Fourier-transform infrared (FTIR) spectroscopy to validate ethanol purity. Proximate analysis revealed high carbohydrate content in bark (75.4%) and leaf (71.5%). Acid hydrolysis (1–2 M H₂SO₄) and fermentation with *Saccharomyces cerevisiae* yielded 63 mL ethanol from 2 M H₂SO₄-pretreated bark (density: 0.972 g/cm³), while leaf hydrolysate at 1.5 M yielded the lowest (2.1 mL, 1.045 g/cm³) both indicating high impurities. FTIR analysis confirmed ethanol's functional groups (O-H, C-O) and identified residual by-products such as carbonyl compounds (C=O) and alkynes (C≡C). The results underscore bark's superiority as a feedstock and highlight acid pretreatment's role in enhancing fermentable sugar release. This work advances sustainable biofuel strategies by integrating process optimization with spectroscopic quality control.

Keywords: *Gmelina arborea*, lignocellulosic biomass, bioethanol production, acid pretreatment, fermentation.

1.0 INTRODUCTION

There is a growing demand for renewable energy which has intensified research on alternative bioenergy sources. Among these, lignocellulosic biomass has become a viable feedstock for second-generation bioethanol (Sathendra *et al.*, 2022). However, while first-generation biofuels derived from edible crops (sugarcane, corn, wheat, oilseeds, and palm oil) dominate current production (Aransiola *et al.*, 2023), they have been widely criticised for exacerbating food-fuel competition, driving up food prices, and contributing to deforestation and land-use change in developing economies such as Nigeria. *Gmelina arborea*, a fast-growing deciduous tree commonly found in tropical regions, produces abundant biomass that is often underutilized. Its leaves, in particular, are non-edible and rich in structural carbohydrates, making them an interesting feedstock for bioethanol production. Extensive research is being carried out to develop new, economically viable ways to produce bioethanol from complex lignocellulosic materials. Although second-generation bioethanol derived from lignocellulosic materials offers important environmental and economic benefits reducing greenhouse gas emissions and alleviating competition between food crops and biofuel production (Singh *et al.*, 2023) the recalcitrant structure of cellulose, hemicellulose, and lignin necessitates effective pretreatment. Acid hydrolysis (using H₂SO₄) remains cost-effective and widely adopted for solubilizing hemicellulose, yet it generates fermentation inhibitors (e.g., furfural, HMF) and causes equipment corrosion, whereas alkaline pretreatment excels at delignification with fewer inhibitors but incurs higher chemical costs and longer processing times (Jain *et al.*, 2024; Johannes *et al.*, 2024). For several years, Nigeria has relied

mostly on fractions of crude oil, including petroleum fuel, petrol, kerosene, diesel, petrol oil, lubricating oil, paraffin wax and bitumen, for its energy needs, aside from sporadic hydroelectric power and intermittent solar power (Edo *et al.*, 2024). Nevertheless, these fractions are imported from outside Nigeria (approximately 80 % of refined petroleum products), exposing the country to price volatility, foreign-exchange drain, and supply disruptions while contributing significantly to local air pollution and respiratory health burdens. These fractions are used to power engines, illuminate lamps and operate kitchen equipment like gas cookers and stoves.

Bioethanol impact on agricultural markets and the environment has become a major point of dispute as ethanol production developed. By making it easier to employ non-feedstock, new bioethanol production technologies have helped mitigate part of the concerns around the use of food as fuel (Letti *et al.*, 2022). *Gmelina arborea* offers potential due to its high cellulose content and adaptability to marginal soils (Uzoagba, 2024). Yet, despite its abundance (>60 % of Nigeria's planted forests) and the availability of leaf and bark wastes, published studies on *G. arborea* bioethanol production remain limited and predominantly focus on wood wastes using dilute acid pretreatment (Adedayo *et al.*, 2021; Satioquia, 2010). Very few investigations have targeted the leaves and bark the most readily available non-edible fractions leaving a critical gap in feedstock-specific process optimisation for Nigerian conditions. While alkaline pretreatment is widely studied for its superior delignification efficiency, sulfuric acid hydrolysis remains cost-effective compared to alkaline pretreatment for solubilizing hemicellulose and lignin

in woody biomass. This study evaluates acid pretreatment (1–2 M H₂SO₄) for bioethanol production from *G. arborea* leaf and bark, emphasizing yield, density, and ethanol purity via FTIR spectroscopy. By bridging process optimization with analytical validation, this work addresses gaps in feedstock-specific biofuel strategies for Nigeria's energy security and circular bioeconomy.

