UMYU Scientifica, Vol. 4 NO. 4, December 2025, Pp 174 – 190
https://doi.org/10.56919/usci.2544.015
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Phytochemical Profiling, Pharmacokinetics, and Antimicrobial Activity of *Azadirachta indica* Leaf Extracts: *In Silico* and *In Vitro* Evaluations

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ABSTRACT
Antimicrobial resistance (AMR) is a critical global health threat, necessitated by the rise of multidrug-resistant "superbugs" and a lack of new antibiotic discovery. Plant-derived phytochemicals, particularly from *Azadirachta indica* (neem), offer a promising reservoir of bioactive molecules with broad-spectrum therapeutic potential. This study aimed to characterize the bioactive profiles of aqueous and methanolic leaf extracts and evaluate their therapeutic potential against viral, bacterial, and fungal targets. Dried *A. indica* leaves were processed into powder and subjected to three-day maceration in methanol and water. Phytochemicals were identified through qualitative screening and Gas Chromatography-Mass Spectrometry (GC–MS) using a SHIMADZU GCMS-QP2010 PLUS system. Pharmacokinetic profiles and drug-likeness were predicted via SwissADME and ADMETLab 3.0. Molecular docking simulations were performed using AutoDock Vina against nine pathogenic targets, while *in vitro* efficacy was determined through well/disk diffusion and MIC/MBC/MFC assays. The aqueous extract provided a significantly higher yield (10.78%) than the methanolic extract (10.58%, $p = 0.013$). GC-MS identified 17 compounds in the methanolic extract (linoleic acid dominant) and 21 in the aqueous extract (oleic acid dominant). Molecular docking revealed that Methyl linolelaidate had the highest affinity for HIV Reverse Transcriptase (-6.9 kcal/mol), while Monopalmitin and Linoleoyl chloride showed strong binding to fungal and bacterial targets (-6.0 kcal/mol). Antimicrobial testing confirmed concentration-dependent activity, with the methanolic extract achieving a 15.6 mm zone of inhibition against *S. epidermidis*. ADMET analysis identified monopalmitin**,** resorcinol, and methyl caprate as the most viable drug candidates. *A. indica* is a potent source of bioactive molecules capable of disrupting microbial membranes and inhibiting key enzymes. While the findings support the therapeutic potential of these compounds, predicted nephrotoxicity for methyl vaccenate and methyl caprate underscores the need for further experimental validation to ensure clinical safety.

ARTICLE HISTORY
Received September 11, 2025
Accepted December 17, 2025
Published December 30, 2025

KEYWORDS
Azadirachta indica, GC-MS, ADMET, Molecular docking, Antimicrobial resistance, Phytochemicals, Lipinski's rule of five.

INTRODUCTION
Antimicrobial resistance (AMR) has emerged as a defining global health crisis of the twenty-first century, accounting for an estimated 1.27 million deaths in 2019 alone (World Health Organisation, 2023). The inappropriate and excessive use of antimicrobials across human, veterinary, and agricultural sectors continues to accelerate resistance, with the most severe clinical and economic burdens borne by low- and middle-income countries (Jindal *et al.,* 2015). AMR threatens the stability of modern medicine by complicating routine infections and escalating risks associated with major surgeries and oncology treatments—a challenge compounded by a stagnant antibiotic development pipeline (Jindal *et al.,* 2015; Ahmed *et al.,* 2024). Projections suggest that unabated resistance could impose an additional USD 1 trillion in healthcare costs by 2050 and cause substantial losses in global gross domestic product (World Bank, 2024).

The declining efficacy of conventional therapies has intensified the search for alternative agents, particularly plant-derived phytochemicals, which serve as prolific reservoirs of structurally diverse antimicrobials (Abdallah *et al.,* 2023). These natural products often exert broad-spectrum activity by destabilizing microbial membranes and disrupting electrochemical gradients, mechanisms that significantly lower the risk of resistance development (Nourbakhsh *et al.,* 2022; Li *et al.,* 2024). Among medicinal flora, *Azadirachta indica* L. (neem) is distinguished by its extensive ethnomedicinal heritage and a rich array of bioactive constituents, including triterpenoids, phenolics, and flavonoids (Orwa *et al.,* 2009; Wylie and Merrell,

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How to cite: Musari, K. A., Umar, Z. D., & Kabir, K. (2025). R Phytochemical Profiling, Pharmacokinetics, and Antimicrobial Activity of *Azadirachta indica* Leaf Extracts: *In Silico* and *In Vitro* Evaluations. *UMYU Scientifica*, 4(4), 174 – 190. https://doi.org/10.56919/usci.2544.015

2022). Limonoids such as azadirachtin and nimbolide, alongside leaf flavonoids like quercetin and β-sitosterol, have demonstrated potent antimicrobial, anti-inflammatory, and antioxidant activities, reinforcing neem's relevance in bridging traditional knowledge and modern drug discovery (Saleem *et al.,* 2018; Bello *et al.,* 2021; Nagini *et al.,* 2021).

In modern pharmacological research, the efficiency of phytochemical recovery is known to be strongly influenced by solvent polarity, necessitating systematic comparisons of extraction strategies to optimize bioactive yields (Momodu *et al.,* 2022; Patel *et al.,* 2024). While Gas Chromatography–Mass Spectrometry (GC–MS) remains indispensable for the comprehensive characterization of these complex plant matrices (Abubakar and Lubabatu, 2025), contemporary discovery pipelines now integrate *in silico* ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling to accelerate early-stage development and reduce clinical attrition (Ameji *et al.,* 2023; Parvathi *et al.,* 2022).

Despite the extensive literature on *A. indica*, there remains a scarcity of research that adopts a holistic framework linking extraction chemistry with predictive pharmacokinetics and experimental validation. This study addresses this deficiency by systematically investigating the influence of solvent polarity—specifically comparing aqueous and methanolic extracts—on the chemical profile and therapeutic potential of *A. indica*. By integrating advanced GC–MS characterization with *in silico* ADMET evaluations and molecular docking, we aim to provide a unified assessment of the herb's efficacy against a diverse panel of viral, bacterial, and fungal pathogens, thereby streamlining the path from traditional use to evidence-based therapeutic application.

METHOD

2.1 Extraction procedure of *Azadirachta indica*

A. indica leaves were shade dried and then crushed to fine powder with a clean, dry mortar and pestle. The powdered leaves were filtered through no. 45 sieve and stored in an airtight container until extraction.

Then fifty grams (50g) of the powdered sample was weighed into two places, which were placed in two separate bottles for extraction with two different solvents. Then plant material were macerated using methanol and aqueous (water) respectively. After closing the two bottles, they were allowed to stand for three days, shaking occasionally were done to achieve full extraction (Abubakar and Haque, 2020). After extraction, methanol solvent was removed by rotary evaporator and the aqueous extract was evaporated in water bath. The samples were then placed in a desiccator to completely dry.

2.2 Phytochemical Screening of *A. indica*

Phytochemical screening was performed on the methanol and aqueous extracts, following the procedures described by Dubale *et al.* (2023); and Abubakar and Haque (2020). The methods were used to screen the presence of saponins, cardiac glycosides, flavonoid, steroids and alkaloids.

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of *A. indica*

A. indica (both methanolic and aqueous) extracts were dried in the oven at 105°C, ground, and extracted with n-hexane prior to GC-MS analysis. Analysis was performed using a SHIMADZU GCMS-QP2010 PLUS Gas chromatography-mass spectrometry. A Perkin Elmer Elite-5 capillary column (30 m × 0.25 mm) with 0.25 µm film thickness composed of 95% dimethyl polysiloxane was used. Gas chromatography (GC) was performed using a gas chromatograph (Agilent 7890B) connected to a mass spectrometer (Agilent MSD5977A) with an HP-5MS column (30 m × 0.25 mm × 0.25 µm). Helium was used as the carrier gas (flow rate 0.5 ml/min; injection volume 1 µl), and the inlet temperature was set to 250°C. The oven temperature was held at 80°C for 4 min, then increased to 200°C and ramped to 280°C at a rate of 20°C/min, maintaining that temperature for 5 min. Overall time was 25 minutes (Omale *et al.*, 2017; Hamisu and Salisu, 2025; Salisu et al., 2025; Usman et al., 2025).

The transfer line of the mass spectrometer (MS) was maintained at 200°C, while the source temperature was set at 180°C, and the GC-MS analysis was carried out with electron impact ionization at 70 eV. Total ion count (TIC) was used for detecting and quantifying compounds. Spectra of individual components were compared to those from the GC-MS library of known compounds (Taylor, 2015).

2.4 ADMET Prediction

The absorption distribution metabolism excretion and toxicity (ADMET) profiles of the specific phytocompounds of *A. indica* revealed by GC-MS analysis, were predicted using two reliable webservers: SwissADME and ADMETLab 3.0 (Xiong *et al.*, 2021; Daina *et al.*, 2017). Both webservers were used to evaluate drug-likeness of these compounds and compliance with Lipinski's rule of five.

SwissADME, accessed via https://www.swissadme.ch, was employed to predict properties such as water solubility, gastrointestinal absorption, blood-brain barrier penetration, and Lipinski's rule of five compliance. ADMETLab 3.0, accessed via https://admetlab3.scbdd.com, was used to assess drug-induced liver injury (DILI) and nephrotoxicity for all phytocompounds.

