

In vitro and *in silico* study of phytoconstituents from *Hyptis suaveolens* L. for antibacterial potential

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The discovery of new antibacterial molecules is crucial given the increasing resistance of bacteria to existing antibiotics. Therefore, there is a need for new, safer antibacterial therapeutics. Even though *H. suaveolens* has a long history of medicinal use, many of its bioactive compounds remain unidentified. Thus, this study aimed to find novel phytochemicals from the methanolic extract of *H. suaveolens* that may be useful against pathogens. *In vitro* antibacterial testing revealed that the 24th fraction showed significant antibacterial activity, and GC-MS analysis identified several phytochemicals. ADMET analysis showed that 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone, diethyl phthalate, n-hexadecenoic acid, squalene, and α -tocopherol acetate have drug-like characteristics. Molecular docking of these five ligands was performed against two target proteins using PyRx AutoDock Vina. The targets included intercellular adhesion protein R from *Staphylococcus aureus* and DNA polymerase III subunit beta from *Escherichia coli*. 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone and diethyl phthalate had the strongest binding affinities and the lowest binding energies. ADMET analysis confirmed that they are drug-like, non-toxic, and non-carcinogenic. These findings suggest that 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone and diethyl phthalate could be useful antibacterial agents for treating infections.

Keywords: Pathogen, GC-MS analysis, ADMET analysis, molecular docking

An increase in antibacterial resistance leads to higher morbidity and mortality due to bacterial infections [World Health Organisation (WHO), 2020]. Multiple drug-resistant (MDR) bacteria are resistant to multiple antibiotics, whereas extensively drug-resistant (XDR) bacteria are resistant to almost all antibiotics¹. As a result, designing and developing new promising antibiotic classes is critical².

H. suaveolens is well-known for its extensive therapeutic characteristics, which contain di- and triterpenes and phenolic compounds, which possess biological activities against other plants, bacteria, viruses, fungi, and larvae³. Many research organisations have studied *H. suaveolens*, as it contains antioxidant, anti-inflammatory, antibacterial, antiviral, and anticancer chemicals⁴. Unique terpenoid and phenolic compounds of *H. suaveolens* can replace conventional drugs to treat resistant and recently discovered bacterial and viral infections⁴. The principal chemical in the ethanolic extract of *H. suaveolens* 5,5-dimethylimidazolidin-2,4-diamine (20.35%) - is a bioactive compound against many diseases⁵. Many drug-resistant organisms, such as

Staphylococcus aureus, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Aspergillus niger*, and *Candida albicans* are susceptible to *H. suaveolens*⁶. Extract of *H. suaveolens* has many properties, like antifungal, analgesic, anti-inflammatory, tissue repair, and antioxidant⁷. The crude leaf extract of *H. suaveolens* could substitute for chemical larvicides⁸.

E. coli and *S. aureus* have been linked to human bacteremic diseases and are resistant to many antibiotics⁹. Treating diseases caused by drug-resistant bacteria with traditional and broad-spectrum antibiotics may unintentionally harm patients due to treatment failure, side effects, and disruption of the normal microbiota¹⁰. Thus, phytochemicals isolated from the methanolic extract of *H. suaveolens* leaves were studied against these two bacteria¹¹.

Phytoconstituents present in the methanolic extract of leaves of *H. suaveolens* were separated by the column chromatography fractionation technique according to their polarity from the plant extracts¹². Phytoconstituents from the 24th fraction of the methanolic extract of leaves of *H. suaveolens* were identified by GC-MS, a widely used method to analyse components in traditional medicinal formulations and medicinal plants¹³. Non-polar phytochemicals, such as volatile oils, fatty acids,

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lipids, and other hydrophobic compounds from plants, are usually analysed using GC-MS¹⁴.