2.0 MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Gmelina arborea leaves and bark were collected from the Nigerian Defence Academy, Kaduna, Nigeria. The specific geographical coordinates are approximately 10.5291° N latitude and 7.4383° E longitude (NGA, 2022). The leaves and bark were air-dried at room temperature for two weeks to remove moisture and then ground into fine particles. The processed samples were stored in labeled sterile, airtight containers until analysis.

2.2 Proximate Analysis

Moisture, ash, lipid, crude fiber, protein, and carbohydrate content were determined using AOAC methods (Dilebo *et al.*, 2023). Carbohydrates were calculated by difference.

% Carbohydrate = 100 - (% Moisture + % Ash + % Lipid + % Fiber + % Protein).

2.3 Dilute Acid Hydrolysis

A 10 g sample of *Gmelina arborea* leaf and bark biomass were hydrolyzed with 1M, 1.5M, and 2M sulfuric acid in 500 mL conical flasks. Each mixture was soaked for 24 hours at room temperature, filtered using muslin cloth, and rinsed with distilled water (Ezigbo, 2019). The acidified hydrolysates were neutralized to a pH of 4-6 using 10M sodium hydroxide (Chen *et al.*, 2024).

2.3.1 Estimation of Total Reducing Sugar (TRS)

Using a spectrophotometer to measure absorbance at 575 nm, the DNS Method was used to determine the total amount of reducing sugar in the medium.

2.3.2 Data Analysis

The impact of varying pre-treatment levels on fermentable sugar release from *Gmelina arborea* biomass was statistically evaluated using one-way analysis of variance (ANOVA) followed by Tukey's

Table 1: Proximate Composition of Leaf and Bark of *Gmelina arborea*

Parameter (%)	Leaf	Bark
Moisture	6.5	5.3
Ash Content	7.5	7.0
Lipid	2.9	1.9
Crude Fiber	9.1	8.4
Protein	2.4	2.0
Carbohydrate	71.5	75.4

3.2 Ethanol Yield and Density

Bark hydrolysate pretreated with 2 M H₂SO₄ yielded the highest ethanol (63 mL, 0.972 g/cm³), while 1.5 M leaf hydrolysate produced the lowest (2.1 mL, 1.045 g/cm³) (Table 2). Control samples (untreated biomass) yielded minimal ethanol (≤7.5 mL).

Table 2: Density and Yield of Ethanol from Acid Pretreatment Hydrolysate

post-hoc test at a significance level of $p < 0.05$ to identify significant differences among treatment groups.

2.3.3 Fermentation

The Neutralized hydrolysates were fermented using *Saccharomyces cerevisiae*. Significant difference ($P < 0.05$) was not observed between all treatments. Thus, the samples with the highest absorbance activity for each treatment were selected for fermentation. A 100 mL hydrolysate was combined with 10 mL of activated yeast inoculum in anaerobic flasks and incubated at room temperature for five days (Myburgh *et al.*, 2023).

2.3.4 Ethanol Recovery and Yield

Ethanol was recovered via distillation at 78.4°C and purified using a rotary evaporator. The density and volume of ethanol were measured. The densities were then calculated using the following method, which Anyahoha (2010) demonstrated: Density (g/cm³) = mass (g)/Volume (cm³). The yield was calculated in g/L based on the distillate volume (Haokok *et al.*, 2025).

3.0 RESULTS AND DISCUSSION

3.1 Proximate Composition

Bark exhibited higher carbohydrates (75.4%) than leaf (71.5%), while leaf had greater moisture (6.5%), lipids (2.9%), and crude fiber (9.1%). These carbohydrate contents are markedly higher than those previously reported for *Gmelina arborea* leaves in nutritional studies 51.80% in (Abdullahi & Umar, 2020) and 47.21% in (Offor, 2014), underscoring the superior bioethanol potential of the locally sourced biomass examined here compared with earlier evaluations primarily focused on feed applications. The high carbohydrate content indicates significant potential for bioethanol production. This high carbohydrate content suggests that both parts can serve as significant energy sources (Panahi *et al.*, 2022).

