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Phytochemical Characterisation and Antimicrobial Activity of *Azadirachta indica* (Neem) Leaf Oil: GC–MS Profiling, FTIR Analysis, and Fumigant Efficacy Against Airborne Microorganisms

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ABSTRACT: *Neem (Azadirachta indica A.Juss.) is among the most medicinally important trees in tropical pharmacopoeia, with documented antibacterial, antifungal, anti-inflammatory, and insecticidal properties attributable to its rich phytochemical content. This study investigated the extraction yield, chemical composition, functional group profile, and fumigant antimicrobial activity of neem leaf oil obtained by Soxhlet extraction with n-hexane. Fifty grams of shade-dried, pulverised neem leaves yielded an average of 7.93 ± 0.30% (w/w) oil across three replicates. Gas Chromatography–Mass Spectrometry (GC–MS) analysis identified 14 compounds, with α-D-glucopyranose (21.13%), 9,12-octadecadienoic acid/linoleic acid (17.98%), n-hexadecanoic acid/palmitic acid (10.27%), octadecanoic acid/stearic acid (8.25%), and oleic acid (7.17%) as the predominant constituents. Fourier Transform Infrared (FTIR) spectroscopy confirmed the presence of ester carbonyl groups, long-chain aliphatic C–H bonds, and hydroxyl/alcohol functionalities, consistent with a fatty acid-rich oil profile. Neem smoke from 50 g of dried leaves completely eliminated airborne bacteria from baseline counts of 15, 9, and 3 CFU/m³ in the Microbiology Laboratory, Preparatory Room, and Instrumentation Room, respectively to zero viable growth post-fumigation. These findings validate the traditional use of neem as a natural disinfectant and confirm the pharmacological significance of its leaf oil.*

Keywords: Azadirachta indica, neem leaf oil, GC–MS, FTIR, antimicrobial, fumigation, fatty acids, phytochemistry

Introduction

Azadirachta indica A.Juss. (family Meliaceae), commonly called neem, Indian lilac, or nimtree, is a fast-growing tropical evergreen indigenous to the Indian subcontinent and naturalised across sub-Saharan Africa, Southeast Asia, and parts of the Middle East (Dhama et al., 2023). It can attain heights of 15–40 metres with a spreading crown diameter of up to 25 metres, and is noted for its tolerance of extreme heat and low rainfall of as little as 18 inches per year (UNEP, 2022). Every part of the tree — leaves, bark, roots, flowers, fruits, and seeds harbours biologically active compounds, earning it the designation of a complete pharmacy in traditional medicine (Biswas et al., 2020).

The pharmacological use of neem predates written records. The Indus civilisation employed neem therapeutically as far back as 4,500 years during the Harappan period, and the plant features prominently in Ayurvedic, Unani, and Homoeopathic systems of medicine (Bandyopadhyay et al., 2020; Kumar and Navaratnam, 2023). Historical manuscripts document its application as an antiseptic against smallpox and other infectious diseases, as well as its utility in treating gastrointestinal disorders, skin conditions, rheumatism, and parasitic infestations. These traditional accounts are increasingly supported by contemporary phytochemical and pharmacological research.

Neem leaf oil, obtained principally through solvent extraction, is a rich reservoir of bioactive molecules. Phytochemical investigations using Gas Chromatography–Mass Spectrometry (GC–MS) have consistently identified a profile dominated by fatty acids notably linoleic, oleic, palmitic, and stearic acids alongside aldehydes, esters, and sugar derivatives (Javed et al., 2021). Linoleic and oleic acids are well-documented modulators of inflammatory pathways and oxidative stress, while palmitic acid contributes to membrane stability and exhibits intrinsic antibacterial properties (Javed et al., 2021). Beyond their individual activities, these compounds may act synergistically to produce the broad-spectrum biological effects attributed to neem leaf preparations.

Complementary characterisation by Fourier Transform Infrared (FTIR) spectroscopy allows identification of the key functional groups such as ester carbonyls, hydroxyl moieties, and long-chain aliphatic chains that underpin these biological activities. Together, GC–MS and FTIR provide a comprehensive chemical fingerprint essential for quality control and standardisation of neem-based products intended for pharmaceutical, nutraceutical, or cosmetic application (Kaur and Saraf, 2010). Despite a rich body of research

on neem seed oil, characterisation of neem leaf oil remains comparatively sparse, particularly in relation to its fumigant antimicrobial potential in indoor environments.

