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Antimicrobial Assessment and *In-silico* Molecular Docking of *Azadirachta indica* (Neem) Leaf Extracts on Some Multidrug Resistance Clinical Isolates

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Authors' contributions

This work was carried out in collaboration among all authors. Author OKT conducted the laboratory experiments, collected and analyzed the data, and contributed to drafting and writing the manuscript. Author KSO coordinated supervision, managed data curation, contributed to writing, reviewing, editing, and visualization, and reviewed the manuscript for intellectual content. Author AMD supervised data collection and validated the results. Author OSM provided critical feedback that helped shape the research direction. Author GTO contributed to the study design, statistical analysis, and preparation of figures and tables. All authors read and approved the final manuscript.

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ABSTRACT

Medicinal plants, as an effective source of traditional medicine, have genuine utility, and about 80% of the suburban population relies on plants as primary health care globally, especially in Africa, due to their availability and fewer complications. This study was designed to determine the antimicrobial effect of *Azadirachta indica extracts* in the context of G6PD deficiency and malaria co-infection on clinical isolates, comprising five (5) bacteria and four (4) fungi, using the agar well diffusion method. Minimum inhibitory and bactericidal concentration, including kinetic growth of the isolates, were determined by macrodilution and spectrophotometry methods. The aim of the study is to determine antimicrobial activities and phytochemical constituents of *Azadirachta indica* (Neem) on multidrug-resistant clinical isolates. The phytochemical and functional group profiling in the extracts were performed using GC–MS standard method and Fourier-Transform Infrared (FTIR) spectrophotometry. In-silico molecular docking analysis of the bioactive compounds in neem extract and oil was determined by computational modelling tools and the molecular Auto Dock software. Data were analysed using one-way ANOVA to compare the mean levels of significance of the parameters, where the level of significance was set as ($P<0.05$), and Duncan Multiple Range Test (DMRT) was used to compare the significance between the groups. The results showed that aqueous neem demonstrates narrow antimicrobial potential, while methanol, including oil of neem extracts, displayed broad antimicrobial potential, and aqueous neem gave the strongest inhibitory zone of 14 mm against *Escherichia coli.* Neem extracts demonstrate broad antifungal potential over amphotericin B, while methanolic neem has a 0.60 mm wide inhibitory zone against *Saccharomyces cerevisiae.* The minimum inhibitory concentration and minimum bactericidal concentration for neem oil were 2.00 mg/ml stronger. Growth kinetics indicated bacteriostatic effects of neem extracts on *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger*. Neem oil and powder contain 14 and 18 phytochemical profiles while α-D-Glucopyranose and n-Hexadecanoic acid were identified at the highest peak area of 21.98 % and 12.31% respectively. In-silico molecular docking identified diethylphthalate as a strong microbial protein inhibitor at -8.5 kcal, low binding energy with *S* aureus 1txt *and E. coli 2ZIP* proteins, while 9,17-octadecadienal (Z), methyl 10-methyl hexadecanoate, and oleic acid contributed to neem oil's antibacterial activity with low binding energy of -8.00 kcal with *S aureus* 1txt *and E. coli* 2ZIP proteins. However, the therapeutic importance of neem in combating pathogenic microbes and their sustainability as an alternative medicine is significant. The findings of this research acknowledge and scientifically validate the use of plants against microbial pathogenic activities.

Keywords: Azadirachta indica; antimicrobial; extracts; fourier-transform infrared; multidrug resistance; medicinal plants.

1. INTRODUCTION

Emergence of multidrug resistance and glucose 6 phosphate dehydrogenase deficiency resulting from infections and the use of synthetic pharmaceutical products for the treatment of infections has become a major public health threat. Glucose-6-phosphate dehydrogenase deficiency (G6PD deficiency) is an inherited genetic disorder characterised by reduced or absent activity of the G6PD enzyme. It is prevalent globally, especially in regions where malaria is endemic (Bello et al., 2024). The multidrug resistance phenomenon is an emerging challenge because the mycobacterium frequently develops resistance against the first-line drugs (Qazi et al., 2022). Medicinal plants as an effective source of traditional medicine have genuine utility, and about 80% of the suburban population relies on plants as primary health care globally, especially in Africa due to their availability and fewer complications (Wylie et al., 2022). Besides, this has been the ancient custom. In spite of the outstanding advancement in synthetic organic pharmacological products of the mother times, 25% of prescribed medicines in Western countries are derived directly or indirectly from plants (Islas et al., 2020). Plants employed in ancient medicine are still understudied with no scientific proof (Ahmed et al*.,* 2023), among these include *Azadirachta indica* (Neem).

Azadirachta indica, commonly known as neem, nim-tree or Indian lilac, is a sacred gift of nature. It is one of the two species in the genus *Azadirachta* and is native to the Indian subcontinent. It is typically grown in tropical and

semi-tropical regions, including Nigeria, and its fruits and seeds are the source of neem oil (Batra et al., 2022). Since the beginning of civilisation, medicinal plants have been an integral element of human society in the fight against disease. *Melia azedarach*, also known as *Azadirachta Indica* A. Juss, is doing well. Because of therapeutic values, Neem has been used for over 4000 years in Ayurvedic medicine (Ibrahim et al., 2023; Wylie & Merrell, 2022). Various ailments of mankind have been treated by the use of neem before any written records were available that had recorded history at its beginning (Su et al*.,* 2023). It has been used in different systems of medicine like Ayurveda and Unani. Every part of Neem, viz., leaf, flower, fruit, seed, kernel bark, root, wood, twig, oil and their purified products, possesses medicinal properties and have been used in traditional remedies (Baby et al., 2022). Oil from the leaves, seeds, and bark possesses a wide spectrum of antibacterial action against Gram-positive and Gram-negative bacteria (WHO, 2019). Thus, screening *Azadirachta indica* (Neem) for its antimicrobial potential and scientific validation of its therapeutic significance is important towards discovering novel antimicrobial drugs with comparatively fewer complications.

However, the aim of the study is to determine antimicrobial activities and phytochemical constituents of *Azadirachta indica* (Neem) on multidrug-resistant clinical isolates.

2. METHODOLOGY

2.1 Study Area

Iree, located in Osun State, Nigeria, is a semi-urban town known for its hilly landscape, thriving agricultural practices, traditional markets, and educational institutions, including Osun State Polytechnic, serving as a hub for commerce, culture, and academic activities.

2.2 Collection of Plant Material

The fresh leaves of the *Azadirachta indica* (neem) were collected from its natural habitat in Iree, Osun State, Nigeria, during early hours of the day and were authenticated by a botanist in the department of Biology, Osun State Polytechnic Iree as *Azadirachta indica.*

2.3 Sample Preparation

The plant sample- *Azadirachta indica* (neem) fresh leaves were thoroughly rinsed under running tap water to remove soil. The rinsed leaves were air-dried at room temperature for 28 days (4 weeks) and blended to a powdery form using an electrical blender and stored in a polythene bag for further analysis following the literature standard methods.

