

GC-MS PROFILING, PHYSICOCHEMICAL CHARACTERIZATION, NMR SPECTROSCOPY AND ANTIBACTERIAL ACTIVITY OF *ANACARDIUM OCCIDENTALE* L. SEED OIL

Olaniyi Edward Famobuwa*, Babatunde Moses Ojo, Enoch Adedayo Adekunbi, Omokehinde Oseni Akinlami, Oluwasetemi Faith Adedigba

Department of Chemistry, Adeyemi Federal University of Education Ondo Nigeria.

Article Info: Received: 15 August 2026, Revised: 05 September 2026, Accepted: 25 September 2026

***Corresponding Author: Olaniyi Edward Famobuwa**

Department of Chemistry, Adeyemi Federal University of Education Ondo Nigeria.

DOI: <https://doi.org/10.5281/zenodo.23065942>



Citation:

Olaniyi Edward Famobuwa*, Babatunde Moses Ojo, Enoch Adedayo Adekunbi, Omokehinde Oseni Akinlami, Oluwasetemi Faith Adedigba. (2026). Gc-ms Profiling, Physicochemical Characterization, Nmr Spectroscopy And Antibacterial Activity Of *Anacardium Occidentale* l. Seed Oil. International Journal of Clinical and Pharmaceutical Innovations, 1(7), 69-74.

ABSTRACT

This study characterized seed oil from *Anacardium occidentale* L. using physicochemical measurements, gas chromatography–mass spectrometry (GC-MS), proton and carbon-13 nuclear magnetic resonance (^1H and ^{13}C NMR) spectroscopy, and antibacterial screening. The oil had an acid value of 5.4 ± 0.6 mg KOH/g, saponification value of 187.4 ± 2.7 mg KOH/g, iodine value of 78.9 ± 1.5 g I₂/100 g, peroxide value of 6.0 ± 1.2 meq O₂/kg, specific gravity of 0.96 ± 0.13 and refractive index of 1.481 ± 0.01 at 40°C. GC-MS profiling demonstrated a complex lipid composition comprising saturated and monounsaturated fatty-acid methyl esters together with minor phenolic, sterol and triterpenoid constituents. Prominent assignments included methyl palmitate (10.153%), methyl 6-octadecenoate (9.9453%), methyl cis-11-eicosenoate (5.0128%), methyl stearate (7.4483%) and methyl oleate. The dataset also contained phenolic lipids, 2-hydroxy-1-(hydroxymethyl)ethyl oleate, squalene, γ -sitosterol and β -amyrin. The ^1H and ^{13}C NMR spectra were consistent with a lipid-rich matrix containing long-chain aliphatic, olefinic, glycerol and ester-carbonyl environments. Antibacterial screening showed the greatest inhibition against *Pseudomonas syringae* (12.50 ± 1.32 mm), followed by *Ralstonia solanacearum* (6.50 ± 1.67 mm). The results indicate that *A. occidentale* seed oil is a chemically diverse plant lipid with potential nutritional, oleochemical and biological relevance.

KEYWORDS: *Anacardium occidentale*; seed oil; GC-MS; NMR; fatty acids; physicochemical properties; antibacterial activity.

INTRODUCTION

Plant seed oils are complex mixtures dominated by triacylglycerols and containing free fatty acids, monoacylglycerols, phytosterols, hydrocarbons, phenolic lipids and other minor constituents. Their composition influences nutritional quality, oxidative stability, processing behaviour and potential industrial applications. GC-MS is widely used for characterization of fatty acids and other oil constituents, particularly after

derivatization, while NMR provides complementary information on the structural environments of lipid molecules.^[1,2]

Anacardium occidentale L. (cashew), family Anacardiaceae, is an economically important tropical plant. Research on the species has documented diverse phytochemicals and antimicrobial properties, although much of the recent antimicrobial literature concerns

leaves, bark or other extracts rather than seed oil.^[4,5] This creates a rationale for characterization of the seed oil as an independent plant-lipid resource.

The present study was therefore designed to characterize *A. occidentale* seed oil by combining physicochemical measurements with GC-MS and NMR spectroscopy and to assess its antibacterial activity against selected test organisms. The study deliberately focuses on *A. occidentale* alone, thereby providing a self-contained chemical and biological profile.

MATERIALS AND METHODS

Plant material and oil sample

Mature *A. occidentale* seeds were collected in southwestern Nigeria, cleaned, dried and processed before oil extraction. The resulting oil was stored in a sealed container until analysis. The sample used for NMR analysis was designated A2.