The antimicrobial activity of neem smoke is of growing interest in resource-limited settings where synthetic disinfectants are costly or unavailable. Airborne microbial contamination in laboratory and healthcare environments constitutes a significant infection risk, and plant-derived fumigants offer an eco-friendly alternative to chemical aerosols. The aim of this study was to evaluate the phytochemical composition and antimicrobial potential of neem (*Azadirachta indica*) leaf oil extracted by the Soxhlet method against airborne microorganisms in selected indoor environments at Osun State Polytechnic, Iree, Osun State, Nigeria.

MATERIALS AND METHODS

Study Area, Plant Collection, and Sample Preparation

This study was conducted at the Microbiology Laboratory, Preparatory Room, and Instrumentation Room of the Department of Science Laboratory Technology, Osun State Polytechnic, Iree, Osun State, Nigeria. Fresh, mature neem (*Azadirachta indica*) leaves were collected from within the Polytechnic premises and authenticated by the Department of Biological Sciences. Leaves were thoroughly washed with distilled water to remove surface debris, then air-dried under shade at 25–30 °C for 7–10 days until constant weight was achieved to prevent degradation of thermolabile bioactive compounds (Isman, 2006). The dried leaves were pulverised into a fine powder using a laboratory grinder and stored in airtight containers until extraction.

Soxhlet Extraction and Percentage Yield

Fifty grams (50 g) of powdered neem leaves were accurately weighed and loaded into a cellulose extraction thimble, which was placed in a Soxhlet extractor attached to a round-bottom flask containing 250–300 mL of analytical-grade n-hexane (Azwanida, 2015; Subapriya and Nagini, 2005). Continuous reflux was maintained at 60–70 °C for 6–8 hours until the solvent in the siphon tube appeared colourless, indicating exhaustive extraction. The solvent–extract mixture was then concentrated under reduced pressure using a rotary evaporator at ≤40 °C to prevent thermal degradation. Residual solvent was removed in a vacuum desiccator, and the crude oil was filtered through Whatman No. 1 filter paper, collected in amber glass bottles, and stored at 4 °C to minimise photo-oxidation. Percentage yield was calculated as: Percentage Yield (%) = [Weight of oil extracted (g) / Initial weight of dry sample (g)] × 100. The extraction was performed in triplicate, and results were reported as mean ± standard deviation.

GC–MS and FTIR Phytochemical Characterisation

GC–MS analysis was performed on a Shimadzu GC–MS QP2020 NX system equipped with an AOC-20i auto-sampler, electron ionisation source (70 eV), quadrupole mass analyser, and DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm), following Muhammad and Kashere (2021). Samples were injected at 250 °C and carried by ultrapure helium (99.999%) at 1.0 mL/min. Eluted compounds were ionised over m/z 40–600, and spectra were processed using Shimadzu LabSolutions GC–MS software with compound identification against the NIST 2017 Mass Spectral Library. FTIR spectroscopy was carried out using a Thermo Scientific Nicolet iS50 FT-IR spectrometer with a diamond ATR (Smart iTR) accessory operating over 4000–400 cm^{-1} at 4 cm^{-1} resolution. The system was calibrated with polystyrene, purged with dry air, and controlled with OMNIC™ software. For each measurement, 1–2 mg of dried extract was applied to the ATR crystal and 32 scans were accumulated. Spectra were processed with atmospheric compensation, baseline correction, and normalisation, and functional groups assigned using standard IR correlation tables.

Isolation of Airborne Microorganisms and Neem Smoke Fumigation

Airborne bacterial populations were assessed using the settle plate method. Sterile Petri dishes containing Nutrient Agar were prepared, solidified, and labelled by location and exposure time. In each of the three rooms : Microbiology Laboratory, Preparatory Room, and Instrumentation Room , the control plates were exposed to ambient air for 20 minutes before neem smoke introduction to establish baseline Total Viable Counts (TVC). For fumigation, 50 g of dried neem leaves were placed on a metal tray and ignited gently; the smoke generated was allowed to fill each room (approximately 3 m × 3 m × 2.5 m) for 10 minutes. Experimental settle plates were then opened and exposed to the smoke-treated air for 20 minutes. All plates were positioned approximately one metre above the ground, covered immediately after exposure, and incubated at 37 °C for 24–48 hours. All tests were performed in triplicate, and TVC was expressed as colony-forming units per cubic metre (CFU/m^3) using mean colony counts.