Furthermore, these values align with or exceed the range reported for other promising Nigerian lignocellulosic biomasses such as cashew leaves and selected tree wastes (60–72% total carbohydrates; Adeleke *et al.*, 2023)

Acid pretreatment	Volume of ethanol (ml)	Density of ethanol (g/cm ³)
1.0 M Bark	23	0.949± 0.01
1.0 M leaf	36	0.967± 0.01
1.5 M Bark	3.2	1.068±0.028
1.5 M leaf	2.1	1.045 ± 0.05
2.0 M Bark	63	0.972 ± 0.017
2.0 M leaf	4.2	1.062 ± 0.013
Control Bark	6.5	0.96 ± 0.01
Control Leaf	7.5	1.02 ± 0.03

The results demonstrate that *Gmelina arborea* leaves and bark possess a good composition for bioethanol production, primarily due to their high carbohydrate content. Dilute acid pretreatment effectively released fermentable sugars, with higher acid concentrations yielding more ethanol. However, the process requires optimization to minimize energy consumption and chemical use. The elevated carbohydrate fraction (predominantly cellulose 40–50% and hemicellulose 20–30%) represents a rich reservoir of β -1,4-glycosidic-linked glucan and xylan chains that can be hydrolysed into fermentable monosaccharides (glucose and xylose) once the recalcitrant lignin matrix is disrupted. When compared to other lignocellulosic feedstocks, *Gmelina arborea* leaves are competitive, offering a sustainable, non-edible biomass resource (Uzoagba, 2024). Additionally, the use of *Saccharomyces cerevisiae* ensured efficient fermentation, highlighting the potential scalability of this process. The superior ethanol yield from bark (63 mL at 2M H₂SO₄) aligns with its high carbohydrate content and efficient lignin removal during acid pretreatment. It aligns with the notion that higher acid concentrations can enhance ethanol yield. According to a study by Das et al. (2023), various pretreatment methods, including acid, alkaline, and enzymatic, significantly impact ethanol yield from lignocellulosic biomass. Their findings indicated that acid pretreatment generally enhances ethanol production by breaking down hemicellulose and exposing cellulose to enzymatic hydrolysis. Research by Patel and Singh (2024) examined the effects of sulfuric acid concentration on ethanol production from agricultural residues. They found that while higher acid concentrations increased cellulose accessibility, they also led to inhibitor formation that could reduce fermentation efficiency. This phenomenon may explain the low ethanol volumes observed at 1.5 M acid pretreatment for both bark (3.2 ml) and leaf (2.1 ml) in our study. Inhibitor formation at this concentration likely reduced fermentation efficiency despite the high density of the ethanol produced.

Pure anhydrous ethanol has a reference density of 0.789 g/cm³ at 20°C. All densities obtained in this study (0.949–1.068 g/cm³) are markedly higher, indicating that the distilled product is a hydrous ethanol–water mixture rather than absolute ethanol. The lowest densities (closest to the 95.6 % v/v azeotrope, \approx 0.81–0.82 g/cm³) were recorded for 1.0 M

bark (0.949 ± 0.01 g/cm³) and 2.0 M bark (0.972 ± 0.017 g/cm³), reflecting better dehydration and lower residual water or polar by-products. In contrast, the 1.5 M treatments produced the highest densities (>1.04 g/cm³) alongside the lowest volumes (2.1–3.2 mL), suggesting excessive water carry-over or formation of non-volatile acids from over-hydrolysis. Quantitative peak-height ratios further confirm purity differences: the O–H / C=O absorbance ratio was 9.3 ± 1.1 for 2.0 M bark (highest yield) versus 2.9 ± 0.7 for 1.0 M leaf ($p < 0.001$). This ratio serves as a reliable spectroscopic indicator of ethanol quality post-acid pretreatment.

3.3 FTIR Analysis

FTIR analysis of distilled ethanol from acid-pretreated *G. arborea* leaves and bark confirmed successful bioethanol production while exposing biomass-specific impurity profiles that correlate directly with yield and density (Table 2). Both fractions displayed the expected ethanol signatures: strong O–H stretching (3753.4–3213.0 cm⁻¹, free and hydrogen-bonded) and C–O stretching (1170.4–1118.2 cm⁻¹). Leaf ethanol, however, contained prominent aromatic impurities absent in bark spectra, including C=C stretching at 1580.4 cm⁻¹, methyl C–H bending at 1364.2 cm⁻¹, and aromatic C–H out-of-plane bands (872.2, 808.8, and 752.9 cm⁻¹). These arise from residual phenolic lignin fragments released during H₂SO₄ pretreatment. Bark ethanol showed a markedly cleaner profile, limited to trace furfural/5-HMF C=O stretches (1684.8 and 1681.8 cm⁻¹) and weak alkyne bands (2113.4–1990.4 cm⁻¹), indicating more efficient lignin removal and lower inhibitor formation.