All the phytoconstituents were evaluated using online tools such as ADMET Lab2 and Swiss ADME for their drug-likeness and toxicity. The primary drawback of drug discovery in clinical trials is the high cost of determining its pharmacokinetic characteristics¹⁵. ADMET analysis is an important initial step of drug discovery¹⁶. The primary motivation for this computer-based approach is to reduce the cost and time of drug design with traditional ADMET profiling¹⁷.

Our study investigated the *in vitro* antibacterial activity, molecular docking, and drug-likeness characteristics of a methanolic extract of the leaves of *H. suaveolens*. The phytochemicals 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone, diethyl phthalate, n-hexadecanoic acid, squalene, and α -tocopherol acetate were proposed as potential hits against selected bacteria.

Materials and Methods

Collection and processing of the plant

H. suaveolens plants were collected from college premises, cleaned with tap water and rinsed with distilled water. The leaves were separated and air-dried at room temperature (RT) for several days. The dried leaves were ground into a powder using a mechanical grinder, and the powder was stored in an airtight bottle for later use.

Extract preparation

To make the extract, 100 grams of leaf powder was dissolved in 300 mL of methanol. The mixture was agitated in an orbital shaker at 40 rpm for 72 hours. Filtration of the mixture was carried out using Whatman No. 1 filter paper. The resulting filtrate was air-dried at RT and stored for subsequent use in the refrigerator¹⁸. A stock solution was prepared by dissolving 100 mg of an extract in 1 mL of dimethyl sulfoxide (DMSO) at 4°C.

Inoculum preparation of bacterial cultures

Escherichia coli (MTCC 443) and *Staphylococcus aureus* (MTCC 3160) were obtained from MTCC Chandigarh. The bacteria were stored at 4°C on an N-Agar slant (Hi-media) for subsequent use. Mueller-Hinton broth was used to pre-cultivate bacteria at 37°C in a rotary shaker and adjusted to a 0.5 McFarland standard (10^8 cells/mL)¹⁹.

Antimicrobial analysis

Following their inoculation into nutrient broth, bacterial cultures were grown for 18 hours at 37°C.

The antimicrobial analysis was assessed using the agar well diffusion method. On sterile Mueller-Hinton agar (Himedia) plates, bacteria were inoculated with a sterile swab. Holes of 8 mm diameter were made using a sterilized cup borer and filled with 100 μ L of methanolic extract prepared in DMSO. Plates were incubated overnight in an incubator at 37°C. After incubation, zones of inhibition were measured²⁰.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was determined using the broth dilution method. The extract was subjected to two-fold serial dilutions in Mueller-Hinton broth. 0.1 mL of pure culture of bacteria [*E. coli* (MTCC 443), and *S. aureus* (MTCC 3160)] was added to all tubes except the negative control and incubated at 37°C for 24 hours. MIC was recorded as the concentration of the extract where no growth of the organisms was detected²¹. Levofloxacin was used as a positive control, while DMSO was used as a negative control. All tests were repeated in triplicate to ensure accuracy.

Minimum bactericidal concentration (MBC)

Mueller-Hinton agar plates were streaked with a loop of culture from the MIC tube that showed no growth and incubated at 37°C for 24 hours. The plates were checked for bacterial growth following incubation²². The lowest concentration of extract that showed no growth was denoted as the MBC, indicating bactericidal activity.

Fractionalization by column chromatography

Column chromatography was performed to separate phytochemicals. The stationary phase was made in a silica gel slurry in hexane. After two-thirds of the column (silica gel, 60 to 120 mesh) had been filled with the slurry, the sample was added. The solvents - hexane, ethyl acetate, and methanol- were allowed to flow down the column in varying ratios. Components were separated according to their own unique affinities towards stationary and mobile phases. The flow rate of the mobile phase is maintained at 1 mL/min. Fragments were gathered every 30 minutes. Using this technique, the chemicals were separated from the mixture. The structural elucidation of each fraction was further investigated²³.