2.5 Molecular Docking Studies

Molecular docking was performed to predict the binding orientations of *A. indica* phytochemicals (identified via GC-MS) against key pathogenic targets. Following the methodology of Butt *et al.* (2020) with modifications, protein structures were retrieved from the RCSB PDB: HIV targets (Reverse Transcriptase, 5VZ6; Integrase, 6WC8; Protease, 4Z4X), *S. epidermidis* ClpP (8QYF), *S.*

aureus DNA Gyrase (7FVT), *E. coli* DNA Gyrase (7DPS), *P. aeruginosa* DNA Gyrase (7PTG), *C. albicans* Chitin Synthase 2 (7STN), and *T. rubrum* KDNase (7P1V).

Proteins were prepared by removing non-complexed ions, adding hydrogen atoms, and applying Gasteiger charges. Ligand 3D structures were sourced from PubChem (SDF format) and refined under identical parameters. Docking simulations were executed using AutoDock Vina within UCSF Chimera, centered on a grid box encompassing known active sites. Binding affinities were recorded, and molecular interactions (e.g., hydrogen bonding) were visualized in 2D using BIOVIA Discovery Studio.

2.6 Antimicrobial Susceptibility Testing (AST)

The antimicrobial efficacy of methanolic and aqueous *A. indica* extracts was evaluated against *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, *C. albicans*, and *T. rubrum*. Isolate identities were confirmed using standard microbiological protocols (Cheesbrough, 2005). Inocula were standardized to a 0.5 McFarland turbidity (approx. 1.5 x 10^8 CFU/mL) in normal saline, prepared by mixing 0.05 mL of 1% $BaCl_2$ with 9.95 mL of 1% H_2SO_4 (Wasihun *et al.,* 2023). Extracts were prepared at a 200 mg/mL stock in DMSO and serially diluted to 100, 50, and 25 mg/mL (Ibrahim and Kebede, 2020).

2.6.1 Antibacterial and Antifungal Assays

Antibacterial activity was determined via the well diffusion method on Mueller-Hinton agar. Plates were inoculated with 0.5 mL of standard culture, and wells were charged with varying extract concentrations before incubation at 37°C for 24 hours (Wasihun *et al.,* 2023).

Antifungal activity was assessed using the disk diffusion method. Sterile 6 mm filter paper disks were saturated with extracts and placed onto Sabouraud Dextrose Agar (SDA) plates inoculated with *C. albicans* and *T. rubrum*. Results were recorded as zones of inhibition (mm) after incubation at 30°C for 48 hours (Berkow *et al.,* 2020).

2.6.2 Determination of MIC, MBC, and MFC

The Minimum Inhibitory Concentration (MIC) was determined using the broth dilution method, defined as the lowest extract concentration showing no visible growth after 24 hours at 37°C (Ibrahim and Kebede, 2020). To determine the Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC), aliquots from tubes showing no growth were sub-cultured onto Nutrient Agar or SDA. The MBC/MFC was recorded as the lowest concentration yielding no colony formation after appropriate incubation.

2.7 Statistical analysis

All statistical analyses were performed with Python (version 3.13.1) and Microsoft Excel (version 2016).

RESULT

3.1 Percentage yields of methanolic and aqueous extracts from the leaves of *Azadirachta indica*

The yield percentages obtained from both methanolic and aqueous extracts of the plant were compared as shown in Table 1. The percentage yield of aqueous extract was 10.78% (5.39g), and was found to be slightly higher and statistically significant than that of methanol extract with 10.58% (5.29g) ($t = -4.30$, $p = 0.013$).

Table 1: Comparison of percentage yields from methanolic and aqueous extracts of *A. indica*

Solvent	Weight of the leaf powder extracted	Weight of the crude extract	Percentage yield
Methanol	50g	5.29g	10.58
Aqueous	50g	5.39g	10.78

Table 2: Phytochemical profile of methanolic and aqueous extracts from *A. indica*

Phtochemical	Methanolic Extract	Aqueous Extract
Saponins	+	+
Cardiac glycoside	+	+
Flavonoid	+	+
Steroid	+	+
Alkaloid	+	+

Key: + = Present; **-** = Absent

3.2 Phytochemical profile of methanolic and aqueous extracts

The results of the phytochemical screening indicated that several bioactive chemical constituents are present in both methanolic and aqueous extracts of *A. indica*. Saponins, cardiac glycosides, flavonoid, steroids and alkaloids were detected in both methanolic extract and aqueous extract (Table 2). This indicates that both solvents resulted in the successful extraction of all tested phytochemicals.

3.3 Gas chromatography-mass spectrometry (GC-MS) result

The GC-MS analysis of the methanolic and aqueous extracts of *A indica* revealed the presence of diverse phytochemical compounds, including fatty acids, alcohols, sugars, and aromatic compounds, indicating the chemical complexity of the plant's bioactive constituents. In the methanolic extract, a total of 17 compounds were identified (Table 3). The most abundant compounds were Linoleic acid (27.38%), and Resorcinol (15.37%). Other

notable constituents included Methylglucose (9.50%), 6-Ethoxy-6-methyl-2-cyclohexenone (8.79%), and Sucrose (2.49%), contributing to the extract's potential bioactivity.

In the aqueous extract, 21 compounds were identified (Table 4). Oleic acid (37.80%) was the most abundant, followed by Z,E-3,13-Octadecadien-1-ol (11.55%). The aqueous extract also contained Glycerin (3.46%) and 3-Methylglucose (7.00%). Additionally, Resorcinol (4.62%) was present in both extracts, though in a higher concentration in the methanolic extract.

Resorcinol, methyl linolelaidate, methyl vaccinate, methyl caprate, linoleoyl chloride and monopalmitin are common compounds identified in both extract. The GC-MS chromatograms of both methanolic and aqueous extracts is shown as Figures 1 and 2 respectively.

Table 3: Phytochemical constituents of methanolic extract identified by GC-MS

Peak No.	Retention Time (min)	Compound Name	Molecular Formula	Molecular Weight	Area %
1	3.894	4-Octene	C_8H_{16}	112	0.60
2	7.758	1,2-Benzenedimethanol	$C_8H_{10}O_2$	138	1.18
3	9.291	Resorcinol	$C_6H_6O_2$	110	15.37
4	11.097	Sucrose	$C_{12}H_{22}O_{11}$	342	2.49
5	14.469	6-Ethoxy-6-methyl-2-cyclohexenone	$C_9H_{14}O_2$	154	8.79
6	14.826	Methylglucose	$C_7H_{14}O_6$	194	9.50
7	15.705	Methyl isohexadecanoate	$C_{17}H_{34}O_2$	270	0.46
8	17.030	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	5.19
9	18.918	Methyl linolelaidate	$C_{19}H_{34}O_2$	294	1.21
10	18.974	Methyl vaccinate	$C_{19}H_{36}O_2$	296	1.15
11	19.340	Methyl caprate	$C_{11}H_{22}O_2$	186	0.26
12	20.144	Linoleic Acid	$C_{18}H_{32}O_2$	280	27.38
13	21.706	Isooctylvinyl ether	$C_{10}H_{20}O$	156	6.19
14	22.709	Erucamide	$C_{22}H_{43}NO$	337	3.62
15	23.571	Brassidic acid	$C_{22}H_{42}O_2$	338	5.55
16	24.113	Monopalmitin	$C_{19}H_{38}O_4$	330	3.18
17	25.731	Linoleoyl chloride	$C_{18}H_{31}ClO$	294	7.85

Table 4: Phytochemical constituents of aqueous extract identified by GC-MS

Peak No.	Retention Time (min)	Compound Name	Molecular Formula	Molecular Weight	Area %
1	3.686	Cyclohexanone	$C_6H_{10}O$	98	0.83
2	3.916	1-hepten	C_7H_{14}	98	1.89
3	4.530	Glycerin	$C_3H_8O_3$	92	3.46
4	6.350	1,3-Dioxolane, 2,4,5-trimethyl	$C_6H_{12}O_2$	116	0.42
5	7.746	4-Methyl-4-hepten-3-one	$C_8H_{14}O$	126	1.31
6	9.383	Resorcinol	$C_6H_6O_2$	110	4.62
7	10.517	3-Pinanol	$C_{10}H_{18}O$	154	0.47
8	11.008	Ethriol	$C_6H_{14}O_3$	134	4.39
9	14.195	3-Methylglucose	$C_7H_{14}O_6$	194	7.00
10	15.711	Methyl tridecanoate	$C_{14}H_{28}O_2$	228	1.08
11	17.053	Palmitic acid	$C_{16}H_{32}O_2$	256	7.03
12	18.923	Methyl linolelaidate	$C_{19}H_{34}O_2$	294	2.58
13	18.983	Methyl vaccenate	$C_{19}H_{36}O2$	296	2.20
14	19.347	Methyl caprate	$C_{11}H_{22}O_2$	186	0.41
15	20.175	Oleic acid	$C_{18}H_{34}O_2$	282	37.80
16	21.697	3-Trifluoroacetoxy-6-ethyldecane	$C_{14}H_{25}F_3O_2$	282	1.05
17	23.108	2-Decyn-1-ol	$C_{10}H_{18}O$	154	1.20
18	23.570	Linoleoyl chloride	$C_{18}H_{31}ClO$	298	5.93
19	23.779	1-Nonadecanol	$C_{19}H_{40}O$	284	1.63
20	24.105	alpha.-Monopalmitin	$C_{19}H_{38}O_4$	330	3.14
21	25.729	Z,E-3,13-Octadecadien-1-ol	$C_{18}H_{34}O$	266	11.55

3.4 ADMET and drug-likeness predictions result

Table 5 shows the ADMET and drug-likeness predictions of the 6 common phytocompounds obtained in the GC–MS of the plant, indicating varied pharmacokinetic properties. The water solubility varied between moderate and high, while resorcinol was highly soluble (Table 5). The GI absorption was mostly found to be high, with the exception of Linoleoyl chloride, which had a low absorption rate (Table 5). Monopalmitin, resourcinol and methyl caprate were the three phytocompounds that were predicted to penetrate the blood-brain barrier (BBB) (Table 5).