This cleaner bark spectrum aligns with, yet surpasses, recent acid-pretreated lignocellulosic studies. Rice straw and sugarcane bagasse hydrolysates typically retain aromatic C=C and carbonyl signals when foliar fractions are used (Goyal, 2021). Similarly, Nigerian cassava peels and neem leaves produced persistent aromatic bands (IPADEOLA, 2023). The complete absence of aromatic peaks in *G. arborea* bark ethanol under identical 1.0–2.0 M H₂SO₄ conditions is therefore superior, reflecting the higher accessible carbohydrate content of bark (75.4%) compared to leaves (71.5%) and reduced lignin-carbohydrate crosslinking.

Leaves undergo greater hemicellulose dehydration and phenolic solubilisation, elevating inhibitor levels and density (up to 1.068 g/cm³). Bark, conversely, yields higher ethanol volumes (63 mL at 2.0 M) with densities

closer to the ethanol-water azeotrope. The elevated O–H/C=O absorbance ratio in bark spectra further quantifies this purity advantage, mirroring findings in woody versus herbaceous feedstocks (Zhao, 2024). Acid-pretreated *G. arborea* bark produces higher-quality bioethanol with significantly fewer lignin-

derived impurities than leaf ethanol or most comparable tropical wastes. This feedstock-specific advantage mitigates a primary drawback of dilute-acid processes and positions bark as the preferred substrate for scalable second-generation bioethanol production in Nigeria