GC-MS analysis of the extract

For the GC-MS analysis, the fractions with the highest antibacterial activity were chosen. A Shimadzu GC TQ8040 system with an Rtx-5MS capillary column of dimension 30 m, 0.25 mm, ID 0.25 μ m was used for analysis. The initial oven

temperature was 100°C, which was held for 2 minutes before being escalated to 120°C at a rate of 5°C/min and kept for 2 minutes, and then increased at the rate of 6°C/min to 300°C and held for 10 min. The experiment lasted 49 min in total. A constant flow of helium at the rate of 1.67 mL/min was maintained. A triple quadrupole mass analyzer with a scan range of 40 m/z to 800 m/z and positive polarity was used.

ADMET analysis

The *in silico* Absorption, Distribution, Metabolism, Excretion, and Toxicity profile of the phytochemicals was analysed using the ADMET lab 2.0²³. The chosen phytochemicals were further assessed for drug-likeness based on the Lipinski Rule of Five.

Molecular docking studies

To determine the best-fit orientation between ligands and proteins, molecular docking was performed using the "key and lock" concept. The prepared protein and ligand files were submitted to PyRx and docked using AutoDock Vina. The BIOVIA Discovery Studio Visualizer tool was used to evaluate the binding energies and molecular interaction of each ligand-protein complex²⁴.

Preparation of ligands and proteins

The antibacterial potential of the compounds found by GC-MS analysis was further analysed using molecular docking. The SDF files of phytochemicals were retrieved from the PubChem database and converted into PDB files by Open Babel integrated within the PyRx platform. The 3D protein structures of intercellular adhesion protein R (PDB ID: 3GEU) from *S. aureus* and DNA polymerase III subunit beta (PDB ID: 4K3P) from *E. coli* were downloaded from the RCSB Protein Data Bank.

Protein-ligand docking

Before docking, protein structures were processed using the BIOVIA Discovery Studio Visualizer program by removing heteroatoms and water molecules from proteins before converting them to .pdb format. Polar hydrogen atoms and Kollman charges were added and then inserted for energy reduction by using conjugate gradient techniques to remove poor protein atom connections. Finally, using

PyRx AutoDock Vina, optimized protein structures were docked against 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone, diethyl phthalate, n-hexadecanoic acid, squalene, and α -tocopherol acetate as ligands²⁵.

Using grid coordinates and grid boxes of specific sizes for every receptor, PyRx AutoDock Vina was utilized to dock each ligand to each receptor. Moreover, BIOVIA Discovery Studio Visualizer software was used for non-bonded interaction calculations and to analyze and visualize docking results.

Results

Antimicrobial activity of crude extract

The methanolic leaf extract of *H. suaveolens* displayed antimicrobial activity against the tested pathogens (Table 1). The zones of inhibition displayed were 7.3 ± 0.61 and 10.6 ± 0.81 mm against *E. coli* and *S. aureus*, respectively. Dimethyl sulfoxide, used as a solvent control, showed no antibacterial activity against selected bacteria, indicating that antimicrobial activity was due to the plant extract only.

Minimum inhibitory concentration

The methanolic leaf extracts of *H. suaveolens* showed an MIC value of 100 μ g/mL for all selected bacteria. DMSO, used as a control, showed no inhibition, while levofloxacin, a standard drug, showed an MIC of 25 μ g/mL against all the bacteria, as summarised in Table 2.

Minimum bactericidal concentration

The methanolic extract of leaves of *H. suaveolens* showed MBC pf 100 μ g/mL against the selected bacteria equal to the MIC values.

Antimicrobial activity of column chromatographic fractions

Fig. 1 shows the antibacterial activity of column chromatographic fractions of the methanolic leaf

Table 1 — Antimicrobial activity of the methanol extract of the 24th fraction of leaves of *H. suaveolens* against selected bacteria

Organisms	Mean value of zone of inhibition \pm SD (mm)	
	Methanolic extract of leaves of <i>H. suaveolens</i>	DMSO as a control
<i>E. coli</i>	7.3 ± 0.61	0
<i>S. aureus</i>	10.6 ± 0.81	0