2.4 Authentication of Clinical Isolates

The clinical isolates (*Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Bacillus subtilis, Bacillus licheniformis, Pseudomonas aeruginosa, Salmonella typhi, Aspergillus niger . Penicillium digitatum, Candida albicans,* and *Saccharomyces cerevisiae* were obtained from Osun State University Teaching Hospital, Osogbo. The bacterial isolates were subjected to staining and some biochemical tests (indole, methyl-red, Voges-Proskauer and Citrate utilisation test) and viewed under the microscope using x100 magnification with oil immersion, while the fungal isolates were subjected to morphological characterisation.

2.5 Preparation of Crude Extract of *Azadirachta indica* (Neem)

The air-dried *Azadirachta indica* (neem) leaves were blended into powder form with an electronic blender. 50g each of the powdered sample was soaked in 200ml of water and methanol, respectively, in a reagent bottle for 7 days to permit full extraction of the active ingredient. After 7 days, the extract was filtered and stored in anair-tight container for further analysis and was then kept in a refrigerator prior to use.

2.6 Extraction of *Azadirachta indica* (neem) *and* Oil

Extraction of the plant and its oil was performed by Soxhlet apparatus with two solvents (ethanol and n-hexane) and distillation apparatus, respectively. 100g *Azadirachta indica* (neem) was separately distilled in a Soxhlet extractor using (Ogunleye et al*.,*2019). The process took 16 hours before it was completed.

2.7 Preparation of Medium

Mass weight of 28 grams of nutrient agar and potato dextrose agar were weighed according to the manufacturer's prescription as described by Tiwari and Jadhav (2021) method using an electronic balance and autoclaved at 121°C for 15 minutes to sterilise the media before use.

2.8 Morphological and Biochemical Characterisation of the Clinical Isolates

Gram staining screening of the isolates was performed according to the method described by (Ali et al*. (*2021) and Famurewa and David (2009), while a biochemical test for the identification of bacterial isolates was carried out using the test described by the standard method.

2.9 Evaluation of Minimum Inhibitory Concentration (MIC)

Different concentrations of the extracts were prepared to obtain 2.5mg/ml, 5.0mg/ml, 7.5mg/ml,10.0mg/ml and 12.5mg/ml, respectively, using Pingali et al*.,* (2020). Three drops of the overnight broth culture of the test organism were inoculated into the dilutions and incubated at 37° C for 24 hours. The lowest concentration of the extracts that inhibited the growth of the test organisms is recorded as the minimum inhibitory concentration (MIC). The MIC was taken as the lowest concentration that prevented visible growth.

2.10 Evaluation of Bactericidal Concentration (MCB) Test

The minimum bactericidal concentration was determined according to the National Committee for Clinical Standards (2020). From the test tubes used in the determination of MIC, the tubes that showed no visible growth were subcultured onto freshly prepared Mueller-Hinton agar and incubated at 37°C for 48hours. The lowest concentration at which the organisms did not recover and grow was taken as the MBC.

2.11 Kinetic Study of the *Azadirachta indica* (neem) Extract

An overnight broth culture of the isolates (5ml) was mixed with fresh nutrient broth (45ml), followed by the addition of 2ml of the aqueous, ethanolic and oil extracts of the samples. The mixture was thoroughly shaken on a mechanical shaker. The optical density (427nm) was determined at 50-minute intervals for 4hours by spectrophotometer using Sarkar et al*.,* (2021) method.

2.12 Phytochemical Analysis of *Azadirachta indica* (Neem) Extract

2.12.1 Instrumentation and methodology of GC-MS analysis

The samples were subjected to GC-MS analysis to determine a representative spectral output of all the ascertainable compounds from the empirical sample. According to the method described by Muhammad & Kashere (2021). After GC-MS separation, all the peaks were compared with the standard structural library of fatty acids to determine the probable fatty acid composition of the samples. The MS scan range was set from 40-800Da. Identification of compounds was obtained by comparing the retention times with those of authentic compounds and with the spectral data obtained from the data library of the corresponding compounds. Quantities of the compounds were represented as relative area percentage derived from the integrator. Identification of phytochemical components was conducted using the database of National Institute of Standards and Technology MS library (NIST-MS library comparing the spectrum obtained through GC-MS, and compounds present in the samples were identified.

2.13 Molecular Docking Analysis of Azadirachta *indica* (Neem) Extracts

The molecular docking analysis of *Azadirachta indica* (neem) extracts typically involves studying the binding interactions of bioactive compounds from these extracts with specific target proteins, often associated with therapeutic effects or pathogen inhibition. according to the method described by Rajendran et al*.,* (2021), this process begins with identifying and isolating the active compounds present in neem such as azadirachtin. These compounds are then structurally characterised, using computational modelling to generate a 3D structure of active compounds to dock microbial protein with molecular docking software such as AutoDock. The software calculates the binding affinity, scoring how well each compound fits within the binding pocket and predicting the stability of the ligand-protein complex based on binding energy. Lower binding energies indicate stronger binding interactions.

2.14 Statistical Analysis

Data were analysed using one-way ANOVA to compare the mean levels of significance of the parameters, where the level of significance was set as ($P<0.05$), and Duncan Multiple Range Test (DMRT) was used to compare the significance between the groups. Excel Plot was used to plot the graphs.

3. RESULTS AND DISCUSSION

Neem aqueous demonstrates narrow spectrum antimicrobial potential and highest inhibitory zone of 14.00 mm was obtained against *Escherichia coli,* followed by *Bacillus subtilis as well as Staphylococcus aureus, Pseudomonas auruginosa* and *Salmonella typhi* ,while methanolic and oil of the neem extracts showed wide antimicrobial spectrum and a high inhibitory zone against gram-positive and gram-negative isolates and Neem extracts demonstrated broad antimicrobial potential over amphotericin B, while methanolic neem has a 0.60 mm wide inhibitory zone against *Saccharomyces cerevisiae.*

Aqueous garlic has a narrow inhibitory effect, while methanol and oil of garlic showed broad antimicrobial spectra and the highest zone of inhibition of 0.80 mm against *Penicillium digitatum* compared to other fungi isolates Resistance of Candida *albicans against* garlic aqueous was also noted, and the differences are not significant ($P < 0.05$). This inhibitory effect of neem extracts varies with the stronger inhibitory activity of methanol and oil of neem leaf extracts on bacteria isolates as reported by Altayb et al*.,* (2022) and Suttiarporn et al*.,* 2020*).* Though resistance against aqueous neem was observed in inhibition zones of both *Proteus mirabilis* and *Bacillus lichenoformis* i.e, gram-positive and gram-negative isolates. The resistance of certain isolates against aqeous neem in this study is in line with (Mudenda et al., 2023) that ethanolic extract of neem exhibits wide and significant antibacterial activity against various clinical

Table 1. Morphological identification of clinical fungi isolates

Organisms	Features
Aspergillus niger	It has powdery texture with dark fuzzy growth and had extremely high growth rate.
Penicillum digitatum	It has brown root with green colouration.
Candida albicans	It produced a germ tube after inoculation in protein medium at 35^0C for 3 hours.
Saccharomyces cerevisiae	It showed unicellular and cylindrical spherical in shape, budding form a protrusion on existing cell, enlarge and then break away.