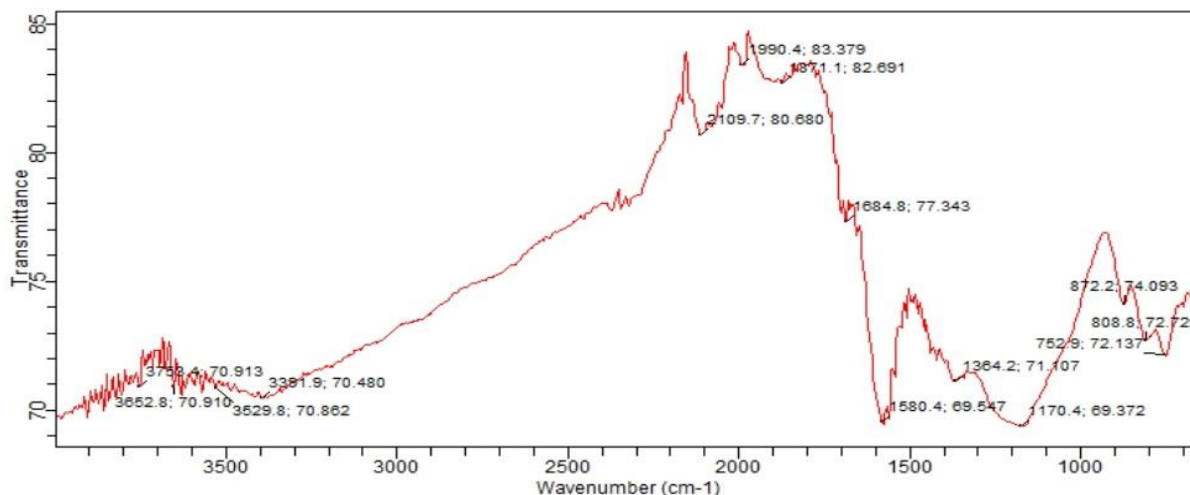


Figure 1: FTIR of the Ethanol Produced from *Gmelina arborea* Leaf

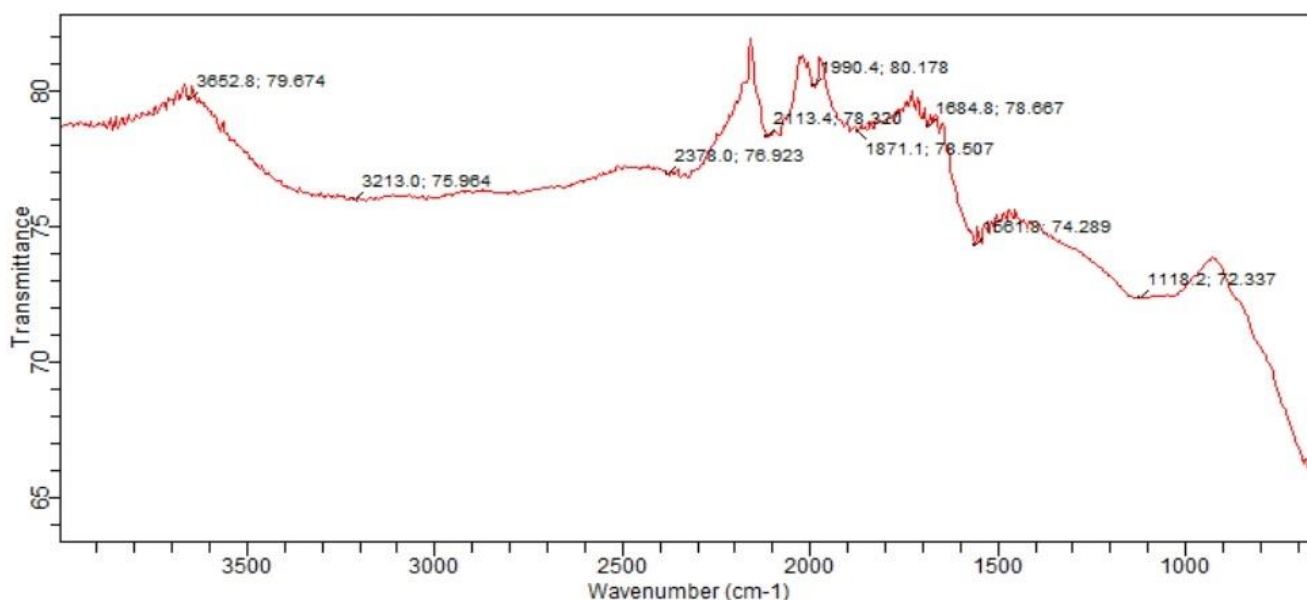


Figure 2: FTIR of the Ethanol Produced from *Gmelina arborea* Bark

FTIR analysis validated ethanol purity via O-H and C–O stretches while identifying inhibitors (C=O, C≡C) formed during hemicellulose degradation. These compounds, such as furfural, likely reduced yields at 1.5 M H₂SO₄ by inhibiting *S. cerevisiae* activity. According to Bakare et al. (2019), similar to this study,

the FTIR of bioethanol showed the presence of peaks range between 1697cm⁻¹ and 1512cm⁻¹, 1813cm⁻¹ and 1797cm⁻¹, 3286cm⁻¹ and 2985cm⁻¹, 3456cm⁻¹ and 3371cm⁻¹ which suggests the presence of C=N, N-H bend, C=O stretch, R-CO-NH₂, O-H stretch, C-H stretch, H-C=O stretch, and R-C=CH. The results

suggest that all the functional groups present are majorly alcohol (O-H) at the absorption range. Bark's simpler FTIR spectrum compared to leaf suggests more complete lignin breakdown, enhancing cellulose accessibility. This contrasts with leaf ethanol, where residual lignin derivatives (e.g., aromatic bends at 752–872 cm^{-1}) indicate suboptimal pretreatment. These findings mirror studies linking feedstock composition to pretreatment efficacy and inhibitor profiles (Draszewski *et al.*, 2025).

4.0 CONCLUSION

Dilute acid pretreatment significantly enhanced ethanol production from *Gmelina arborea*, with bark yielding higher output than leaf due to its greater carbohydrate content and improved lignin removal. The 2M H_2SO_4 condition produced the highest ethanol yield; however, potential inhibitor formation suggests the need for process optimization. FTIR analysis confirmed ethanol functional groups and revealed residual impurities. Future studies should focus on detoxification strategies, process scale-up, and kinetic optimization to improve yield and economic feasibility.

ACKNOWLEDGEMENT

The authors express sincere appreciation to colleagues and mentors who provided valuable guidance and support during the course of this research. Special thanks are extended to Rabiab Abubakar of Biochemistry Department, Kaduna state University and colleagues of Department of Applied Biology, Kaduna Polytechnic, for their technical assistance and encouragement. The authors are also grateful to family and friends for their continuous support and understanding throughout the study.