Oil extraction

The seed material was subjected to the solvent-extraction procedure used in the study, after which the solvent was removed to obtain the seed oil.

Physicochemical analysis

Acid value, saponification value, iodine value, peroxide value, specific gravity and refractive index were determined using standard procedures. Results are reported as mean $\pm$ standard deviation.

GC-MS analysis

The oil was derivatized for GC-MS analysis of fatty-acid constituents. Constituents were tentatively identified by

comparison of acquired mass spectra with spectral-library entries. The master dataset reports retention time, molecular formula, molecular mass, chromatographic area percentage and library-match quality. In accordance with accepted GC-MS reporting practice, library matches are treated as tentative identifications unless confirmed by authentic standards or orthogonal spectroscopic evidence.^[1]

NMR spectroscopy

The *A. occidentale* sample was analysed in CDCl₃ at 25 °C on an Agilent VNMR400 spectrometer. The ¹H spectrum was acquired on 26 July 2026 and the ¹³C spectrum under the same solvent/temperature conditions. The ¹³C dataset contained resonances from approximately 14 ppm to 179.6 ppm, including extensive aliphatic signals, oxygenated carbons, unsaturated carbons and ester/carboxyl carbonyl signals.

Antibacterial assay

Antibacterial activity was evaluated by the agar-diffusion procedure used in the study. Ciprofloxacin (500 mg preparation) served as the reference antibiotic. Zones of inhibition were measured in millimetres and reported as mean $\pm$ standard deviation. Because diffusion of hydrophobic oils through agar can be limited, inhibition-zone results are interpreted as screening data and not as direct equivalents of MIC values.^[3]

Statistical analysis

Results are presented as mean $\pm$ standard deviation. Where inferential testing was performed, $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Table 1: Physicochemical properties of *A. occidentale* seed oil.

Parameter	Result
Acid value (mg KOH/g)	5.4 $\pm$ 0.6
Saponification value (mg KOH/g)	187.4 $\pm$ 2.7
Iodine value (g I ₂ /100 g)	78.9 $\pm$ 1.5
Peroxide value (meq O ₂ /kg)	6.0 $\pm$ 1.2
Specific gravity	0.96 $\pm$ 0.13
Refractive index (40 °C)	1.481 $\pm$ 0.01

The acid value indicates measurable free fatty-acid content, while the saponification value is consistent with an oil containing predominantly long-chain fatty-acyl groups. The iodine value indicates moderate unsaturation and agrees with the GC-MS detection of several monounsaturated constituents. The peroxide value

indicates the presence of primary oxidation products but does not, by itself, establish advanced rancidity. Such parameters are strongly affected by seed maturity, extraction, handling and storage and was therefore be interpreted for the particular sample analysed.

GC-MS composition

Table 2: Principal and diagnostically useful GC-MS assignments in *A. occidentale* seed oil.

RT (min)	Compound	Formula	MW	Area %	Quality
10.614	Methyl tetradecanoate	C ₁₅ H ₃₀ O ₂	242.40	0.0396	98
11.7103	Methyl pentadecanoate	C ₁₆ H ₃₂ O ₂	256.43	0.0277	97
12.6662	Methyl 9-hexadecenoate	C ₁₇ H ₃₂ O ₂	268.44	0.6611	98
13.109	Methyl hexadecanoate (methyl palmitate)	C ₁₇ H ₃₄ O ₂	270.46	10.153	97
15.9519	Methyl oleate (9-octadecenoate, Z)	C ₁₉ H ₃₆ O ₂	296.49	41.4671	99

16.0963	Methyl 6-octadecenoate	C ₁₉ H ₃₆ O ₂	296.49	9.9453	99
16.3287	Methyl stearate	C ₁₉ H ₃₈ O ₂	298.51	7.4483	99
17.9751	Linoleic acid	C ₁₈ H ₃₂ O ₂	280.45	0.1484	95
18.4168	Methyl cis-11-eicosenoate	C ₂₁ H ₄₀ O ₂	324.55	5.0128	99
20.9503	(Z)-3-(pentadec-8-en-1-yl)phenol	C ₂₁ H ₃₄ O	302.50	5.2745	89
23.9389	2-Hydroxy-1-(hydroxymethyl)ethyl oleate	C ₂₁ H ₄₀ O ₄	356.54	0.9525	99
25.5973	Squalene	C ₃₀ H ₅₀	410.73	0.0681	98
31.3741	γ-Sitosterol	C ₂₉ H ₅₀ O	414.72	0.4584	99
31.5513	β-Amyrin	C ₃₀ H ₅₀ O	426.72	0.0909	97