RESULTS

Percentage Yield of Neem Leaf Oil

Soxhlet extraction of 50 g of shade-dried, powdered neem leaves produced oil yields of 7.60%, 8.20%, and 8.00% across the three replicates, giving a mean yield of 7.93 ± 0.30% (w/w), as detailed in Table 1. The low standard deviation across replicates reflects the reproducibility of the extraction protocol under controlled laboratory conditions.

Table 1: Percentage Yield of Neem Leaf Oil by Soxhlet Extraction

Sample	Weight of Dried Leaves (g)	Weight of Oil Extracted (g)	Percentage Yield (%)
Replicate 1	50.00	3.80	7.60
Replicate 2	50.00	4.10	8.20
Replicate 3	50.00	4.00	8.00
Mean ± SD	50.00 ± 0.2	3.97 ± 0.15	7.93 ± 0.30

Airborne Microbial Counts Before and After Neem Smoke Fumigation

Baseline TVC values prior to fumigation differed among the three rooms, reflecting differences in occupancy and activity level (Table 2). The Microbiology Laboratory recorded the highest pre-fumigation count (15 CFU/m³), followed by the Preparatory Room (9 CFU/m³) and the Instrumentation Room (3 CFU/m³). Following exposure to neem smoke, no viable bacterial colonies were detected in any of the three locations, representing complete (100%) elimination of culturable airborne bacteria.

Table 2: Total Viable Bacterial Count of Indoor Air Before and After Neem Smoke Fumigation

Location	Before Neem Smoke (CFU/m³)	After Neem Smoke (CFU/m³)
Microbiology Laboratory	15	No viable growth
Preparatory Room	9	No viable growth
Instrumentation Room	3	No viable growth

GC–MS Phytochemical Profile of Neem Leaf Oil

GC–MS analysis identified 14 compounds in the neem leaf oil, spanning fatty acids, sugar derivatives, aldehydes, esters, and long-chain hydrocarbons (Table 3). The dominant compounds were α-D-glucopyranose (21.13%), 9,12-octadecadienoic acid, linoleic acid (17.98%), n-hexadecanoic acid, palmitic acid (10.27%), octadecanoic acid, stearic acid (8.25%), and oleic acid (7.17%). Minor constituents included glycerine (5.47%), 14-methyl-8-hexadecyn-1-ol (7.00%), 9,17-octadecadienal (7.15%), and glycidyl palmitate (2.95%), among others.

Table 3: GC–MS Phytochemical Profile of *Azadirachta indica* (Neem) Leaf Oil

Peak	RT (min)	Compound Name	Mol. Formula	Mol. Mass	Peak Area (%)	% Comp.	Mass Frag. m/z
1	3.81	Glycerine	C3H8O3	92	4.33	5.47	43, 61, 92
2	4.80	2H-Pyran-2-one, tetrahydro-6-methyl-	C6H10O2	114	4.49	3.24	42, 70, 114

Peak	RT (min)	Compound Name	Mol. Formula	Mol. Mass	Peak Area (%)	% Comp.	Mass Frag. m/z
3	5.50	α-D-Glucopyranose	C6H12O6	180	21.98	21.13	45, 67, 180
4	8.32	14-Methyl-8-hexadecyn-1-ol	C17H32	252	6.63	7.00	43, 68, 252
5	10.78	Octadecanoic acid (Stearic acid)	C18H36O2	284	7.56	8.25	43, 73, 284
6	12.64	9,17-Octadecadienal, (Z)-	C18H32O	264	6.80	7.15	41, 60, 264
7	13.18	n-Hexadecanoic acid (Palmitic acid)	C18H26O	256	9.14	10.27	43, 109, 258
8	15.25	9,12-Octadecadienoic acid, methyl ester	C19H34O2	294	3.37	4.59	41, 67, 294
9	17.28	Oleic acid	C18H34O2	282	7.91	7.17	41, 55, 282
10	19.24	Methyl 10-methylhexadecanoate	C18H36O2	284	4.07	2.65	74, 143, 284
11	20.50	9,12-Octadecadienoic acid (Z,Z)- (Linoleic acid)	C18H32O2	280	16.12	17.98	67, 81, 280
12	20.82	7-Pentadecyne	C15H28	208	0.72	0.13	68, 81, 208
13	23.25	cis-9-Hexadecenal	C16H30O	238	2.91	1.97	41, 55, 238
14	24.98	Glycidyl Palmitate	C19H36O3	312	3.84	2.95	98, 115, 312