Table 2. Biochemical identification of clinical bacteria isolates

Organism	Indole Test	M.R Test	V.P Test	Citrate Test	Gram Rxn	Shape
Staphylococcus aureus	-	-	+	-	+	Cocci (spherical)
Proteus mirabilis	-	+	-	+	-	Bacilli (rod-shaped)
Escherichia coli	+	+	-	-	-	Bacilli (rod-shaped)
Bacillus subtilis	-	-	+	+	+	Bacilli (rod-shaped)
Bacillus licheniformis	-	-	+	+	+	Bacilli (rod-shaped)
Pseudomonas aeruginosa	-	-	-	+	-	Bacilli (rod-shaped)
Salmonella typhi	-	+	-	+	-	Bacilli (rod-shaped)

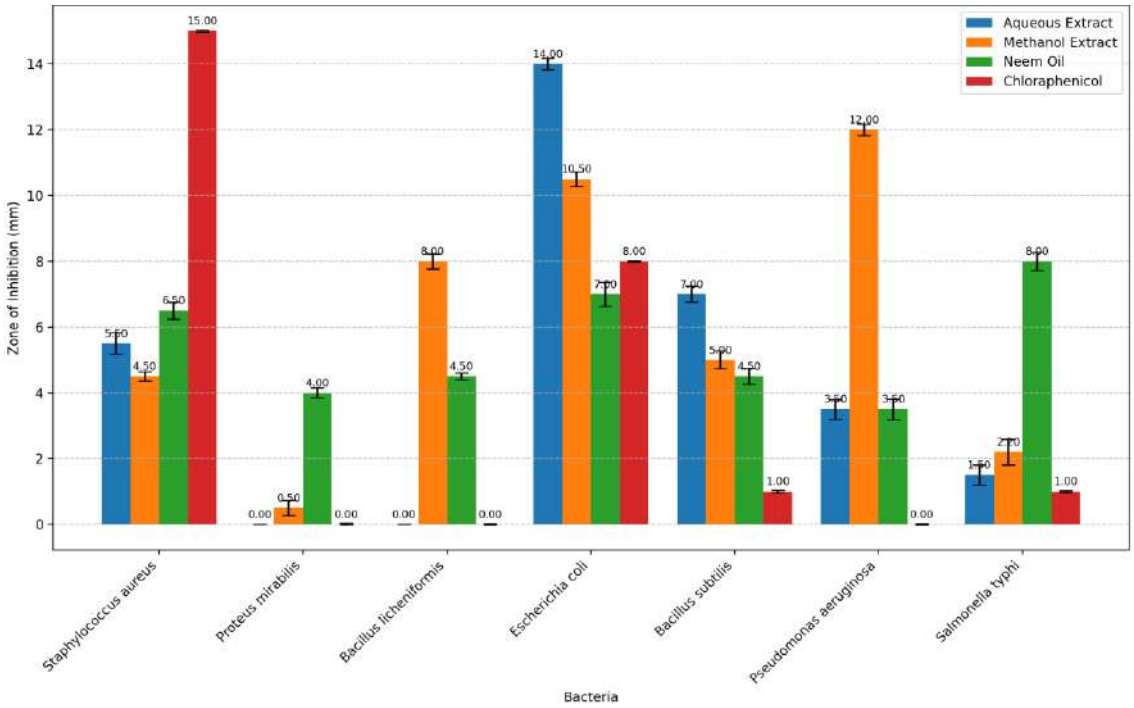


Fig. 1. Antimicrobial effect of *Azardiracta indica* (Neem) extracts on clinical bacteria pathogens

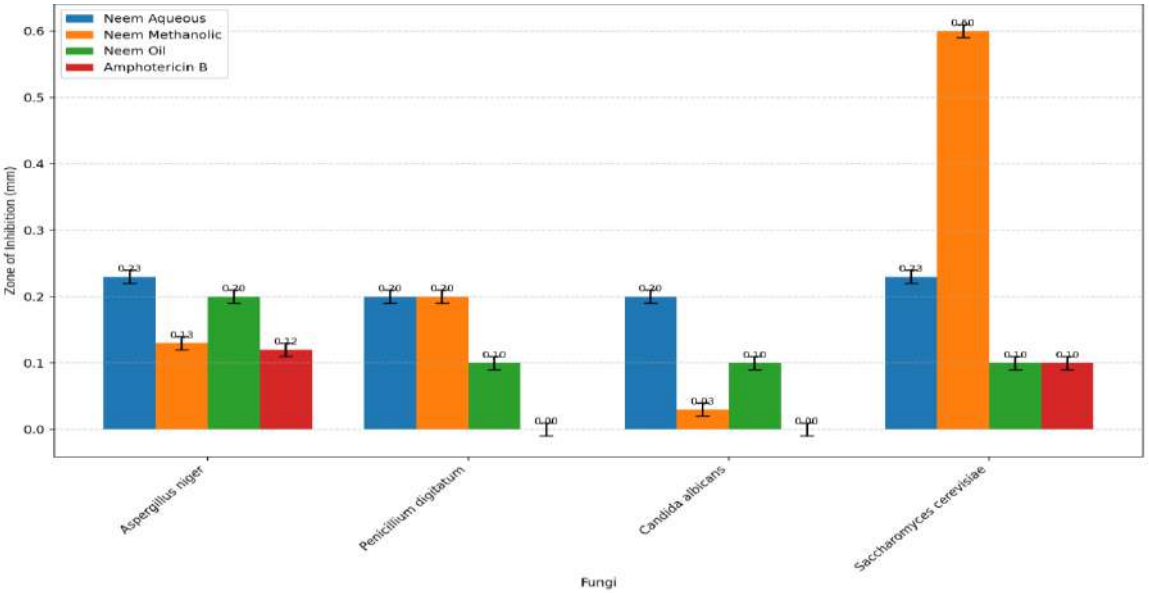


Fig. 2. Antimicrobial effect of neem extracts on clinical fungi isolates

isolates and (Baby et al., 2022) which states that neem oil has a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms which is attributed to the presence of azardirachtin that acts as anti-microbial active compound in the neem. Neem extracts demonstrate broad antifungal potential over amphotericin B, while methanolic neem has 0.60 mm wide inhibitory zone against *Saccharomyces cerevisiae.* The findings agree with Kumar et al., (2020) and are attributed to the antimycotic effect of neem extract.

Table 3. Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration of Azadirachta indica (Neem) extract against bacteria isolates

	Neem Aqueous		Neem Methanolic		Neem Oil	
Isolates	**MIC ± SEM (mm)**	**MBC ± SEM (mm)**	**(MIC ± SEM) (mm)**	**(MBC ± SEM) (mm)**	**(MIC ± SEM) (mm)**	**(MBC ± SEM) (mm)**
S. aureus	10.00 ± 0.29^{c}	12.50 ± 0.35^{c}	5.00 ± 0.15^{b}	10.00 ± 0.28^{b}	2.50 ± 0.10^{a}	2.50 ± 0.10^{a}
P. mirabilis	2.50 ± 0.08^{a}	10.00 ± 0.28^{b}	7.50 ± 0.20^{c}	10.00 ± 0.30^{b}	2.50 ± 0.09^{a}	2.50 ± 0.08^{a}
E. coli	12.50 ± 0.35^{c}	10.00 ± 0.30^{b}	10.00 ± 0.26^{b}	12.50 ± 0.34^{c}	2.50 ± 0.10^{a}	2.50 ± 0.10^{a}
B. subtilis	2.50 ± 0.09^{a}	12.50 ± 0.36^{c}	5.00 ± 0.15^{b}	10.00 ± 0.28^{b}	2.50 ± 0.10^{a}	2.50 ± 0.09^{a}
P. aeruginosa	2.50 ± 0.10^{a}	5.00 ± 0.14^{b}	5.00 ± 0.16^{b}	10.00 ± 0.25^{c}	2.50 ± 0.08^{a}	2.50 ± 0.07^{a}
B. licheniformis	2.50 ± 0.09^{a}	7.50 ± 0.20^{b}	5.00 ± 0.14^{b}	10.00 ± 0.27^{c}	2.50 ± 0.09^{a}	2.50 ± 0.08^{a}
S. typhi	10.00 ± 0.27^{c}	10.00 ± 0.25^{b}	5.00 ± 0.13^{b}	10.00 ± 0.26^{b}	2.50 ± 0.10^{a}	2.50 ± 0.10^{a}