Table 2 — Minimum inhibitory concentration of methanol extract of 24th fraction of leaves of *H. suaveolens* against selected bacteria

Organisms	Mean Minimum inhibitory concentration \pm SD (μ g/mL)		
	Methanolic extract of leaves of <i>H. suaveolens</i>	DMSO as a control	Levofloxacin as standard
<i>E. coli</i>	100 ± 0	0	25 ± 0
<i>S. aureus</i>	100 ± 0	0	25 ± 0

extract of *H. suaveolens* against selected bacteria. Most fractions exhibited antibacterial activity against at least one of the bacteria. Fraction numbers 21–24 and 36 showed antibacterial activity against both bacteria. The active fractions showed greater antibacterial activity against *S. aureus* than against *E. coli*. Among all fractions, the 24th fraction showed the largest inhibition zones, measuring 4.5 ± 0.57 mm against *E. coli* and 7.3 ± 0.57 mm against *S. aureus*. Furthermore, fractions 7–10 and 13–16 were selectively inhibited only by *S. aureus*.

GC-MS analysis of the methanol extract of *H. suaveolens* leaves

GC-MS analysis of the 24th column fraction of the methanolic leaf extract of *H. suaveolens* exhibited the presence of 50 peaks (Fig. 2). Mass spectra of compounds were matched with the NIST library for compound identification. Retention time, molecular

formula, molecular weight, and name of the identified compound obtained from the library are summarised in Table 3. The list of identified compounds with their name, structure, chemical class, and reported biological activity is presented in Table 4. The major components present in methanolic extract are 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone with RT 30.183 and a peak area of 12.75%; diethyl phthalate with RT 14.069 and peak area of 3.41%; 9-eicosene, (E)- with RT 18.963 and a peak area of 0.85%; n-hexa-decanoic acid with RT 21.993 and a peak area of 4.35%; hepta-siloxane, hexadecamethyl- with RT 32.267 and a peak area of 0.94%; squalene with RT 33.624 and a peak area of 12.72%; (+/-) α -tocopherol acetate with RT 37.598 and a peak area of 2.30%; sulfurous acid, cyclohexyl methyl octadecyl with RT 40.865 and a peak area of 18.27%; sulfurous acid, 2-ethylhexyl octadecyl ester with RT 42.862 and a peak