Table 5: ADMET and drug-likeness predictions of the phytocompounds from *A. indica*

S/N	Compound	Water Solubility	GI Absorption	BBB Penetration	Lipinski Rule	DILI	Nephrotoxicity
1	Linoleoyl chloride	Moderately	Low	No	Yes, 1 violation: MLogP>4.15	0.0	0.054
2	Methyl linolelaidate	Moderately soluble	High	No	Yes, 1 violation: MLogP>4.15	0.003	0.343
3	Methyl vaccenate	Moderately soluble	High	No	Yes, 1 violation: MLogP>4.15	0.006	0.429
4	Monopalmitin	Moderately soluble	High	Yes	Yes	0.034	0.344
5	Resorcinol	Very soluble	High	Yes	Yes	0.17	0.072
6	Methyl caprate	Soluble	High	Yes	Yes	0.179	0.428

Table Legend: ADMET and Drug-likeness Predictions of the Phytocompounds

1. S/N: Serial Number of the compounds in the list.
2. Compound: Name of the phytochemical compound under investigation.
3. Water Solubility: Indicates the ability of the compound to dissolve in water, categorized as poorly, moderately, highly, or very soluble.
4. GI Absorption: Predicts the absorption of the compound through the gastrointestinal (GI) tract, classified as high or low.
5. BBB Penetration: Indicates whether the compound can cross the blood-brain barrier (BBB), with "Yes" meaning it penetrates the BBB and "No" meaning it does not.
6. Lipinski Rule: Evaluates drug-likeness based on Lipinski's rule of five. A violation (e.g., MLogP > 4.15) suggests reduced likelihood of good oral bioavailability.
7. DILI: Drug-Induced Liver Injury (DILI) risk prediction, with higher values indicating increased likelihood of hepatotoxicity.
8. Nephrotoxicity: Predicts the potential of the compound to cause kidney toxicity, with higher values indicating greater risk.

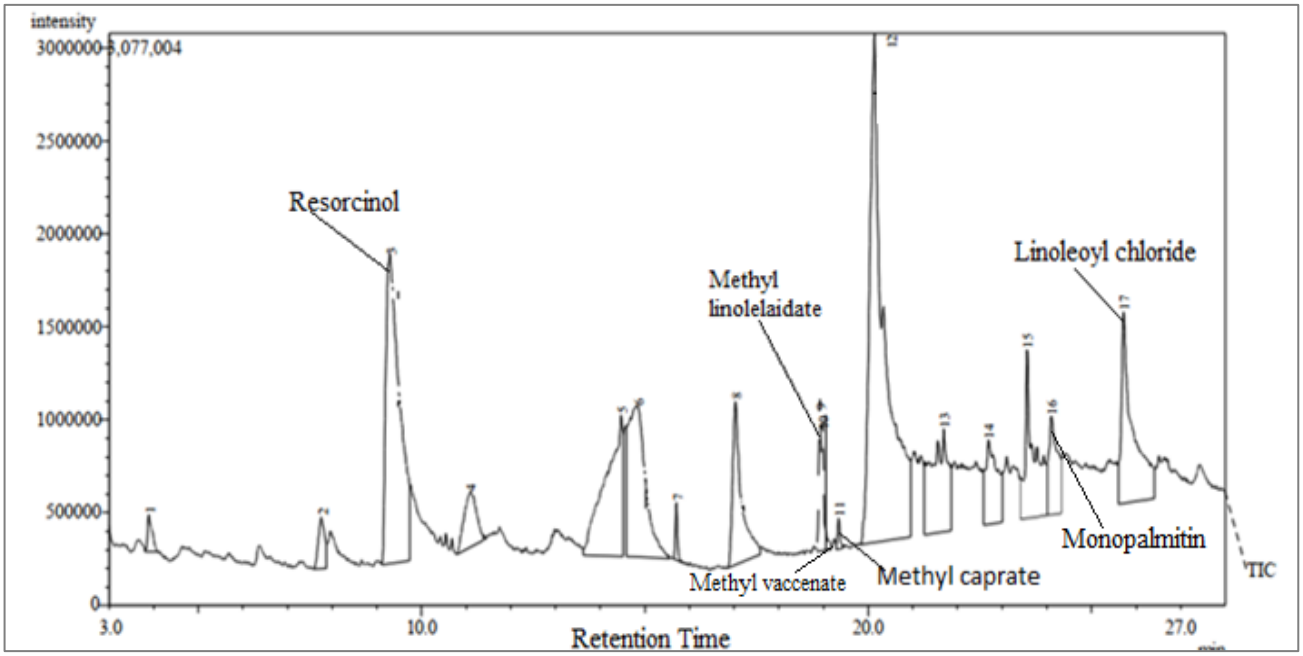


Figure 1: GC–MS chromatographic profile of *A. indica* methanolic extract

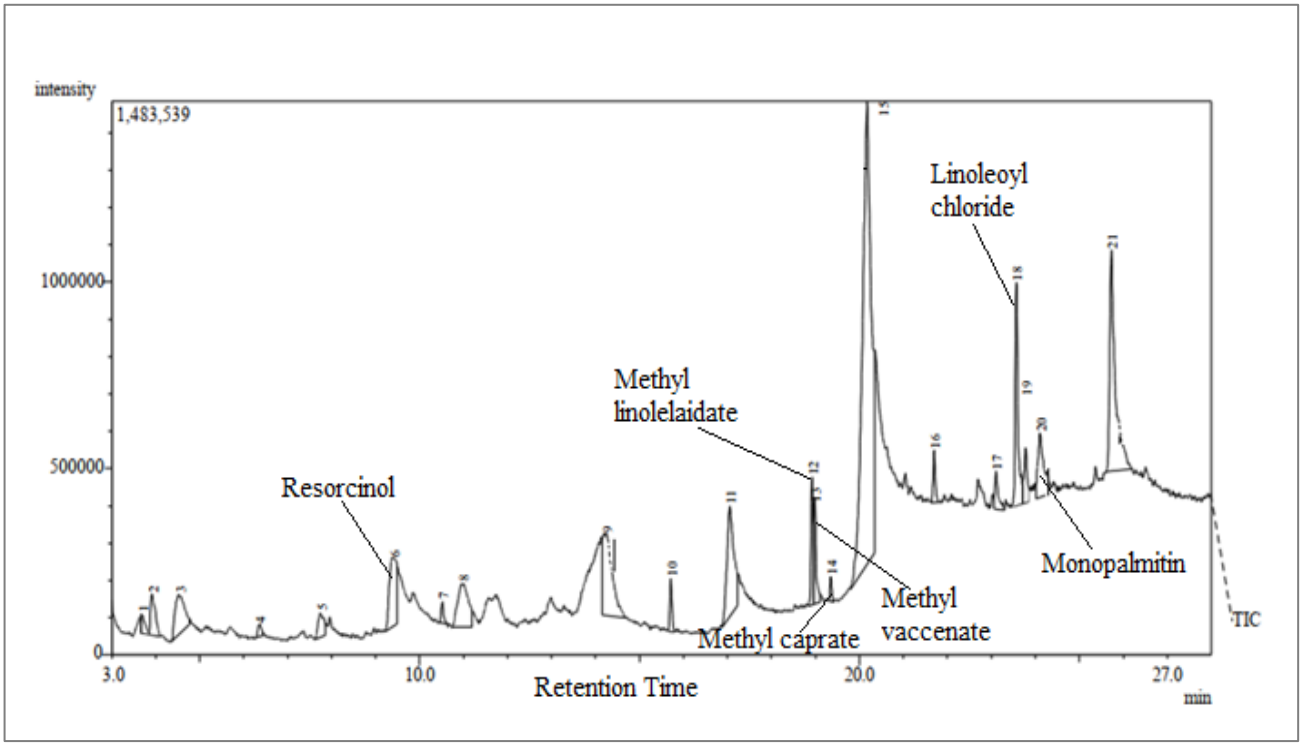


Figure 2: GC–MS chromatographic profile of *A. indica* aqueous extract

Table 6: Molecular docking scores (kcal/mol) of selected *Azadirachta indica* phytocompounds against HIV enzymes and representative bacterial and fungal target proteins.