Legend

a, b, c, d = Duncan significance group at P < 0.05
Means with same letter are not significantly different
Means with different letters are significantly different
SEM = Standard Error of Mean (after ±)

Table 4. Minimum inhibitory and minimum fungicidal concentration of Azadirachta indica (neem) extract against the clinical fungi isolates

Isolate	**Aqueous Neem**		**Methanolic Neem**		**Neem Oil**	
	MIC ± SEM (mm)	**MBC ± SEM (mm)**	**MIC ± SEM (mm)**	**MBC ± SEM (mm)**	**MIC ± SEM (mm)**	**MBC ± SEM (mm)**
Aspergillus niger	5.00 ± 0.15^{c}	5.00 ± 0.15^{b}	2.50 ± 0.08^{a}	7.50 ± 0.22^{c}	2.50 ± 0.09^{a}	2.50 ± 0.08^{a}
P. digitatum	5.00 ± 0.14^{c}	5.00 ± 0.13^{b}	2.50 ± 0.10^{a}	10.00 ± 0.30^{c}	2.50 ± 0.09^{a}	2.50 ± 0.08^{a}
C. albicans	5.00 ± 0.15^{c}	10.00 ± 0.28^{c}	2.50 ± 0.08^{a}	2.50 ± 0.09^{a}	2.50 ± 0.07^{a}	2.50 ± 0.08^{a}
S. cerevisiae	7.50 ± 0.20^{c}	7.50 ± 0.21^{c}	2.50 ± 0.09^{a}	7.50 ± 0.22^{c}	2.50 ± 0.08^{a}	2.50 ± 0.08^{a}

Legend

a, b, c, d = Duncan significance group at P < 0.05
Means with same letter are not significantly different
Means with different letters are significantly different
SEM = Standard Error of Mean (after ±)

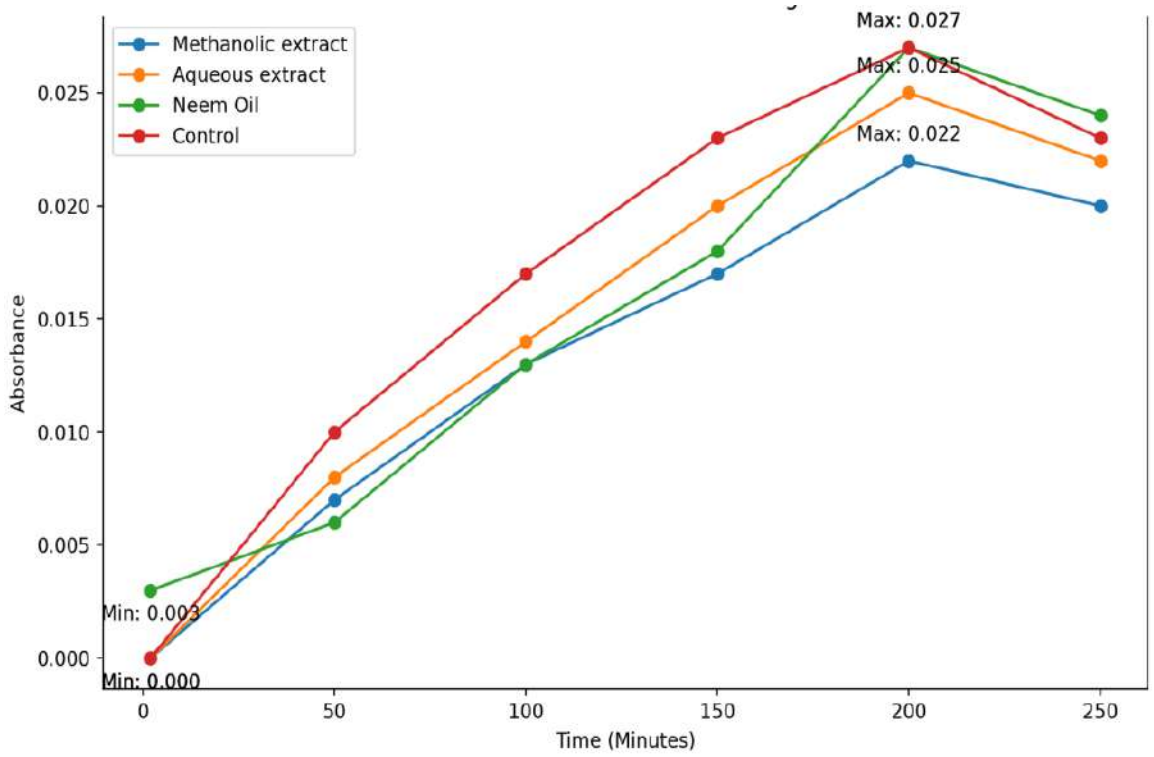


Fig. 3. Kinetic growth curve of *Azadirachta indica* (neem) aqeous, methanolic and oil extracts against Staphylococcus aureus