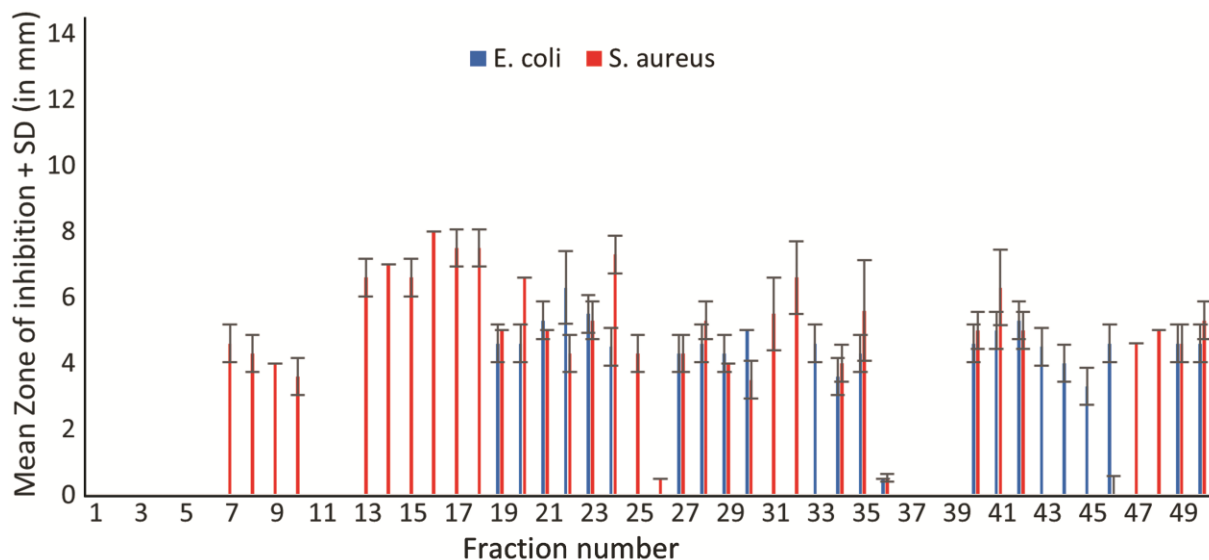


Fig. 1 — Antimicrobial activity of fractions obtained from column chromatography of the methanol extract of the leaf of *H. suaveolens* against selected bacteria.

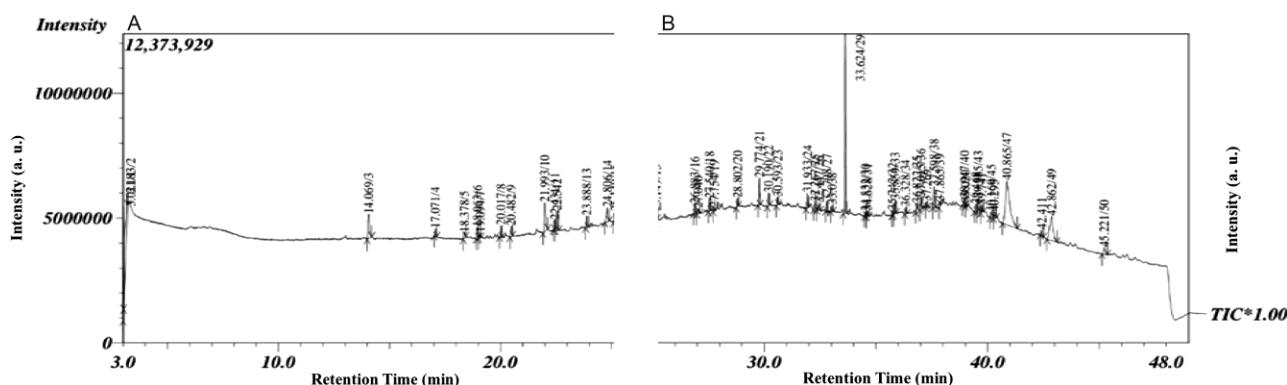


Fig. 2 —GC-MS analysis of the 24th fraction from the methanol extract of *H. suaveolens* leaves.

Table 3 — GC-MS analysis of the 24th fraction from the methanol extract of leaves of *H. suaveolens*