FTIR Functional Group Analysis

FTIR spectroscopy of the neem leaf oil revealed characteristic absorption bands consistent with a fatty acid-rich composition. A strong absorption band at approximately 1740 cm^{-1} confirmed the presence of ester carbonyl (C=O) groups, indicative of triglycerides and fatty acid esters. Aliphatic C–H stretching vibrations at 2850–2920 cm^{-1} confirmed the presence of long-chain hydrocarbons, while a broad O–H stretching band at ~3400 cm^{-1} indicated hydroxyl groups associated with fatty acids and alcohols. These spectral features are consistent with the GC–MS-identified constituents and with published FTIR profiles for *Azadirachta indica* oil (Kaur and Saraf, 2010; Biswas et al., 2002).

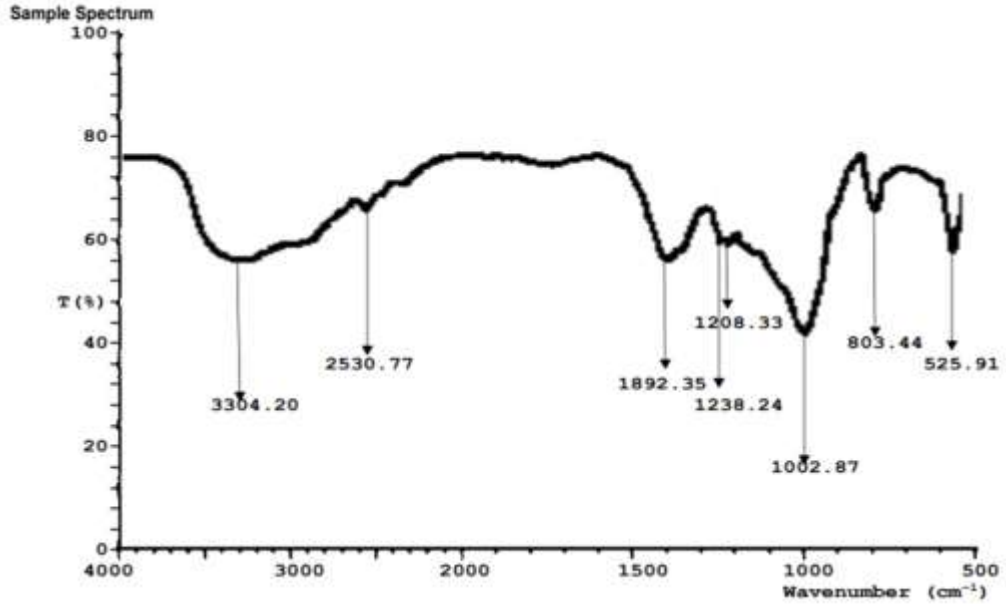


Figure 1: FTIR Analysis of *Azadirachta indica* (Neem) Oil

Discussion

The mean oil yield of 7.93 ± 0.30% (w/w) obtained from shade-dried *Azadirachta indica* leaves by Soxhlet extraction with n-hexane falls squarely within the range of 6–10% reported for neem leaf extracts using non-polar solvents in comparable studies (Azwanida, 2015; Subapriya and Nagini, 2005). This value is also consistent with the maximum neem leaf oil yield of 7.68 ± 0.11% documented using encapsulation-assisted extraction methods (Food Chemistry: X, 2024), confirming that the Soxhlet approach achieves competitive recovery under standard laboratory conditions. As anticipated, leaf yields were markedly lower than those routinely recorded for seed kernels, which produce considerably higher oil quantities owing to their substantially greater storage lipid content (Biswas et al., 2002). The narrow standard deviation across replicates (0.30%) reflects procedural consistency and underscores the reproducibility of the extraction protocol. Factors known to introduce inter-batch variability including particle size, solvent volume, and drying technique were carefully controlled, consistent with recommendations by Azwanida (2015).