The GC-MS profile is dominated by fatty-acid derivatives, with methyl oleate, methyl palmitate, methyl 6-octadecenoate and methyl stearate among the most prominent assignments. The presence of multiple monounsaturated components is consistent with the measured iodine value. The occurrence of phenolic lipids is also noteworthy because such compounds are characteristic minor constituents of some Anacardiaceae-derived lipid materials and may contribute to biological activity.

The detection of γ-sitosterol, squalene and β-amyrin indicates an unsaponifiable fraction in addition to the major glyceride-derived fatty-acid components. GC-MS of seed and vegetable oils is particularly useful for simultaneous observation of fatty acids, phytosterols and other minor constituents.^[1] In GC-MS analysis of oils, it is common practice to consider a measured RI to “match” the literature value if the difference (ΔRI) is within ±10 units; this tolerance threshold is supported by studies of this seed oil.^[9]

NMR characterization

Table 3: Representative interpretation of the A2 ¹³C NMR spectrum.

Observed ¹³ C region (ppm)	Interpretation
~14	Terminal methyl carbons
22–37	Long-chain aliphatic CH ₂ /CH carbons
62–69	Oxygenated glycerol carbons
110–135	Unsaturated and minor aromatic/phenolic carbon environments
172–180	Ester/free fatty-acid carbonyl carbons

The ¹H NMR spectrum showed the characteristic aliphatic envelope of long-chain fatty-acyl groups, signals in the olefinic region consistent with unsaturated chains, and resonances associated with glyceride ester

environments. The ¹³C spectrum contained extensive aliphatic resonances, oxygenated carbons and carbonyl carbons. These observations are consistent with the GC-MS evidence for a fatty-acid-rich oil.

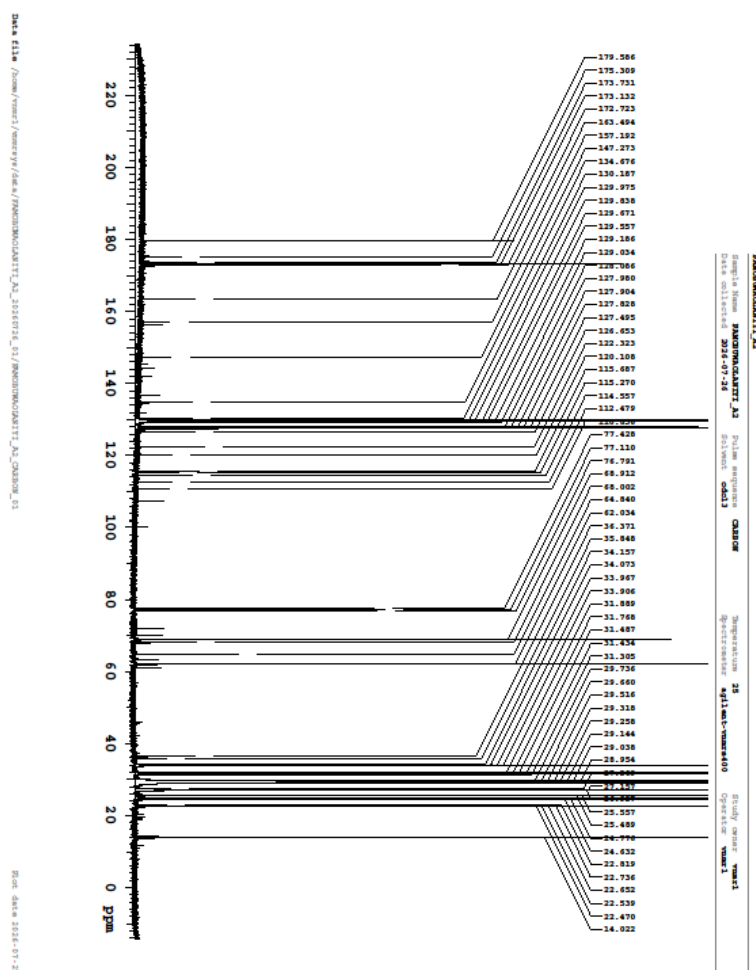
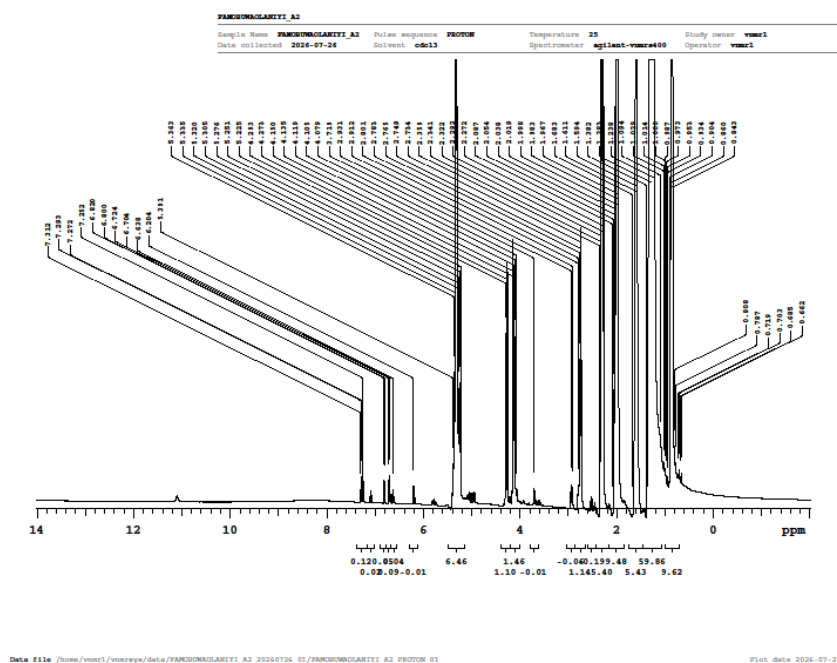
Antibacterial activity