Peak#	R.Time	Area	Area %	Height	Name
1	3.021	4040819	3.02	4293010	9-Oxabicyclo[6.1.0]nonan-4-ol
2	3.183	17070590	12.75	1060083	1,2-Dihydro-2-oxo-4-quinolyl 2-pyridyl ketone
3	14.069	4570094	3.41	970264	Diethyl Phthalate
4	17.071	1008644	0.75	313850	Heptasiloxane, hexadecamethyl-
5	18.378	699090	0.52	228515	Tetradecanoic acid
6	18.963	1132424	0.85	444278	9-Eicosene, (E)-
7	19.094	613204	0.46	214945	Hexadecane
8	20.017	1312888	0.98	464059	Heptasiloxane, hexadecamethyl-
9	20.482	1254698	0.94	423491	1-Hexadecanol
10	21.993	5822407	4.35	1173842	n-Hexadecanoic acid
11	22.431	937068	0.70	396179	1-Tricosene
12	22.542	2620065	1.96	771562	Heptasiloxane, hexadecamethyl-
13	23.888	1384896	1.03	473175	n-Heptadecanol-1
14	24.806	2716895	2.03	577521	Heptasiloxane, hexadecamethyl-
15	25.149	1218863	0.91	370307	Octadecanoic acid
16	26.883	1139376	0.85	391999	Heptasiloxane, hexadecamethyl-
17	27.040	851381	0.64	213104	3,7,11-trimethyldodeca-2,6,10-trien-1-yl palmitate
18	27.540	1360617	1.02	502293	Sulfurous acid, cyclohexylmethyl tridecyl ester
19	27.754	319371	0.24	153189	[1,1'-Biphenyl]-2,3'-diol, 3,4',5,6'-tetrakis (1,1-dimethylethyl)
20	28.802	903434	0.67	345020	Heptasiloxane, hexadecamethyl-
21	29.774	2512423	1.88	1095069	Carbonic acid, 2-Ethylhexyl undecyl ester
22	30.190	1530709	1.14	530204	Bis(2-Ethylhexyl) phthalate
23	30.593	904228	0.68	323457	Heptasiloxane, hexadecamethyl-
24	31.933	1683104	1.26	611335	Sulfurous acid, cyclohexylmethyl tetradecyl ester
25	32.267	1263641	0.94	306129	Heptasiloxane, hexadecamethyl-
26	32.447	818265	0.61	292409	2,4,4,6,6,8,8-Heptamethyl-1-nonene
27	32.798	1036799	0.77	428319	1,3-Benzenedicarboxylic acid, bis(2-Ethylhexyl) ester
28	33.038	777533	0.58	256781	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol
29	33.624	17018793	12.72	7186763	Squalene
30	34.532	468736	0.35	181998	Cholesta-3,5-diene
31	34.628	638864	0.48	226526	Tetracosane
32	35.742	409682	0.31	179481	Hexadecane, 1-iodo-
33	35.859	1458522	1.09	553565	Sulfurous acid, cyclohexylmethyl pentadecyl ester
34	36.328	715937	0.53	265680	Eicosyl heptyl ether
35	36.822	662071	0.49	266640	Octacosane, 2-methyl-
36	37.015	1488821	1.11	516355	Cholesterol
37	37.269	583465	0.44	230645	9-Hexadecenoic acid, octadecyl ester, (Z)-
38	37.598	3082905	2.30	852373	(.+/-)-.alpha.-tocopherol acetate
39	37.865	441625	0.33	201757	Tetratetracontane
40	38.947	1083943	0.81	296305	Pentatriacontane
41	39.020	437615	0.33	163007	Ethanol, 2-(9-octadecenyloxy)-, (Z)-
42	39.468	1122805	0.84	290213	9-Hexadecenoic acid, octadecyl ester, (Z)-
43	39.585	2195456	1.64	551850	Sulfurous acid, cyclohexylmethyl octadecyl ester
44	39.741	880599	0.66	284466	Hexadecanoic acid, hexadecyl ester
45	40.160	1315097	0.98	226457	Heptyl triacontyl ether
46	40.295	1389304	1.04	245416	Sulfurous acid, butyl cyclohexylmethyl ester
47	40.865	24450861	18.27	1782559	Sulfurous acid, cyclohexylmethyl octadecyl ester
48	42.411	802473	0.60	235316	cis-9-Hexadecenoic acid, heptyl ester
49	42.862	10046351	7.51	975381	Sulfurous acid, 2-Ethylhexyl octadecyl ester
50	45.221	1645907	1.23	269299	Sulfurous acid, cyclohexylmethyl tetradecyl ester
		133843358	100.00	33606441	

Table 4 — Chemical compounds identified by GC-MS of the 24th fraction of leaves extract from *H. suaveolens* and their biological activities