Phytocompound	HIV Reverse Transcriptase	HIV Integrase	HIV Protease	*S. aureus* Gyrase	*S. epidermidis* ClpP	*E. coli* Gyrase	*P. aeruginosa* Gyrase	*C. albicans* Chitin Synthase 2	*T. rubrum* KDNase
Linoleoyl chloride	-6.5	-4.0	-4.7	-4.9	-5.7	-5.5	-5.4	-6.0	-4.6
Methyl caprate	-6.5	-3.7	-4.4	-4.4	-4.6	-4.7	-5.0	-5.1	-4.3
Methyl linolelaidate	-6.9	-4.8	-4.3	-5.0	-5.0	-5.4	-5.2	-5.6	-4.6
Methyl vaccenate	-6.1	-4.8	-4.6	-4.7	-5.4	-5.2	-5.1	-5.6	-4.6
Monopalmitin	-4.3	-3.4	-4.4	-5.2	-5.3	-5.2	-5.1	-6.0	-4.9
Resorcinol	-5.8	-4.1	-4.0	-4.6	-5.3	-4.7	-4.6	-5.3	-4.9

With regards to the Lipinski rule of five, three compounds met all of its criteria; however, Linoleoyl chloride, Methyl linolelaidate, and Methyl vaccenate were exceptions with one violation (high partition coefficient, MLogP > 4.15) (Table 5). The hepatotoxicity risk (DILI) was found to be low for all candidate compounds, with Linoleoyl chloride showing no fit in, while Methyl caprate had the highest DILI value (0.179) (Table 5). Nephrotoxicity scores were diverse: Methyl vaccenate presented the highest nephrotoxicity risk (0.429), on the contrary Linoleoyl chloride scored the lowest (0.054) (Table 5).

3.5 Molecular Docking Results

3.5.1 HIV Protein Docking

Molecular docking of six *A. indica* phytocompounds against HIV enzymes revealed varying binding affinities, measured in kcal/mol (Table 6). Methyl linolelaidate exhibited the highest binding affinity for Reverse Transcriptase with a binding energy of -6.9 kcal/mol, followed by Linoleoyl chloride and Methyl caprate at -6.5 kcal/mol. Monopalmitin showed the weakest affinity for this target at -4.3 kcal/mol.

For Integrase, Methyl linolelaidate and Methyl vaccenate recorded the strongest binding energies of -4.8 kcal/mol, while Monopalmitin displayed the weakest affinity at -3.4 kcal/mol. In the case of Protease, Linoleoyl chloride showed the highest interaction (-4.7 kcal/mol) and Resorcinol recorded the lowest affinity (-4.0 kcal/mol).

3.5.2 Bacterial and Fungal Protein Docking

Against bacterial targets (Table 6), Linoleoyl chloride demonstrated the most consistent and strongest binding,

Table 7: Residue-level interaction profiles of *Azadirachta indica* phytochemicals with viral, bacterial, and fungal target proteins, showing hydrogen bonding, π–σ, alkyl, van der Waals, and unfavorable contacts.

Ligand	Target Protein / Enzyme	Interaction Type	Interacting Residues
Methyl linolelaidate	Reverse Transcriptase	π–σ	TRP226
		Alkyl	LYS220, PHE224, VAL105, LEU231, TYR185, LEU97, PRO92
Methyl linolelaidate	Integrase	π–σ	TRP76
		Alkyl	ALA42, ALA73, LEU46, TYR43
		van der Waals	GLU40
Methyl linolelaidate	Protease	Alkyl	ALA127, VAL146, LEU175, ILE131, LEU153, VAL183
Linoleoy chloride	*S. aureus* Gyrase	Alkyl	PHE947, PHE960, ALA138
Linoleoy chloride	*S. epidermidis* ClpP	Hydrogen bond	VAL417
		Alkyl	LEU472, LEU496, ARG493, PRO471, MET445, ILE468
Linoleoy chloride	*E. coli* Gyrase	Hydrogen bond	SER121
		Unfavorable bump	HF6301
		Alkyl	VAL167, VAL43, VAL71, ALA47, ILE78, ILE94, VAL120
Linoleoy chloride	*P. aeruginosa* Gyrase	Alkyl	ALA220, ILE245, ILE261
Monopalmitin	*C. albicans* Chitin Synthase-2	Hydrogen bond	ARG397, GLU395, TRP439
		Alkyl	TYR309, ILE287, ALA332, VAL328
Monopalmitin	*T. rubrum* KDNase	Hydrogen bond	ASP62, ASN60, ARG61, ARG364
		Alkyl	ARG299, ARG298, TYR355

specifically -4.9 kcal/mol for *S. aureus* gyrase, -5.7 kcal/mol for *S. epidermidis* ClpP, -5.5 kcal/mol for *E. coli* gyrase, and -5.4 kcal/mol for *P. aeruginosa* gyrase. Methyl linolelaidate also showed significant binding to *E. coli* gyrase at -5.4 kcal/mol.

Regarding fungal targets (Table 6), Linoleoyl chloride and Monopalmitin shared the highest affinity for *C. albicans* chitin synthase 2 (-6.0 kcal/mol). For *T. rubrum* KDNase, Monopalmitin and Resorcinol exhibited the strongest binding energy at -4.9 kcal/mol.

3.5.3 Residue-Level Interaction Profiles

Detailed interaction analysis (Figures 3, 4 & 5) identified specific amino acid residues and bond types involved in ligand-protein complexes (Table 7):

- Methyl linolelaidate interacted with Reverse Transcriptase via a π–σ bond (TRP226) and multiple Alkyl bonds (LYS220, PHE224, VAL105, LEU231, TYR185, LEU97, PRO92). Its interaction with Integrase involved a π–σ bond (TRP76) and van der Waals forces (GLU40).
- Linoleoyl chloride formed Hydrogen bonds with VAL417 in *S. epidermidis* ClpP and SER121 in *E. coli* gyrase. It also showed an unfavorable bump with residue HF6301 in E. coli gyrase.
- Monopalmitin established Hydrogen bonds with ARG397, GLU395, and TRP439 in *C. albicans* chitin synthase 2, and with ASP62, ASN60, ARG61, and ARG364 in *T. rubrum* KDNase.

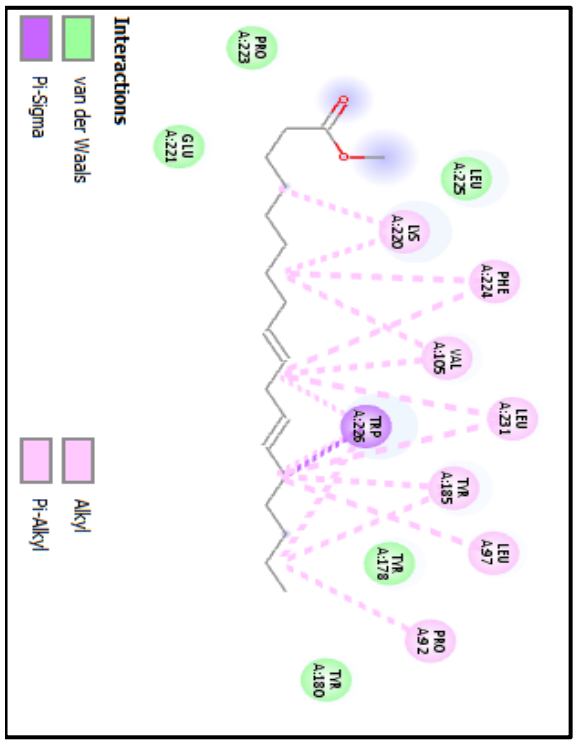
3.6 Antimicrobial Susceptibility Results

3.6.1 Antibacterial Activity

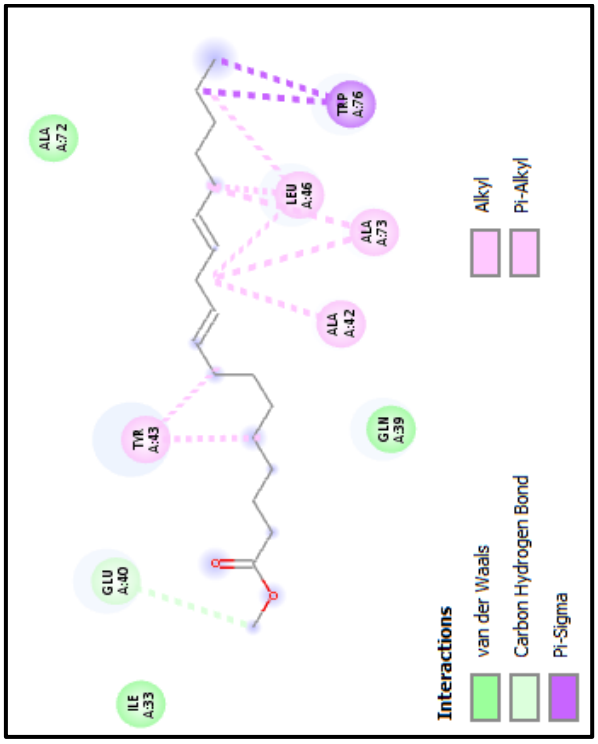
The maximum zone of inhibition recorded (Figure 6) was 15.6 mm against *S. epidermidis* using 200 mg/mL of methanolic extract. For the aqueous extract, the highest activity was 14.1 mm against *P. aeruginosa* at the same concentration. No activity was observed at 25 mg/mL across all organisms.