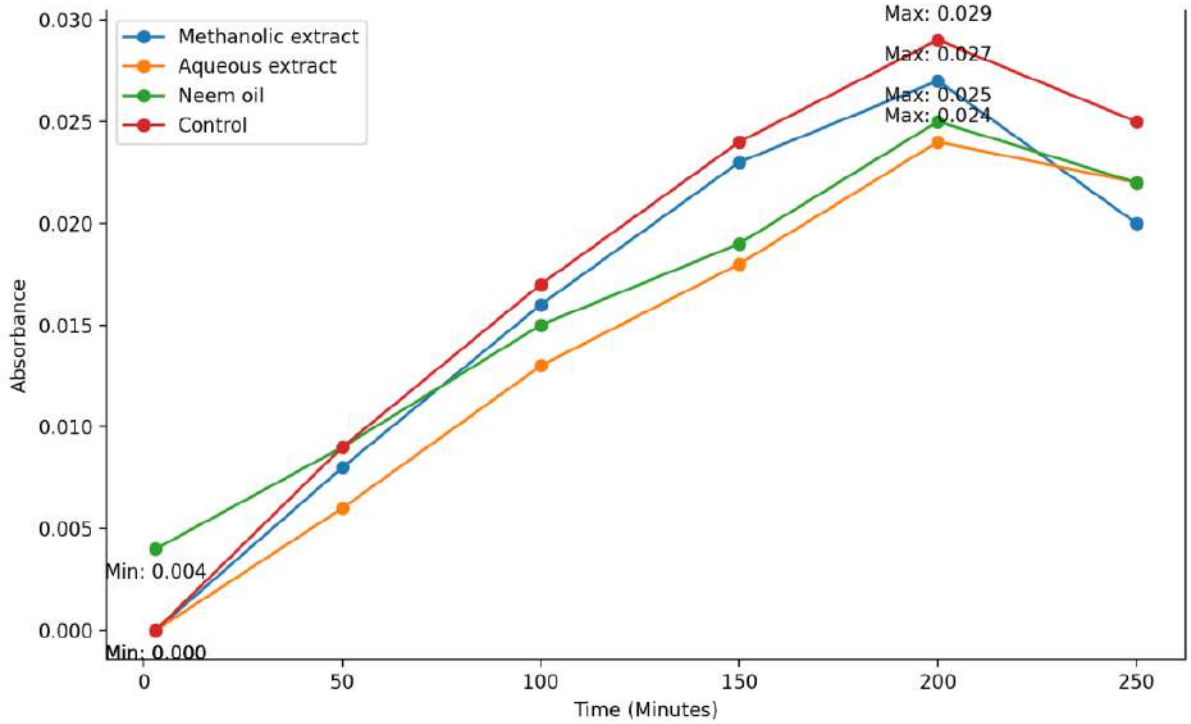


Fig. 4. Kinetic growth curve of Azadirachta indica (neem) aqeous, methanolic and oil extracts against *Escherichia coli*

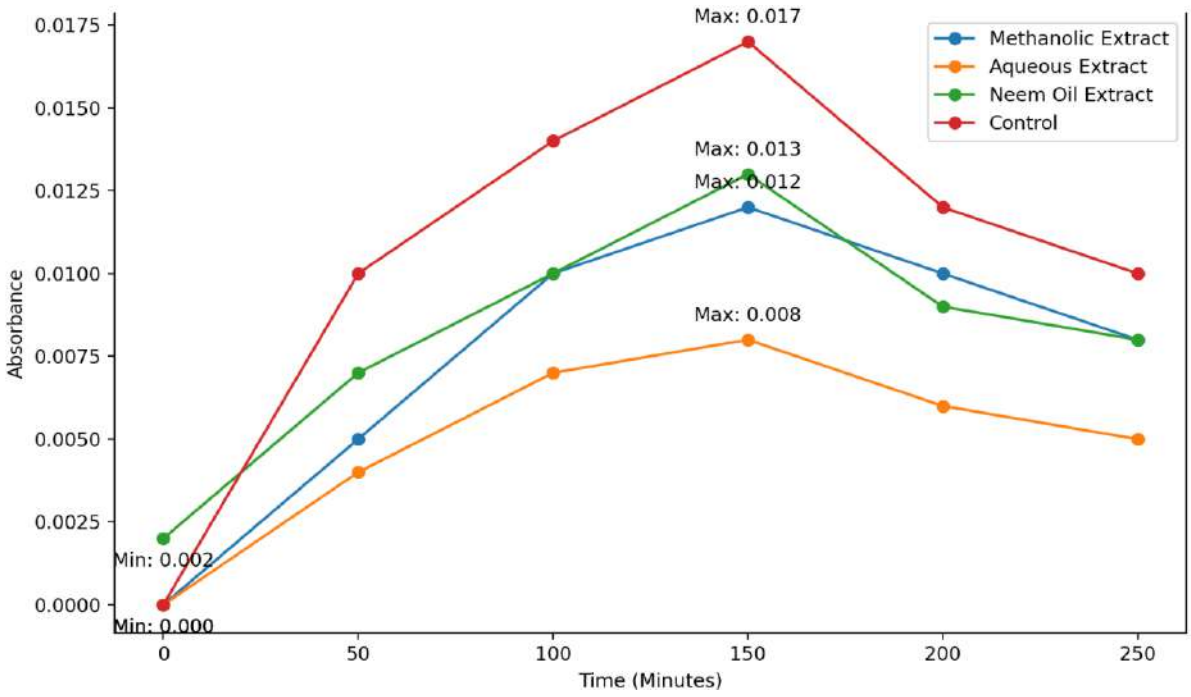


Fig. 5. Kinetic growth curve of Azadirachta indica (neem) aqeous, methanolic and oil extracts against *Candida albicans*

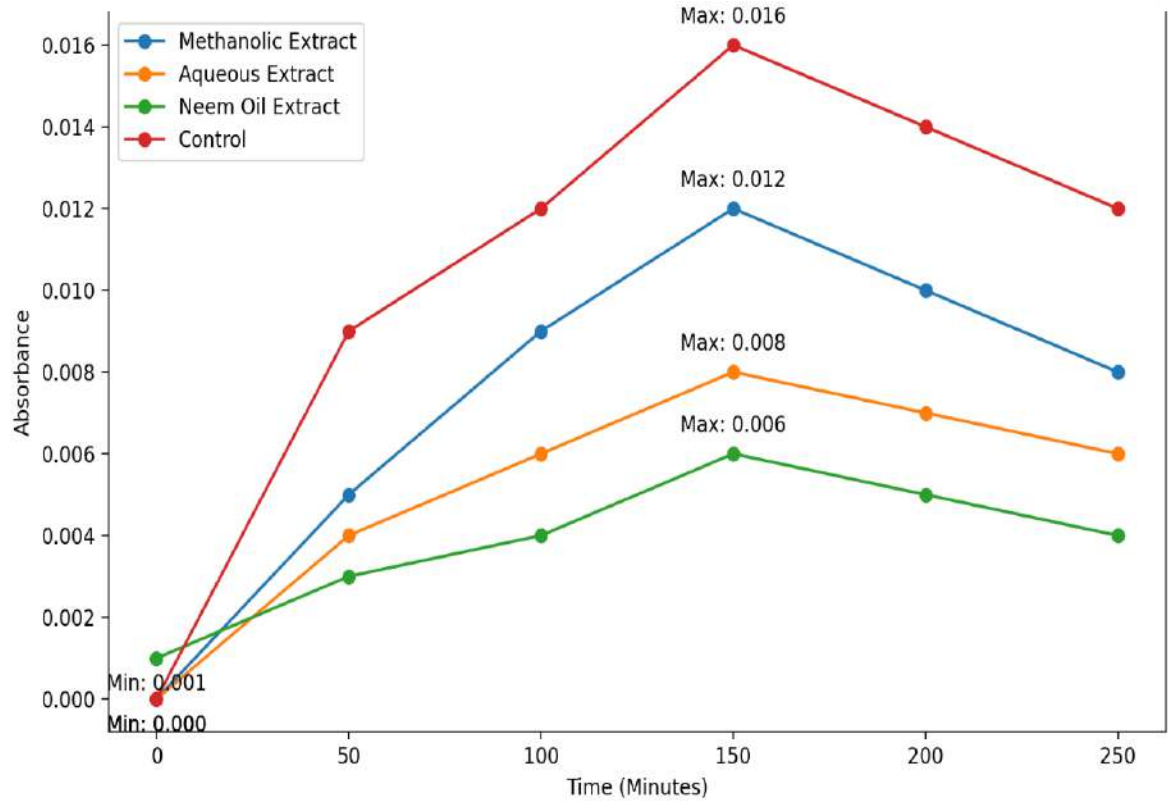
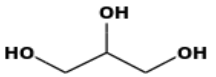
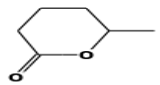
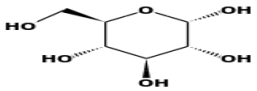
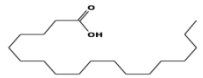
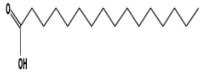