Peak#	Name	Chemical class (Classified by Classyfire)	Biological activities
1	9-Oxabicyclo[6.1.0]nonan-4-ol	Secondary alcohol	No activity reported
2	1,2-Dihydro-2-oxo-4-quinolyl 2-pyridyl ketone	Hydroquinolones	No activity reported
3	Diethyl Phthalate ²⁶	Benzoic acid esters	antimicrobial, acetylcholinesterase, and neurotoxic activity
4	Heptasiloxane, hexadecamethyl ³⁷	Siloxanes	Antimicrobial, Antifungal, health-related products, cosmetics, paints, varnishes, surface treatments and cookware
5	Tetradecanoic acid ²⁸	Long-chain fatty acids	Antibacterial
6	9-Eicosene, (E) ²⁹	Unsaturated aliphatic hydrocarbons	Antifungal and antioxidant
7	Hexadecane ³⁰	Alkanes	Antimicrobial and antioxidant
8	Heptasiloxane, hexadecamethyl ²⁷	Siloxanes	Antimicrobial, Antifungal, health-related products, cosmetics, paints, varnishes, surface treatments and cookware
9	1-Hexadecanol ³¹	Long-chain fatty alcohols	Antimicrobial
10	n-Hexadecanoic acid ³²	Long-chain fatty acids	anticancer, antimicrobial, antioxidant, and antihemolytic activity
11	1-Tricosene	Unsaturated aliphatic hydrocarbons	Not Reported
12	Heptasiloxane, hexadecamethyl ²⁷	Siloxanes	Antimicrobial, Antifungal, health-related products, cosmetics, paints, varnishes, surface treatments and cookware
13	n-Heptadecanol-1	Long-chain fatty alcohols	Not Reported
14	Heptasiloxane, hexadecamethyl ²⁷	Siloxanes	Antimicrobial, Antifungal, health-related products, cosmetics, paints, varnishes, surface treatments and cookware
15	Octadecanoic acid	Long-chain fatty acids	Not Reported
16	Heptasiloxane, hexadecamethyl ²⁷	Siloxanes	Antimicrobial, Antifungal, health-related products, cosmetics, paints, varnishes, surface treatments and cookware
17	3,7,11-trimethyldodeca-2,6,10-trien-1-yl palmitate	Fatty acid esters	Not Reported
18	Sulfurous acid, cyclohexylmethyl tridecyl ester	Organooxygen compounds	Not reported
19	[1,1'-Biphenyl]-2,3'-diol, 3,4',5,6'-tetrakis (1,1-dimethylethyl)-	Biphenols	Not reported
20	Heptasiloxane, hexadecamethyl ²⁷	Siloxanes	Antimicrobial, Antifungal, health-related products, cosmetics, paints, varnishes, surface treatments and cookware
21	Carbonic acid, 2-ethylhexyl undecyl ester	Carbonic acid diesters	Not Reported
22	Bis(2-ethylhexyl) phthalate ³⁴	Benzoic acid esters	Antibacterial
23	Heptasiloxane, hexadecamethyl ²⁷	Siloxanes	Antimicrobial, Antifungal, health-related products, cosmetics, paints, varnishes, surface treatments and cookware
24	Sulfurous acid, cyclohexylmethyl tetradecyl ester	Organooxygen compounds	Not Reported
25	Heptasiloxane, hexadecamethyl ²⁷	Siloxanes	Antimicrobial, Antifungal, health-related products, cosmetics, paints, varnishes, surface treatments and cookware

(Contd.)

Table 4 — Chemical compounds identified by GC-MS of the 24th fraction of leaves extract from *H. suaveolens* and their biological activities (Contd.)