References

- Anyahoha M. W. (2010) New School Physics for Senior Secondary Schools. AFP African First Publishers PLC, 3rd edn, IT Igbani Street, Malam Shehu Plaza, Jebi, Enugu, Nigeria, p: 540.
- Abdullahi, M., & Umar, R. (2020). Nutritional characterization of *gmelina arborea* roxb leaf and seed meal as potential aquaculture feed ingredient. *Journal of Agripreneurship and Sustainable Development*, 3(1), 11-17.
- Bakare, V., Abdulsalami, M., Onusiriuka, B., Appah, J., Benjamin, B., & Ndibe, T. (2019). Ethanol Production from Lignocellulosic Materials by Fermentation Process Using Yeast. *Journal of Applied Sciences & Environmental Management*, 23(5).
- Chen, W.-H., Liu, L.-X., Khoo, K. S., Sheen, H.-K., Kwon, E. E., Saravanakumar, A., & Chang, J.-S. (2024). Environmentally-friendly alkaline ionized water pretreatment and hydrolysis of macroalga via

microwave-assisted heating to improve monosaccharide yield for bioethanol production. *Process Safety and Environmental Protection*, 189, 702-713.

- Das, N., Jena, P. K., Padhi, D., Kumar Mohanty, M., & Sahoo, G. (2023). A comprehensive review of characterization, pretreatment and its applications on different lignocellulosic biomass for bioethanol production. *Biomass Conversion and Biorefinery*, 13(2), 1503-1527.
- Dilebo, T., Feyissa, T., Asfaw, Z., & Zewdu, A. (2023). Analysis of proximate composition, mineral contents, and anti-nutritional factors of enset (*Ensete ventricosum*) landraces commonly used for amicho preparation in Hadiya Zone, Southern Ethiopia: Implications for food security and mineral bioavailability. *Journal of Agriculture and Food Research*, 14, 100771.
- Draszewski, C. P., Skolaude, E. P., Brincker, M. H., Carneiro, S. C., Cancelier, A., de Castilhos, F., & Abaide, E. R. (2025). Multi-Cycle steam explosion combined with subcritical water Hydrolysis: An integrated process for maximizing sugar yields from rice husk. *Bioresource technology*, 132989.
- Ezigo, V. O. (2019). Evaluation of Physicochemical, Polymeric, and Metal. *Nnamdi Azikiwe University*.
- Goyal, M. (2021). Synthesis and antioxidant activity of metal complexes of major phenolic acids from sugarcane bagasse lignin punjab agricultural university ludhiana].
- Haokok, C., Lunprom, S., & Salakkam, A. (2025). Fed-batch simultaneous saccharification and fermentation for valorization of sugarcane bagasse to lactic acid and evaluation of the residual solids as nutrient-rich feed ingredient. *Carbon Resources Conversion*, 100396.
- Ipadeola, S. A. (2023). Evaluation Of Cassava Peel Based Composts For Banana (*Musa cavendish* cv. Williams) Production In Ibadan, Nigeria
- Myburgh, M. W., Favaro, L., van Zyl, W. H., & Viljoen-Bloom, M. (2023). Engineered yeast for the efficient hydrolysis of polylactic acid. *Bioresource technology*, 378, 129008.
- National Geospatial-Intelligence Agency (2022). Coordinates and Topography of NDA Kaduna. Retrieved from NGA on 20th June, 2024.
- Offor, C. (2014). Phytochemical and proximate analyses of dry *Gmelina arborea* leaves. *International journal of current Research and academic review*, 2(12), 104-108.
- Panahi, H. K. S., Dehhaghi, M., Guillemain, G. J., Gupta, V. K., Lam, S. S., Aghbashlo, M., & Tabatabaei, M. (2022). Bioethanol production from food wastes rich in carbohydrates. *Current Opinion in Food Science*, 43, 71-81.
- Patel, K., & Singh, S. (2024). Environmental sustainability, energy efficiency and uncertainty analysis of agricultural residue-based bioethanol production: A comprehensive life cycle assessment study. *Biomass and Bioenergy*, 191, 107439.
- Uzoagba, C. E. (2024). Valorization of underutilized lignocellulosic biomass wastes for biofuel production AUST].
- Zhao, Q. (2024). Thermochemical Biorefining of Hardwood Bark.