Three-way ANOVA indicated that extract concentration had a highly significant effect on the zone of inhibition ($F(3, 32) = 21.02$, $p < 0.001$). Organism type also showed a significant effect ($p = 0.039$), but extract type (aqueous vs. methanol) did not ($p = 0.475$). Post-hoc analysis using

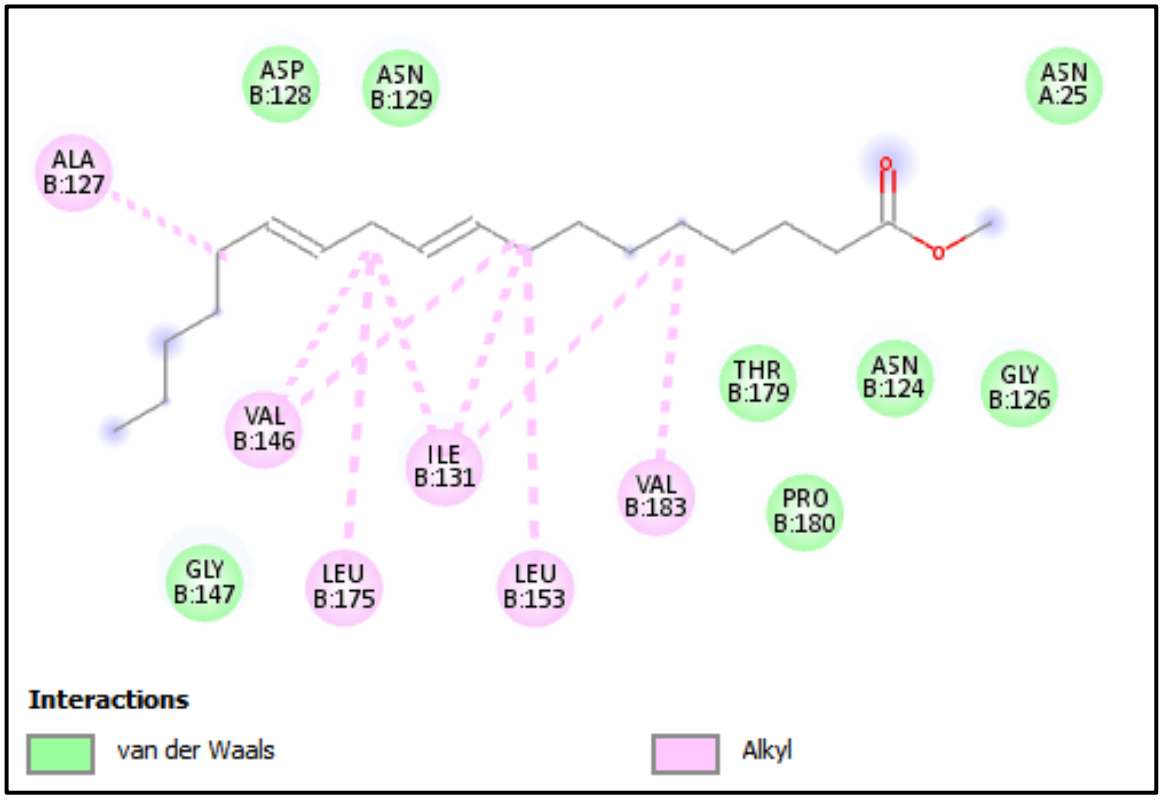
Tukey's HSD confirmed that antibacterial activity differed significantly with decreasing concentration ($p = 0.0004$), though no significant differences were found between specific bacterial species. MIC and MBC values showed that methanolic extracts (Figure 7) generally had lower inhibitory concentrations, with *P. aeruginosa* exhibiting the lowest MBC (50 mg/mL). One-way ANOVA revealed no statistically significant differences in MIC or MBC values between bacterial species ($p = 0.314$ and $p = 0.258$, respectively) or extract types.



With Reverse Transcriptase



With Integrase



With Protease

Figure 3: 2D representation of molecular interactions of Methyl Linolaidate (an *A. indica* phytochemical) with key HIV proteins