The fumigant bactericidal effect of neem smoke was absolute, reducing airborne bacterial counts from 15, 9, and 3 CFU/m³ to zero in the Microbiology Laboratory, Preparatory Room, and Instrumentation Room, respectively. This finding strongly corroborates reports by Asif (2022), who demonstrated significant antimicrobial activity of neem preparations against both Gram-positive and Gram-negative airborne pathogens. The pre-fumigation differences in baseline counts across rooms are consistent with Tiwari et al. (2023), who attributed higher airborne microbial loads in laboratory and handling rooms to increased human traffic and substrate availability. The complete elimination of viable bacteria post-fumigation aligns with Kudom et al. (2021), who attributed neem's biocidal activity to volatile aldehydes, limonoids, and fatty acid vapours that disrupt bacterial cell membranes and inhibit key metabolic enzymes. In contrast to synthetic chemical disinfectants which can cause respiratory irritation, environmental persistence, and antimicrobial resistance neem smoke offers an eco-friendly, biodegradable alternative suitable for low-resource indoor environments (Javed et al., 2021).

GC–MS analysis revealed α-D-glucopyranose as the most abundant compound (21.13%), a finding also reported by Javed et al. (2021) in neem leaves extracted with ethyl acetate and n-hexane, where polar sugar residues were carried over into non-polar extracts. The predominance of fatty acids is the most pharmacologically significant aspect of the GC–MS profile. Linoleic acid (17.98%), the principal fatty acid constituent, is a polyunsaturated omega-6 fatty acid well-established for its anti-inflammatory and antioxidant properties through modulation of prostaglandin synthesis and reactive oxygen species (Javed et al., 2021). This prevalence is in agreement with Biswas et al. (2002), who identified linoleic acid as a core component of *Azadirachta indica* leaf lipid fractions across different geographic origins, suggesting it is a conserved metabolite rather than an environmentally contingent constituent.

Palmitic acid (10.27%) and stearic acid (8.25%) are saturated fatty acids with demonstrated antibacterial activity against a range of pathogens and are known to enhance membrane stability in biological and formulated systems (Javed et al., 2021). Their co-occurrence with oleic acid (7.17%) is a monounsaturated fatty acid with well-characterised skin-protective, wound-healing, and anti-inflammatory effects mirrors the composition reported in neem leaf extracts from Iraq and India (Javed et al., 2021). Minor volatile

compounds including long-chain aldehydes (9,17-octadecadienal; 7.15%), methyl esters, and 2H-pyran-2-one derivatives further reflect the chemical diversity typical of comprehensive neem GC–MS profiles and have been individually linked to antioxidant and insecticidal synergism in botanical studies (Azwanida, 2015). These findings are consistent with Biswas et al. (2002) but contrast with neem seed oil profiles dominated by azadirachtin and other high-molecular-weight limonoids highlighting a fundamental compositional divergence between leaf and seed fractions that is important for product-specific applications.

FTIR spectroscopy corroborated the GC–MS data, confirming ester carbonyl groups at ~1740 cm^{-1}, long-chain aliphatic C–H stretching at 2850–2920 cm^{-1}, and a broad O–H band at ~3400 cm^{-1}. These bands are in excellent agreement with Kaur and Saraf (2010), who characterised identical functional groups as the molecular basis for neem oil's biological activities, and with Biswas et al. (2002), who attributed the triglyceride-rich ester profile to the predominance of fatty acid glycerides. The convergence of GC–MS and FTIR data pointing to a fatty acid-dominated composition with esters, alcohols, and aldehydes provides a mutually reinforcing chemical fingerprint that strengthens confidence in the phytochemical characterisation and supports potential applications of neem leaf oil in dermatological formulations, natural disinfectant products, and anti-inflammatory therapeutics.

Conclusion

This study confirmed that Soxhlet extraction of *Azadirachta indica* leaves yields oil rich in bioactive fatty acids notably linoleic, palmitic, stearic, and oleic acids as established by GC–MS, with FTIR spectroscopy corroborating the presence of ester, hydroxyl, and long-chain aliphatic functionalities. Neem smoke demonstrated complete bactericidal efficacy against airborne microorganisms across three distinct indoor environments, validating its use as a natural fumigant disinfectant. These findings collectively affirm the pharmacological relevance of neem leaf oil and its potential for development into pharmaceutical, nutraceutical, and cosmetic formulations. Future research should investigate the antimicrobial spectrum against fungi, viruses, and antibiotic-resistant bacterial strains, optimise extraction conditions for higher bioactive yield, and examine the effects of seasonal and geographic variation on phytochemical composition.

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