Fig. 6. Kinetic growth curve of Azadirachta indica (neem) aqeous, methanolic and oil extracts against *Aspergillus niger*

Table 5. Phytochemical analysis of *Azadirachta indica* (neem) oil

Peak	RT	Name of Gas	M. Formula	M. Mass	Peak Area (%)	% Comp	Mass frag m/z	Structure
1	3.81	Glycerine	$C_3H_8O_3$	92	4.33	5.47	43, 61, 92	
2	4.80	2H-Pyran-2-one, tetrahydro-6-methyl-	$C_6H_{10}O_2$	114	4.49	3.24	42, 70, 114	
3	5.50	α-D-Glucopyranose	$C_6H_{12}O_6$	180	21.98	21.13	45, 67, 180	
4	8.32	14-Methyl-8-hexadecyn-1-ol	$C_{17}H_{32}$	252	6.63	7.00	43, 68, 252	
5	10.78	Octadecanoic acid	$C_{18}H_{36}O_2$	284	7.56	8.25	43, 73, 284	
6	12.64	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264	6.80	7.15	41, 60, 264	
7	13.18	n-Hexadecanoic acid	$C_{18}H_{26}O$	256	9.14	10.27	43, 109, 258	
8	15.25	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	3.37	4.59	41, 67, 294	
9	17.28	Oleic acid	$C_{18}H_{34}O_2$	282	7.91	7.17	41, 55, 282	
10	19.24	Methyl 10-methylhexadecanoate	$C_{18}H_{36}O_2$	284	4.07	2.65	74, 143, 284	

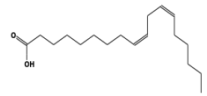
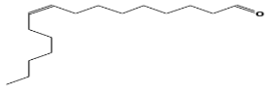
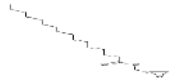
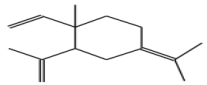
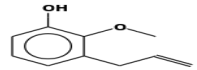
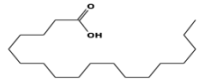
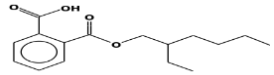
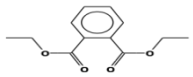
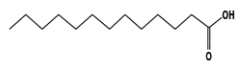
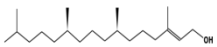
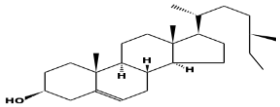
Peak	RT	Name of Gas	M. Formula	M. Mass	Peak Area (%)	% Comp	Mass frag m/z	Structure
11	20.50	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	16.12	17.98	67, 81, 280	
12	20.82	7-Pentadecyne	$C_{15}H_{28}$	208	0.72	0.13	68, 81, 208	
13	23.25	cis-9-Hexadecenal	$C_{16}H_{30}O$	238	2.91	1.97	41, 55, 238	
14	24.98	GlycidylPalmitate	$C_{19}H_{36}O_3$	312	3.84	2.95	98, 115, 312	

Table 6. Phytochemical analysis of*Azadirachta indica* (neem) powder

Peak	RT	Name of Gas	M. Formula	M. Mass	Peak Area (%)	% Comp	Mass frag m/z	Structure
1	5.16	p-Xylene	C_8H_{10}	106	7.60	6.84	51, 91, 106	
2	5.75	Oxime-, methoxy-phenyl-	$C_8H_9NO_2$	151	1.55	2.11	51, 91, 151	
3	6.00	γ-Elemene	C_9H_{12}	204	4.20	3.73	91, 91, 120	
4	7.25	n-Hexadecanoic acid	$C_{10}H_{16}$	256	12.31	11.63	43, 73, 256	

Peak	RT	Name of Gas	M. Formula	M. Mass	Peak Area (%)	% Comp	Mass frag m/z	Structure
5	7.50	Phenol, 2-methoxy-3-(2-propenyl)-	$C_{10}H_{12}O_2$	164	1.81	2.52	41, 47, 142	
6	9.03	Octadecanoic acid	$C_{18}H_{36}O_2$	284	11.59	12.98	43, 73, 284	
7	9.50	Octadecanoic acid, methyl ester	$C_{19}H_{34}O_2$	294	7.97	7.41	39, 115, 116	
8	10.25	Mono(2-ethylhexyl) phthalate	$C_{16}H_{22}O_4$	278	5.80	6.21	70, 149, 278	
9	11.25	Diethyl Phthalate	$C_{12}H_{14}O_4$	222	10.40	11.24	65, 149, 222	
10	12.00	Tridecanoic acid	$C_{13}H_{26}O_2$	214	7.25	3.21	43, 73, 214	
11	12.80	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	7.97	8.10	43, 79, 292	
12	14.75	Phytol	$C_{20}H_{40}O$	296	4.35	3.73	43, 71, 296	
13	16.00	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	7.61	8.02	43, 81, 296	
14	16.25	8,11,14-Eicosatrienoic acid	$C_{20}H_{34}O_2$	306	2.70	3.14	41, 63, 306	
15	17.80	Tricosane	$C_{23}H_{48}$	324	1.27	1.64	43, 57, 324	

Peak	RT	Name of Gas	M. Formula	M. Mass	Peak Area (%)	% Comp	Mass frag m/z	Structure
16	19.16	Nonacosane	$C_{29}H_{60}$	408	2.17	3.10	43, 57, 408	
17	19.50	γ-Sitosterol	$C_{29}H_{50}O$	414	1.99	2.18	43, 55, 414	
18	23.25	Tetratriacontane	$C_{34}H_{70}$	478	1.09	2.14	43, 57, 478	