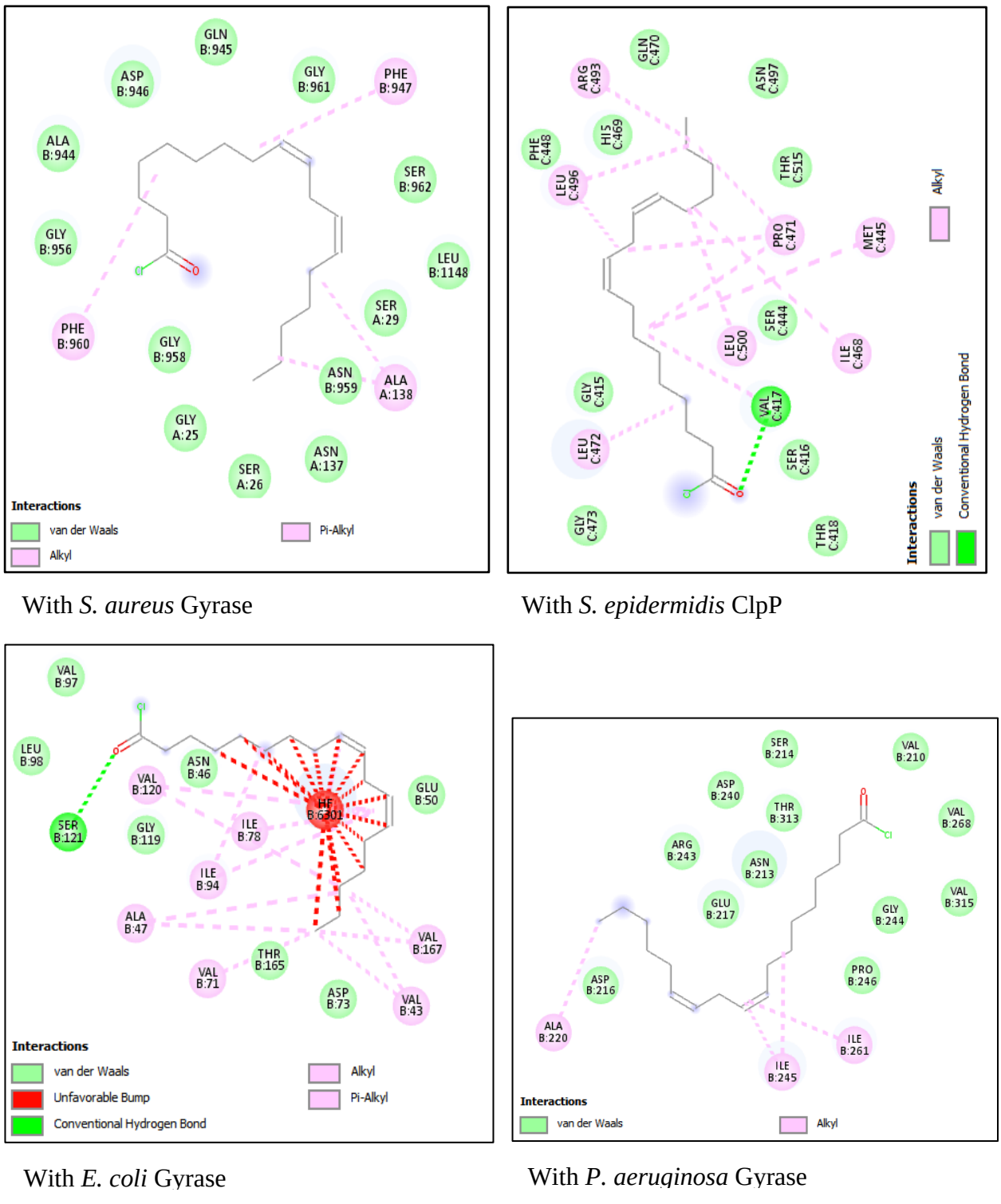


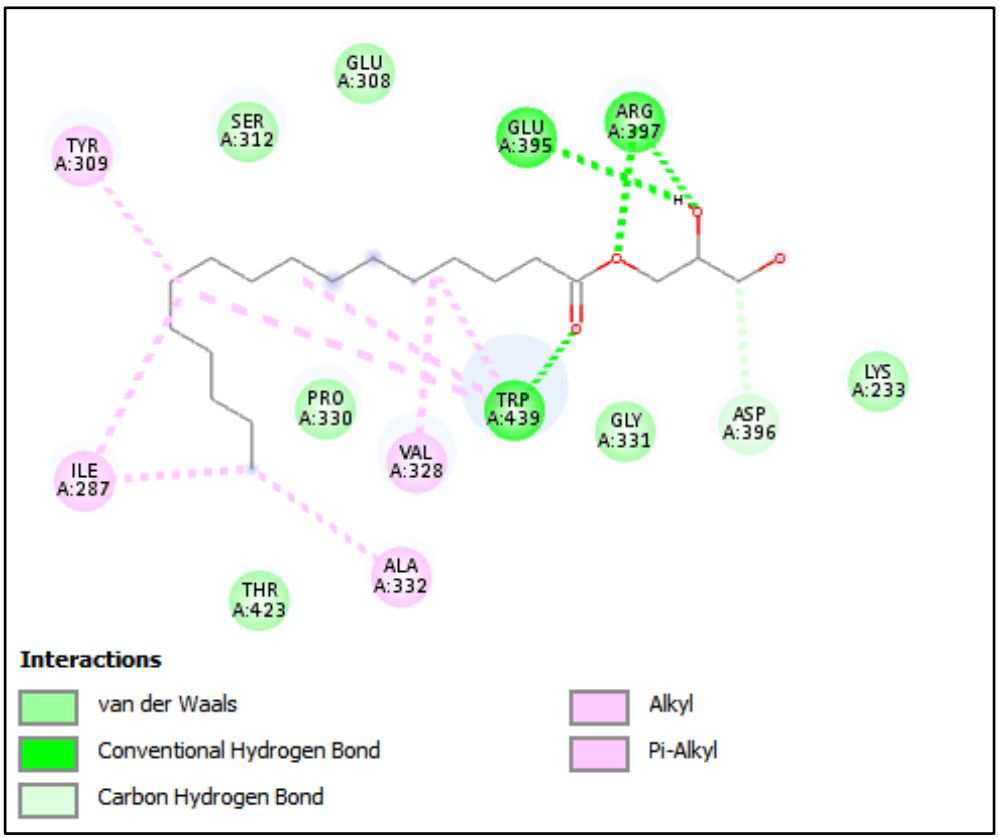
Figure 4: 2D representation of molecular interactions of Linoleoy Chloride (an *A. indica* phytochemical) with various bacterial proteins

3.6.2 Antifungal Activity

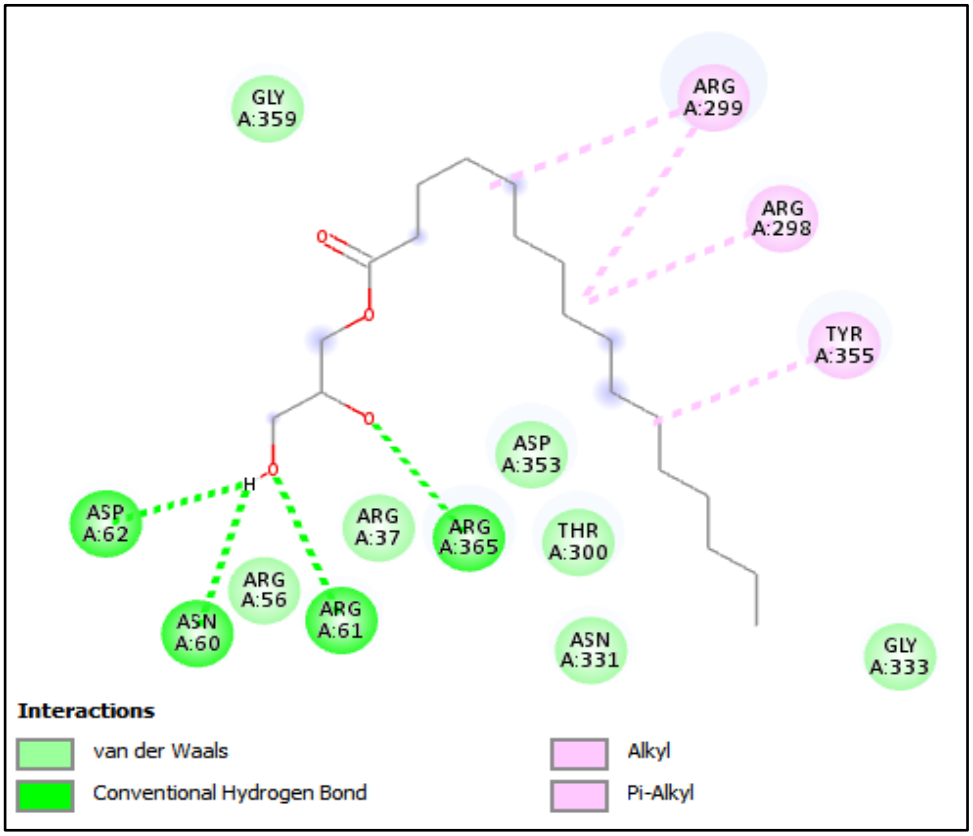
The methanolic extract achieved a 14 mm zone of inhibition against *C. albicans* at 200 mg/mL, while the aqueous extract reached 10 mm. *T. rubrum* was not inhibited by the methanolic extract at any concentration but showed sensitivity to the aqueous extract (11.6 mm at 200 mg/mL) (Figure 8).

Two-way ANOVA for antifungal zones of inhibition showed no significant main effects for organism type ($p = 0.170$) or extract type ($p = 0.818$). However, a significant moderate positive correlation ($r = 0.6238$, $p = 0.0098$) was found between extract concentration and the zone of inhibition.

For *C. albicans*, the MIC of the methanolic extract was 50 mg/mL and the MFC was 200 mg/mL. For *T. rubrum*, the aqueous extract revealed both MIC and MFC at 100 mg/mL (Figure 9). Statistical analysis showed no significant main effects for fungi type or extract type regarding MIC and MFC values.



With *C. albicans* Chitin Synthase 2



With *T. rubrum* KDNase

Figure 5: 2D representation of molecular interactions of Monopalmitin (an *A. indica* phytochemical) with fungal proteins

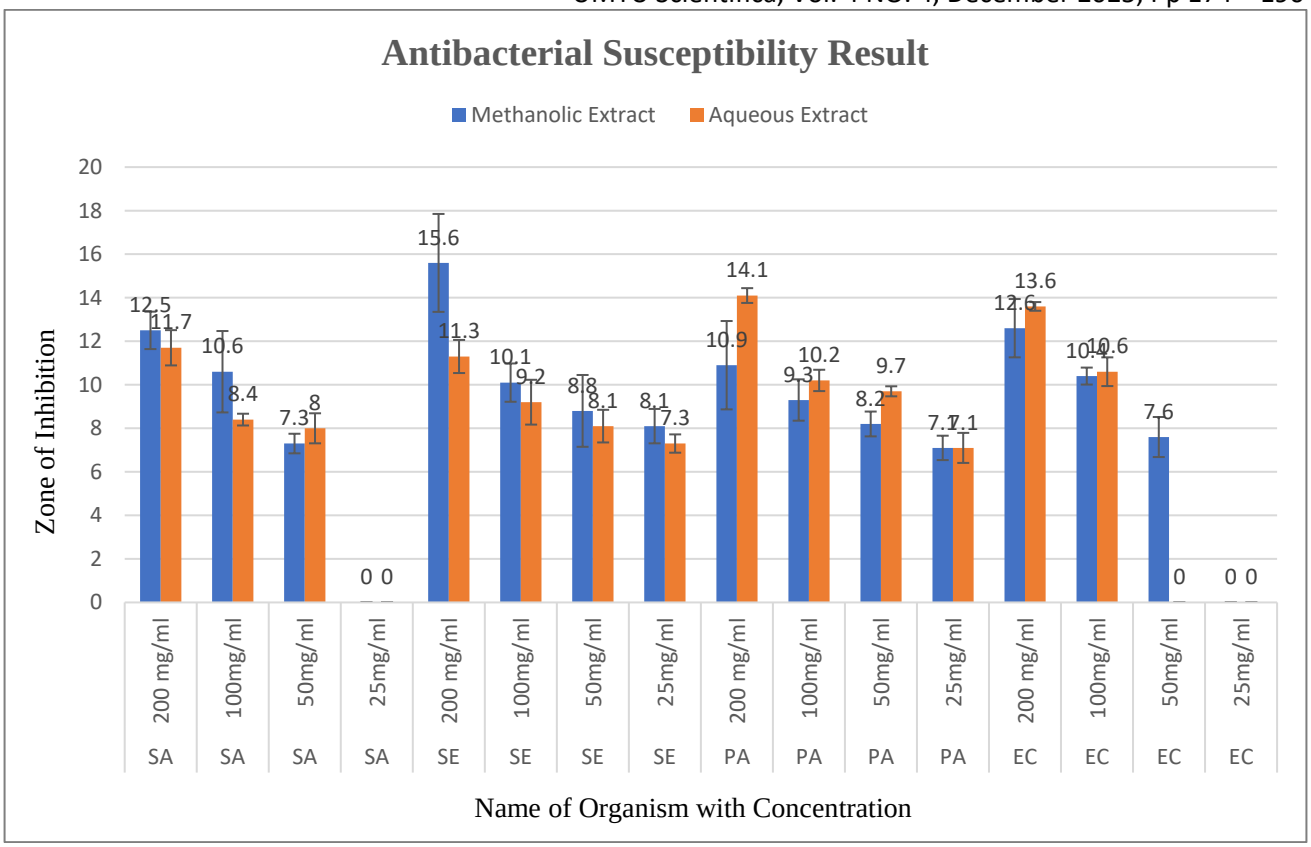


Figure 6: Antibacterial Activity of Methanolic and Aqueous Extracts of *A. indica* against Selected Bacteria: SA = *Staphylococcus aureus;* SE = *S. epidermidis;* PA = *P. aeruginosa;* EC = *E. coli*