Table 4: Antibacterial activity of A. occidentale seed oil.

Organism	A. occidentale oil (mm)	Ciprofloxacin (mm)
Pseudomonas syringae	12.50 ± 1.32	28.00
Salmonella typhi	5.62 ± 2.13	28.00
Bacillus cereus	3.50 ± 3.34	30.00
Escherichia coli	5.50 ± 1.87	26.00
Staphylococcus aureus	2.75 ± 1.36	34.00
Streptococcus faecalis	2.17 ± 1.32	28.00
Ralstonia solanacearum	6.50 ± 1.67	30.00

The largest inhibition zone was observed against P. syringae (12.50 ± 1.32 mm), followed by R. solanacearum (6.50 ± 1.67 mm). The activity was lower than that of ciprofloxacin in every assay. The observed activity may reflect additive or synergistic effects among fatty acids and minor constituents.^[10] Recent studies have

demonstrated antimicrobial properties of A. occidentale extracts, supporting continued investigation of the species as a source of antibacterial metabolites.^[4,5] Nevertheless, MIC/MBC assays are required before claims of strong antibacterial potency or therapeutic usefulness can be made.^[3]



CONCLUSION

A. occidentale seed oil exhibited moderate unsaturation, measurable oxidative status and a diverse chemical profile dominated by saturated and monounsaturated fatty-acid derivatives. GC-MS also indicated phenolic lipids, phytosterol, squalene and triterpenoid constituents, while CNMR supported the presence of long-chain lipid and ester-carbonyl environments. The oil showed measurable antibacterial activity, particularly against *P. syringae*. These findings support further investigation of the oil as a potential nutritional and oleochemical resource and justify mechanistic antimicrobial studies using MIC, MBC and purified constituents.

Author contributions

Famobuwa OE.: conceptualization, data analysis, proofreading, Adekunbi EA.: experiments, literature writing, Ojo BM.: manuscript writing, data analysis, plant materials, AkinlamiOO: literature writing, and experiments.

Funding

There was no funding received.

Data availability

All data generated or analyzed are available within the manuscript.

Ethics Declarations

Ethics approval

Not applicable, as the study does not pertain to any studies involving humans or animals.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interest

The authors declare no competing interests.

Plant ethics statement

The fruits comprising the pulp and the seed, were gathered from Igele Market in Ondo, Ondo State, Nigeria. The plant' fruits grown in Bolorunduro area of Ondo East local government council were harvested on the day of purchase (20th May, 2026). The plant materials were identified by Dr. R.O. Sanni of the Department of Botany of Adeyemi Federal University of Education and a voucher specimen was deposited at AFUED herbarium. The collection of the plants used in this study complies with local or national guidelines.