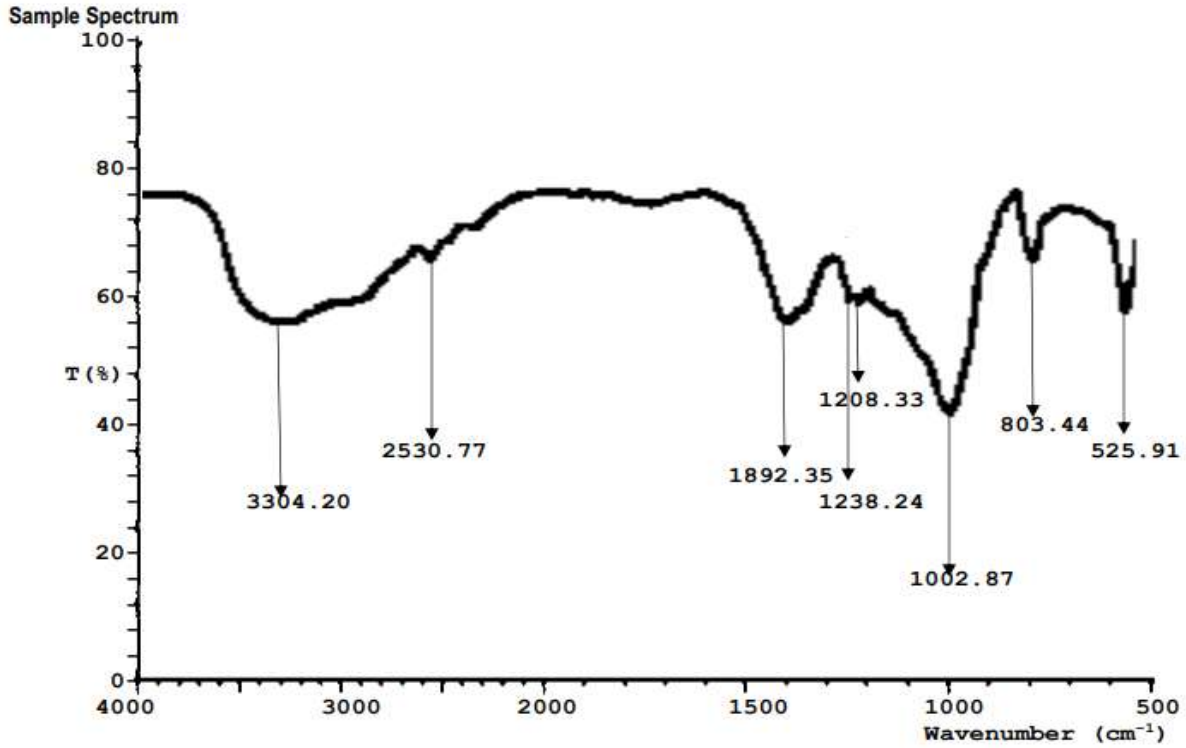


Fig. 7. FTIR analysis of *Azadirachta indica* (neem) oil

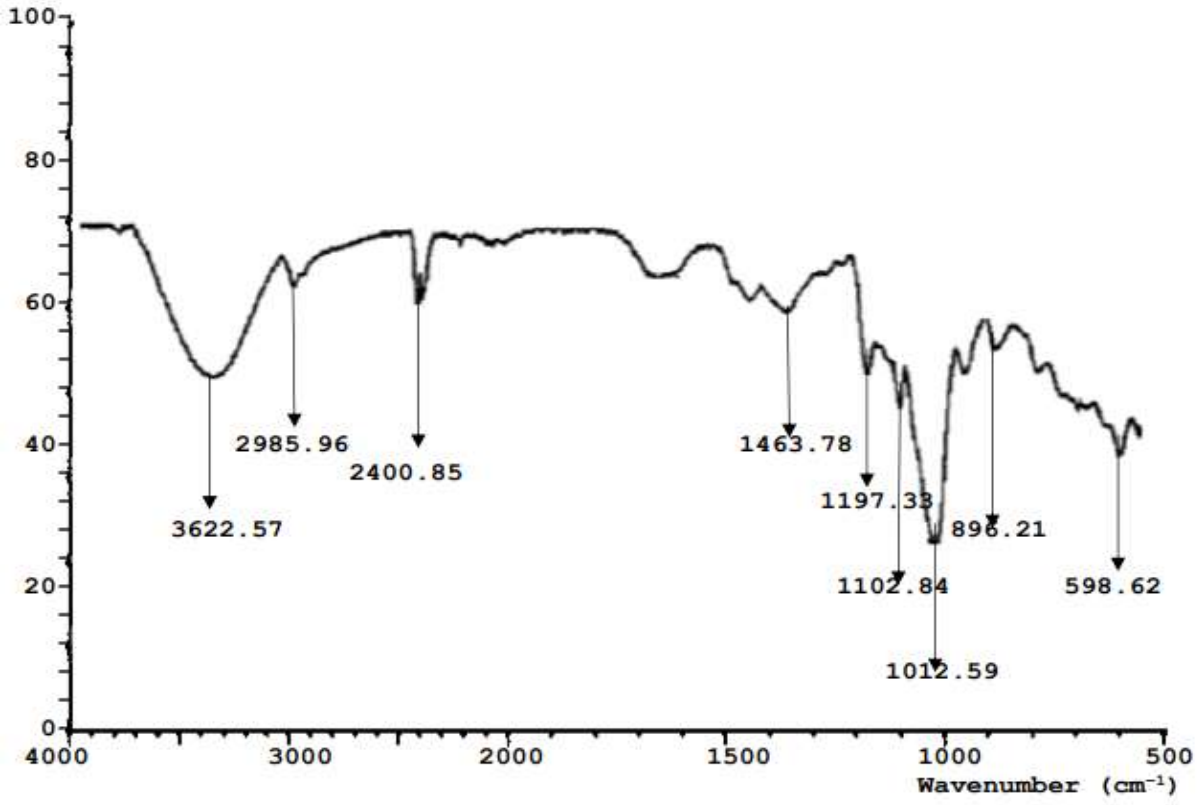


Fig. 8. FTIR analysis of *Azadirachta indica* (neem) powder

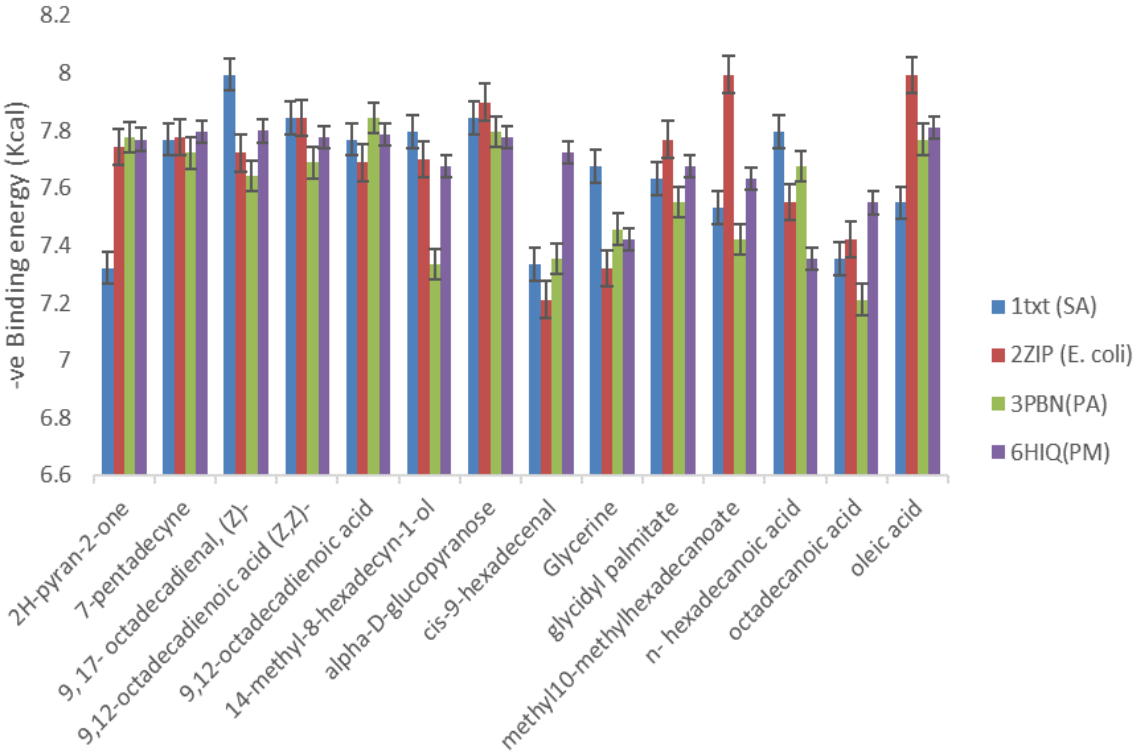


Fig. 9. The molecular docking analysis of*Azadirachta indica* (neem) oil

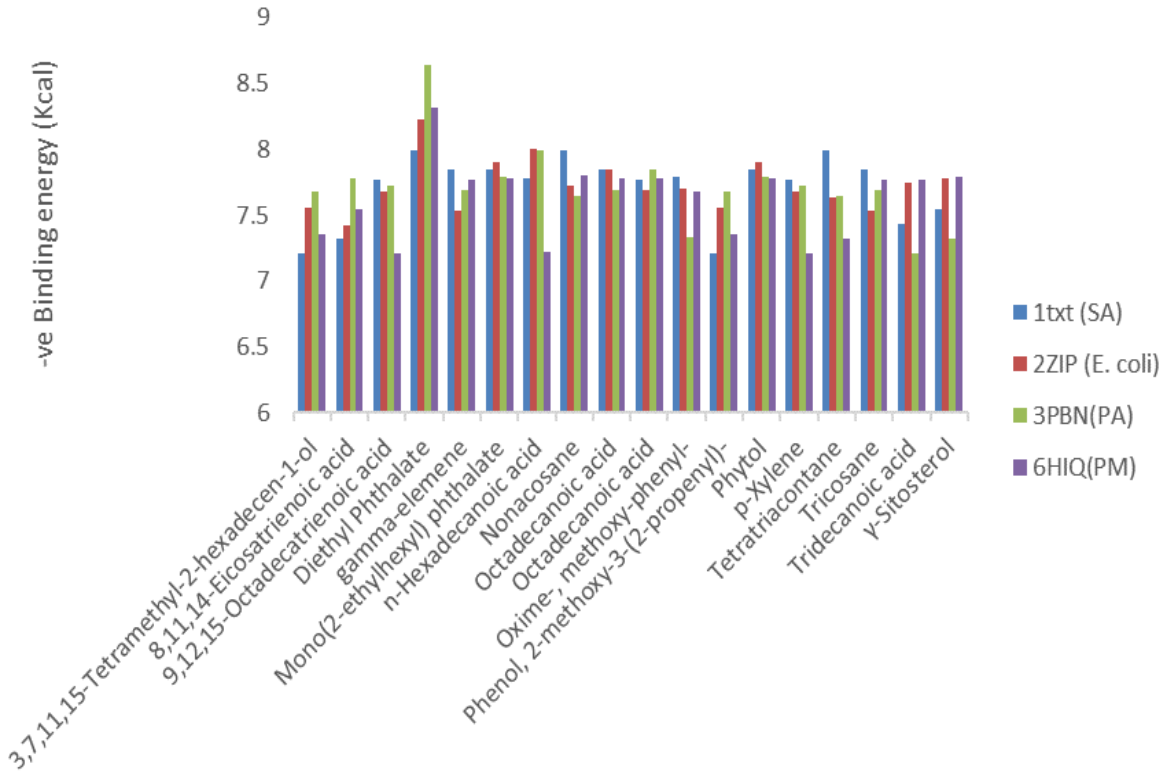


Fig. 10. The molecular docking analysis of *Azadirachta indica* (neem) powder

MIC and MBC of *Azardiracta aindica* extracts revealed that MIC and MBC of neem oil and MIC of methanolic extract were 2.00 mg/ml stronger than the minimum inhibitory and minimum bactericidal concentration of aqueous and methanol of neem extracts against the bacterial isolates as reported by (Suttiarporn et al*.,* 2020). This is due to the bioactive compounds in neem extracts that are more permeable in neem oil and can easily penetrate the isolates' cell wall.

Figs. 3 - 6 showed that aqueous, methanol and oil of neem extracts exhibited bacteriostatic influence against *Staphylococcus aureus and Escherichia. coli* with optical density indicating cell growth below the control agent cell growth of 0.027 nm and 0.028 nm, as well as *Candida albicans* and *Aspergillus niger* cells, with growth of 0.029 nm and 0.033 nm, respectively. This finding is in accordance with (Kumar et al*.,* 2022) who suggested that neem is effective against a plethora of pathogens and causes a reduction in pathogenic microbes from the gut as a result of the azardiractin, nimbidin and nimbin components of neem leaf. The results of phytochemical further confirm the presence of bioactive compounds in neem and high-level activities against pathogens due to enormous varieties of phytochemical profiling of the extracts, which revealed a wide spectrum of 18 and 14 identified phytochemicals in neem powder and oil.

Neem oil and powder confirm the presence of α-D-Glucopyranose and n-Hexadecanoic acid at the highest peak area of 21.98 % and 12.31% respectively. These phytochemicals account for more than 135 compounds wide range of active compounds isolated from different parts of neem, as described by (De Paulo et al., 2023), and several reviews have also been published on the chemistry and structural diversity of these compounds according to (Egho and Ilondu, 2023). Neem oil and extracts contain broad band hydroxyl group,strong carboxylic group including weak ester and alkene