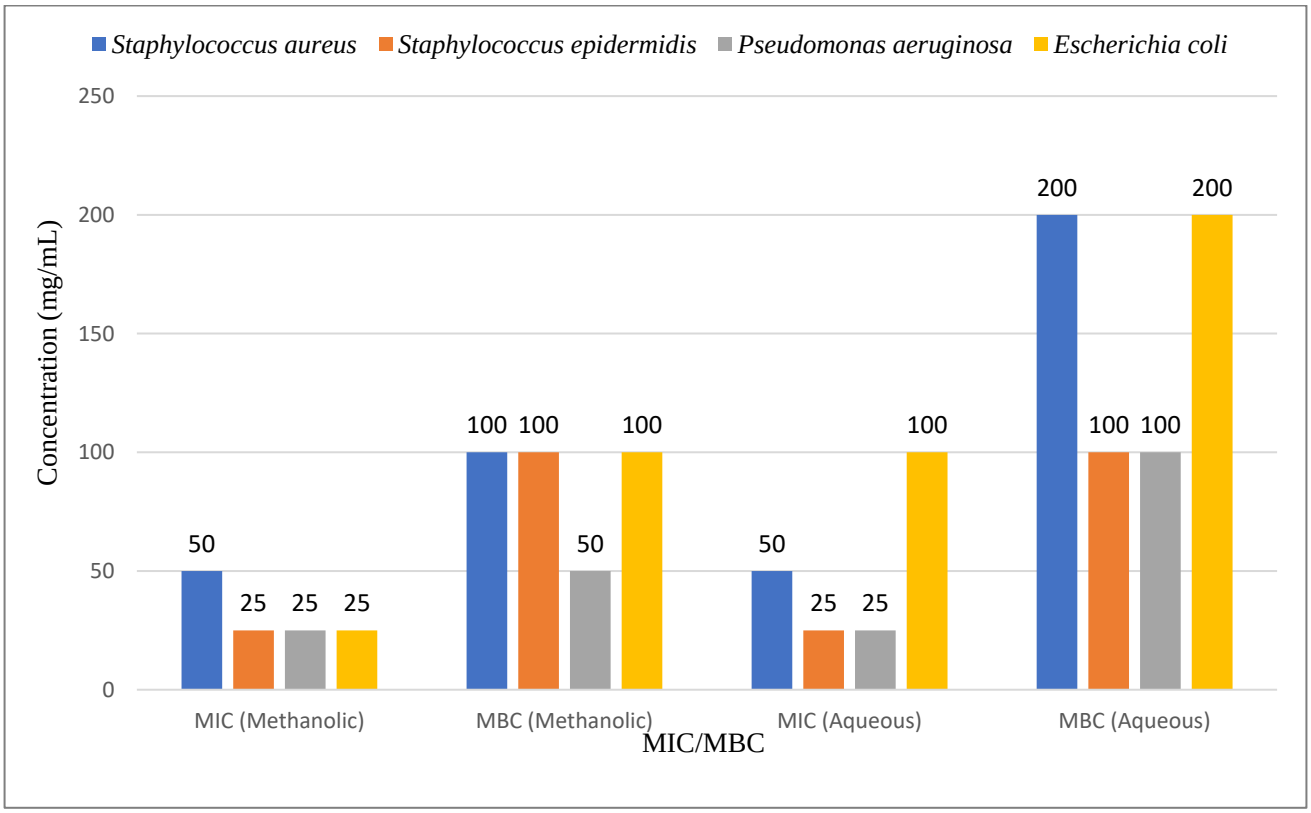


Figure 7: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *A. indica* Against Various Isolates

DISCUSSION

The results of this study demonstrate that the aqueous extract of *A. indica* produced a significantly higher percentage yield (10.78%) than the methanolic extract (p = 0.013). This aligns with previous reports by Sadat *et al.* (2018), who noted a similar advantage for water as an extraction solvent (14.4% aqueous vs. 9.4% methanolic). While the yield in the current study was slightly lower than

that of Sadat *et al.* (2018), this discrepancy may be attributed to their use of ultrasonic-assisted extraction (UAE) compared to the conventional methods employed here. Furthermore, while Francine *et al.* (2015) and Hikaambo *et al.* (2022) reported varying yields, ranging from 8.5% to 22.2% for aqueous extracts—the collective evidence reinforces that water is a highly effective solvent for *A. indica*. These variations likely reflect differences in plant source, leaf state (fresh vs. dried), and environmental factors.

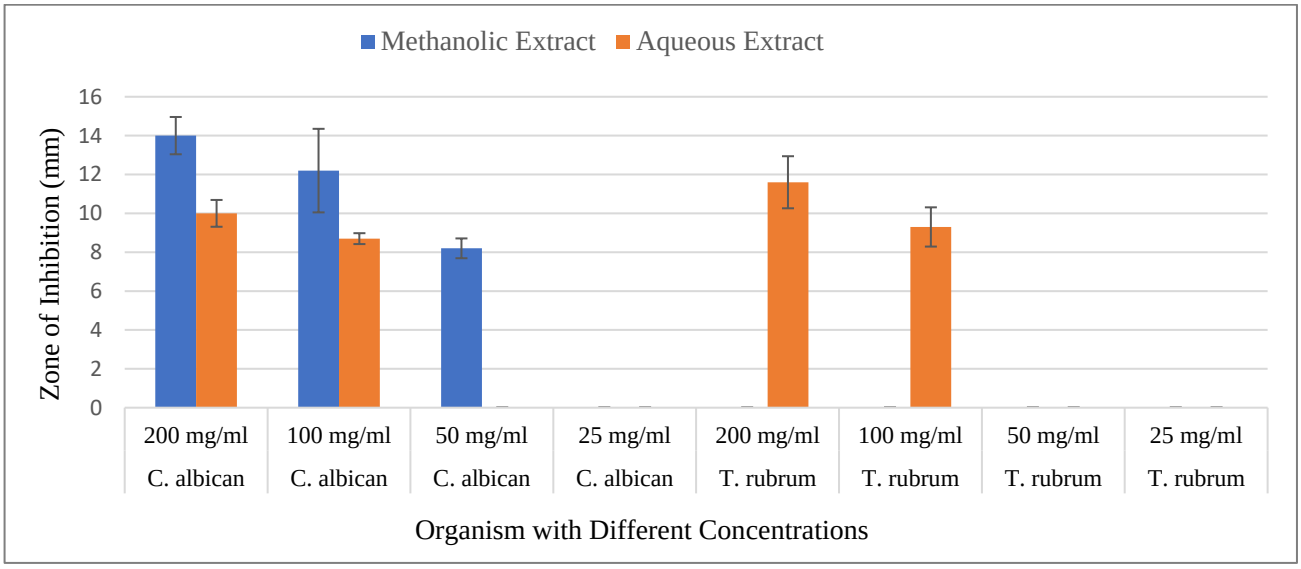


Figure 8: Antifungal Activity of Methanolic and Aqueous Extracts of *A. indica* against Selected Fungi

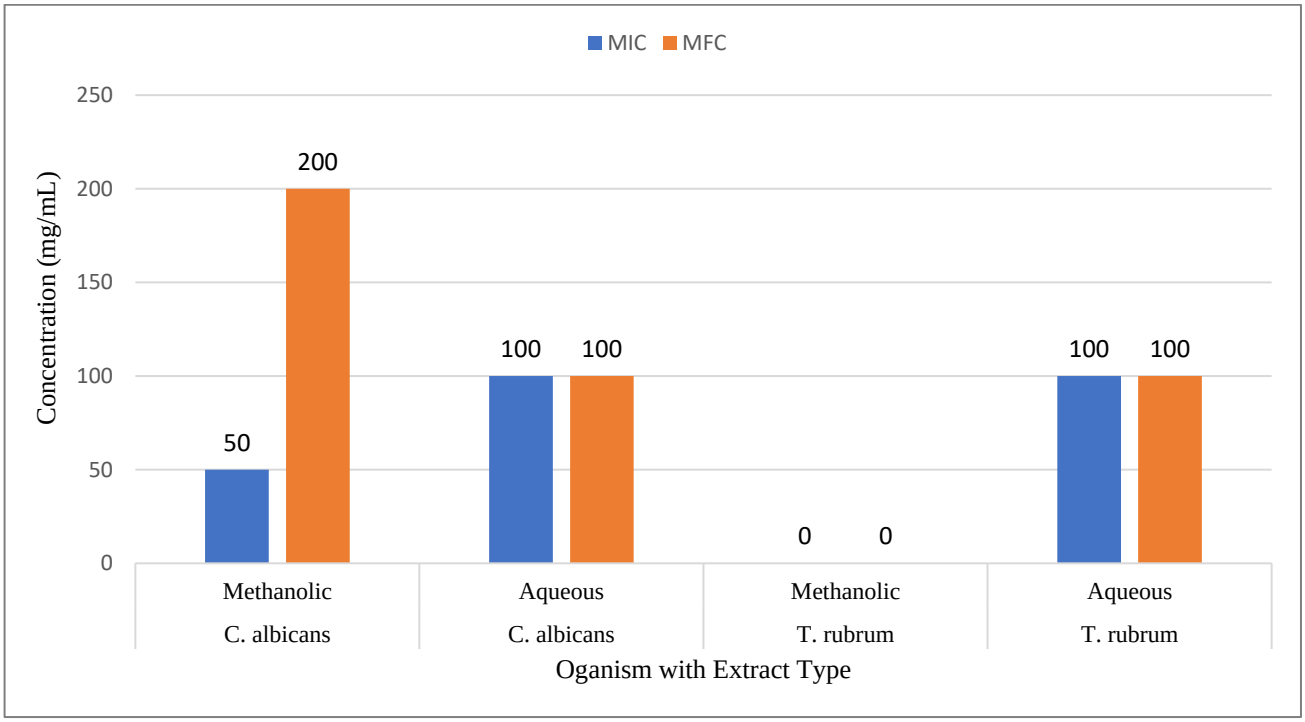


Figure 9: Minimum Inhibitory Concentrartion (MIC) and Minimum Fungicidal Concentrartion (MFC) of *A. indica* against Selected Fungi

Phytochemical screening revealed the presence of saponins, cardiac glycosides, flavonoids, steroids, and alkaloids in both extracts. While Ramadass and Subramanian (2018) reported an absence of terpenoids and steroids in ethanol and chloroform extracts, our study successfully identified these compounds, suggesting that solvent polarity and extraction methodology are critical to the resulting profile. These findings are consistent with Khanal (2021), who emphasized that the phytochemical composition of *A. indica* varies significantly depending on the plant part used.