REFERENCES

1. Mota MFS; Waktola HD; Nolvachai Y; Marriott PJ. Gas chromatography–mass spectrometry for characterisation, assessment of quality and authentication of seed and vegetable oils. *TrAC T. Anal. Chem.*, **2021**; 143: 116238. doi:10.1016/j.trac.2021.116238.
2. Muhammad Abdul Rahim; Hudda Ayub; AqeelaSehrish; Saadia Ambreen; Faima Atta Khan; Nizwa Itrat; Anum Nazir; Aurbab Shoukat; Amna Shoukat; Afaf Ejaz; Fatin Ozogul; Elena Bartkiene; Joao Miguel Rocha. Essential components from plant source oils: a review on extraction, detection, identification, and quantification. *Molecules*, **2023**; 28: 6881. doi:10.3390/molecules28196881.
3. Hulankova R. Methods for determination of antimicrobial activity of essential oils in vitro—A review. *Plants*, **2024**; 13: 2784. doi:10.3390/plants13192784.
4. Osungunna MO; Akinwumi FO; Odediran SA; Onifade DI; Imokhai AO; Olaoye HA; Ologbenla VA; Omotayo NC. Evaluation of antimicrobial activity of *Anacardium occidentale* leaf extracts against multiple antibiotic resistant bacterial isolates. *Discover Bacteria*, **2025**; 2: 3. doi:10.1007/s44351-025-00010-0.
5. Extraction of *Anacardium occidentale*: a kinetic, thermodynamic, phytochemical identification, and antibacterial study. *Food Bioscience*, **2024**; 62: 105336. doi:10.1016/j.fbio.2024.105336.
6. Dagaci MZ; Hassan LG; Umar KJ; Paiko YB; Lakan II. Physicochemical analysis of *Blighia sapida* (Sapindaceae) seed oil from Zago, Agaie Local Government Area of Niger State. *Jewel Journal of Scientific Research*, **2025**.
7. Tsado DB; Muhammad NM; Ahmed IY; Olusanya JJ. Physicochemical properties and fatty acid profiles of oils from *Blighia sapida* aril, *Dacryodes edulis* pulp, and *Cyperus esculentus* tubers. *J. Chem. A. Sci.*, **2025**; 1(2): 1–13. doi:10.60787/jcas.vol1no2.56.
8. Olumoroti IA; Oyelaran AO; Bolaji B. Production and characterization of ackee apple (*Blighia sapida*) seeds and African star apple (*Chrysophyllum albidum*) seeds oil mixtures and their biodiesel. *ABUAD J. Eng. Res. Dev.*, **2024**; 7(1). doi:10.53982/ajerd.2024.0701.10-j.
9. Achraf khaldi; Kheira mehdi; Amina mazeri; Abdelkader Elhadj Berrezig. Comparative gc-ms analysis of *Anvillea radiata* coss & dur and *Cotula cinerea* del essential oils and their mixture: chemical profiling and classification. *Bull. Chem. Soc. Ethiop.*, **2026**; 40(7): 1501-1513.
10. Tran Phuong Ha; Phan Thi Diem Tran; Vu Thi Thanh Tam; Ton That Huu Dat; Le Canh Viet; Cuong. Chemical composition and Biological activities of *Illicium parvifolium* Leaf Essential oil. *Bull. Chem. Soc. Ethiop.*, **2026**; 40(10): 2255-2264.
11. AOAC International. Official Methods of Analysis of AOAC International. 22nd ed. Rockville, MD: AOAC International; **2023**.
12. ISO 660:2020. Animal and vegetable fats and oils—Determination of acid value and acidity.
13. ISO 3961:2018. Animal and vegetable fats and oils—Determination of iodine value.

14. ISO 3960:2017. Animal and vegetable fats and oils—Determination of peroxide value.
15. ISO 3657:2020. Animal and vegetable fats and oils—Determination of saponification value.
16. ISO 6885:2016. Animal and vegetable fats and oils—Determination of anisidine value.
17. World Health Organization. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues. Geneva: WHO.
18. Famobuwa, O.E., Adekunbi, E.A., Ojo, B.M. and Ogungbemi, T.S (2025). Comparative physicochemical properties of Anacardium occidentale and Blighia sapida seed oils. European Journal of Pharmaceutical and Medical Research, 12(9): 202-206.