bonds, while neem crude extracts exhibits hydroxyl, aliphatic, aromatic, ester and nitrogen-containing groups, corresponding to flavonoids, tannins, and alkaloids responsible for its antimicrobial and anti-inflammatory effects as well as microbial cell disruption and death. This finding agrees with (Ali et al*.,* 2021) that supports neem medicinal and antimicrobial properties of the ethanol and phenol by interfering with the cell membrane and disruption of cell resulting to cell death as well as carboxylic acid, with a strong band which interfere with microbial metabolism, protein synthesis, and cell growth as reported by (Yadav et al*.,* 2024).

Molecular docking analysis reveals the binding affinity of neem extracts, Figs. 9 to 10, where the binding affinity with microbial protein reflects the oil's effectiveness in inhibiting the protein's function. Peaks or troughs on the graph showing strong affinity of 9, 17-Octadecadienoic, Methyl 10-methylhexadecanoate and oleic acid at a negative low binding energy of - 8.00kcal with *S.aureous* 1txt *and E. coli* 2*ZIP protein* indicating the potential antibacterial efficacy of neem oil. The docking of neem extracts showed that Diethylphalate has a strong affinity at a low negative binding energy of -8.5 kcal with *S. aureus* 1txt protein, suggesting strong antimicrobial potential, which could be indicative of potent antibacterial or bioactive properties. This conforms with (Chhavi et al*.*, 2022) and the study noted *Azardiracta indica* as one of the richest sources of salanin in nature, which is well recognised for its interference with microbial metabolism, enzyme activation and biomolecule synthesis (CLSI, 2020 a,b).

4. CONCLUSION

The antimicrobial activity of neem against clinical isolates demonstrates the therapeutic significance of plants in combating pathogenic microbes, and its bacteriostatic and fungistatic potential enhances the plant's spectrum antimicrobial potential. The findings of this research acknowledge and scientifically validate the local use of neem against microbial activities.

5. RECOMMENDATION

Plant extracts should be standardised by the appropriate bodies to regulate their safe use, proper preservation and sustainable use of such plant resources, considering prevalence of multidrug-resistant pathogens worldwide.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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