GC-MS analysis further identified bioactive compounds with established antimicrobial and antiviral relevance, such as linoleoyl chloride, methyl caprate, and resorcinol (An *et al.,* 2021). Although both solvents yielded similar qualitative profiles, the methanolic extract showed higher abundances of phenolic and moderately nonpolar constituents, such as resorcinol (Hrichi *et al.,* 2020). Conversely, methyl vaccenate was more abundant in the aqueous extract. Compounds like methyl caprate and monopalmitin have demonstrated the ability to disrupt microbial lipid membranes, particularly in Gram-positive

bacteria, making them potential candidates for antiviral adjuvants (Jahanger *et al.,* 2022).

GC–MS analysis further revealed several phytochemicals with established antimicrobial relevance, with variations in composition and abundance reflecting solvent polarity effects (Sun *et al.,* 2025). Compounds such as linoleoyl chloride, methyl caprate, methyl linolelaidate, methyl vaccenate, monopalmitin, and resorcinol have been previously associated with antimicrobial and antiviral activities (An *et al.,* 2021). Six compounds were common to both extracts, though resorcinol and fatty acid derivatives were more abundant in the methanolic extract, suggesting more efficient extraction of phenolic and moderately nonpolar compounds by methanol (Hrichi *et al.,* 2020). This may partly explain the comparatively enhanced antimicrobial activity observed for the methanolic extract.

Several identified compounds, including methyl caprate and monopalmitin, are known to disrupt microbial lipid membranes and exhibit antibacterial effects, particularly against Gram-positive organisms. Although evidence for their antiviral activity remains largely theoretical or computational, their lipid-based structures suggest potential roles as antiviral agents or adjuvants against enveloped viruses (Olaleye *et al.,* 2018; Jahanger *et al.,* 2022).

ADMET and drug-likeness analyses provided insights into the pharmacokinetic and toxicity profiles of the selected compounds. Water solubility, a key determinant of oral bioavailability, was higher for compounds such as resorcinol and methyl caprate, which correlated with improved predicted absorption profiles (Jiang *et al.,* 2016; Rocha *et al.,* 2023; Huang *et al.,* 2023). Poorly soluble compounds, including linoleoyl chloride, may require formulation strategies to enhance bioavailability (Yu *et al.,* 2020). Predicted gastrointestinal absorption suggested that several compounds could be suitable for oral administration, while limited blood–brain barrier penetration restricted the CNS applicability of some phytochemicals.

Lipinski's Rule of Five analysis indicated that highly lipophilic compounds, such as linoleoyl chloride and methyl linolelaidate, violated standard drug-likeness thresholds, potentially affecting oral absorption (Elkamili *et al.,* 2023). However, known therapeutic agents that similarly violate these criteria demonstrate that such limitations do not necessarily preclude clinical relevance (Reese *et al.,* 2023).

Toxicity predictions suggested generally low hepatotoxic risk, though moderate nephrotoxicity scores observed for methyl vaccenate and methyl caprate indicate the need for caution. As these findings are based on in silico models, experimental validation remains essential to confirm safety and therapeutic viability.

Therefore, ADMET profiling identified monopalmitin, resorcinol, and methyl caprate as promising candidates for further drug development due to favorable absorption and drug-likeness properties. Nonetheless, potential toxicity concerns and limited BBB penetration underscore the need for further optimization and experimental validation before clinical application.

The molecular docking analysis demonstrates that *A. indica*–derived phytocompounds possess appreciable binding affinities toward key viral, bacterial, and fungal targets, indicating their potential to inhibit pathogen replication and growth. The ligands showed favorable interactions with HIV reverse transcriptase, integrase, and protease, with Methyl linolelaidate exhibiting the strongest overall affinity. In reverse transcriptase, Methyl linolelaidate formed a π–σ interaction with TRP226 and multiple alkyl contacts with LYS220, PHE224, VAL105, LEU231, TYR185, LEU97, and PRO92. Against integrase, it established a π–σ interaction with TRP76, alkyl interactions with ALA42, ALA73, LEU46, and TYR43, and a van der Waals contact with GLU40. In protease, its binding was dominated by alkyl interactions with ALA127, VAL146, LEU175, ILE131, LEU153, and VAL183. Collectively, these interaction patterns indicate a stable hydrophobic binding mode across HIV targets, supporting the potential inhibitory role of Methyl linolelaidate.

These findings are consistent with earlier reports that neem phytochemicals possess multitarget therapeutic potential with comparatively lower toxicity and added antioxidant and immunomodulatory benefits (Kai *et al.,* 2023; Mohanty *et al.,* 2023). Nonetheless, challenges remain, particularly for HIV, where latent reservoirs (Cossarini *et al.,* 2023) and high mutation rates (Gardner, 2020) complicate durable therapeutic success. Moreover, translation from in silico and in vitro efficacy to in vivo and clinical application is resource-intensive (Bailon *et al.,* 2022; Frattari *et al.,* 2023). Overall, this study establishes moderate-to-high binding of *A. indica* phytocompounds to viral, bacterial, and fungal targets, with Methyl linoleate, Linoleoyl chloride, and Monopalmitin emerging as promising leads warranting further in vivo and clinical evaluation.

The computational predictions were supported by antimicrobial susceptibility testing. Both methanolic and aqueous extracts of *A. indica* exhibited concentration-dependent antibacterial activity, with maximal inhibition at 200 mg/mL. The methanolic extract showed the highest overall activity (e.g., 15.6 mm against *S. epidermidis*), while the aqueous extract was most effective against *P. aeruginosa* (14.1 mm). These trends align with Hikaambo *et al.* (2022), who reported enhanced activity at higher concentrations and greater efficacy of aqueous extracts against *P. aeruginosa*. Variability across studies persists; Gupta *et al.* (2013) and Susmitha *et al.* (2013) reported greater potency of ethanolic extracts against *E. coli*, whereas no significant differences between extract types were observed here, underscoring the influence of extraction solvent and strain-specific susceptibility. Consistent with Herrera-Calderon *et al.* (2019) and Altayb *et al.* (2022), the present findings confirm the broad-spectrum and dose-dependent antibacterial activity of neem, attributed to bioactive constituents such as limonoids, triterpenoids, and flavonoids.

Regarding antifungal activity, methanolic extracts were most effective against *C. albicans* (≥14 mm at 200 mg/mL), while aqueous extracts produced moderate inhibition (10 mm). These observations accord with Mahmoud *et al.* (2011) and Herrera-Calderon *et al.* (2019). In contrast, *T. rubrum* was resistant to methanolic extracts but moderately susceptible to aqueous extracts (maximum 11.6 mm at 200 mg/mL), consistent with the strain-specific responses reported by Noites *et al.* (2023). The lack of methanolic activity against *T. rubrum* may reflect differential fungal susceptibility and extraction-dependent bioavailability, as suggested by Hashem *et al.* (2024).

Collectively, these results validate the antibacterial and antifungal potential of *A. indica*, particularly against *C. albicans*, while highlighting variable efficacy against *T. rubrum*. The observed concentration–response relationship further substantiates neem's antimicrobial promise and supports its development as a natural alternative to synthetic agents.

CONCLUSION

This study demonstrates that *A. indica* is a rich source of bioactive compounds with significant antimicrobial potential. Both aqueous and methanolic extracts contained key secondary metabolites, with the aqueous extract yielding slightly more material. GC–MS analysis identified shared compounds, among which monopalmitin, resorcinol, and methyl caprate exhibited favorable drug-likeness properties, although predicted nephrotoxicity in some candidates warrants caution. *In silico* analyses revealed that methyl linolelaidate, linoleoyl chloride, and monopalmitin possess strong and target-specific affinities toward HIV enzymes, bacterial proteins, and fungal enzymes, respectively. These computational predictions were corroborated by *in vitro* assays, which confirmed broad-spectrum, concentration-dependent antibacterial and antifungal activity, with methanolic extracts showing superior efficacy against bacterial strains and *Candida albicans*. Collectively, these findings position *A. indica* as a promising source of antimicrobial leads and underscore the need for further in vivo and clinical studies to validate safety, pharmacokinetics, and therapeutic efficacy.

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