Peak#	Name	Chemical class (Classified by Classyfire)	Biological activities
26	2,4,4,6,6,8,8-Heptamethyl-1-nonene	Branched unsaturated hydrocarbons	Not reported
27	1,3-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester ³³	m-Phthalate esters	Antimicrobial and antifouling
28	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	Triterpenoids	Not Reported
29	squalene ³³	Triterpenoids	Antimicrobial, Antioxidant, Antitumour, Cancer preventive, and Pesticide
30	Cholesta-3,5-diene	Cholestane steroids	Not reported
31	Tetracosane	Alkanes	Not Reported
32	Hexadecane, 1-iodo ³⁵	Organo iodides	antimicrobial, antioxidant, anticancer
33	Sulfurous acid, cyclohexylmethyl pentadecyl ester	Organooxygen compounds	
34	Eicosyl heptyl ether	Dialkyl ethers	Not reported
35	Octacosane, 2-methyl-	Branched alkanes	Not Reported
36	Cholesterol ³⁶	Cholestane steroids	anticancer activity, anti-cardiac activity, anti-inflammatory activity, antimicrobial activity, anti-psychotic activity, antioxidant activity, and drug-loaded activity
37	9-Hexadecenoic acid, octadecyl ester, (Z)-	Fatty acid esters	Not reported
38	(+/-) α tocopherol acetate ³⁷	Quinone and hydroquinone lipids	Antioxidant, antibacterial
39	Tetratetracontane ³⁸	Alkanes	Antibacterial
40	Pentatriacontane ³⁸	Alkanes	Antibacterial
41	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	Ethers	Not reported
42	9-Hexadecenoic acid, octadecyl ester, (Z)-	Fatty acid esters	Not reported
43	Sulfurous acid, cyclohexylmethyl octadecyl ester	Organooxygen compounds	Not Reported
44	Hexadecanoic acid, hexadecyl ester	Fatty acid esters	Not reported
45	Heptyl triacontyl ether	Ethers	Not Reported
46	Sulfurous acid, butyl cyclohexylmethyl ester	Organooxygen compounds	Not reported
47	Sulfurous acid, cyclohexylmethyl octadecyl ester	Organooxygen compounds	Not Reported
48	cis-9-Hexadecenoic acid, heptyl ester	Fatty acid esters	Not Reported
49	Sulfurous acid, 2-ethylhexyl octadecyl ester	Organooxygen compounds	Not Reported
50	Sulfurous acid, cyclohexylmethyl tetradecyl ester	Organooxygen compounds	Not Reported

area of 7.51% (area 1.18%); and linolenic acid, 2-hydroxy-1-(hydroxymethyl) at RT 32.607 (peak area 0.82%). The prominent bioactive compounds present are sulfur-containing esters, fatty acids, terpenoids, phthalates, and heterocyclic compounds.

ADMET analysis of selected phytoconstituents from *H. suaveolens* leaves

The physicochemical, ADMET, and drug-like properties of 5 selected phytochemicals of *H. suaveolens* are presented in Table 5. From the drug

likeness criteria, 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone and diethyl phthalate fully pass the drug likeness rules, representing strong potential as orally bioavailable drug molecules. Total polar surface area is 62.82^oA and 52.6^oA, and log p-values are 1.8 and 2.6 for 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone and diethyl phthalate, respectively, indicating that these chemicals are more water soluble than the other three chemicals. All five selected chemicals showed the same absorption patterns.

Table 5 — Predicted ADMET properties of selected phytoconstituents from *H. suaveolens* and standard drug levofloxacin (Contd.)

	Environmental Toxicity					
Bioconcentration Factor	0.77	0.578	1.298	3.131	2.688	0.327
IGC50 (Growth inhibition concentration)	3.688	3.585	5.142	4.38	5.375	2.299
	Drug Likeness					
Lipinski	Accepted	Accepted	Accepted	Accepted	Accepted	Accepted
Pfizer	Accepted	Accepted	Rejected	Rejected	Rejected	Accepted
GSK	Accepted	Accepted	Rejected	Rejected	Rejected	Accepted
Golden Triangle	Accepted	Accepted	Accepted	Rejected	Rejected	Accepted

Table 6 — Binding affinity of selected phytochemicals from *H. suaveolens* and levofloxacin against target proteins

Ligand	3geu - Intermolecular adhesion protein			4k3p - DNA polymerase III subunit beta		
	Affinity (kcal/mol)	Distance from the best mode		Affinity (kcal/mol)	Distance from the best mode	
		RMSD l.b.	RMSD u.b.		RMSD l.b.	RMSD u.b.
1,2-Dihydro-2-oxo-4-quinolyl 2-pyridyl ketone	-7.1	0	0	-6.2	0	0
Diethyl phthalate	-5.2	0	0	-4.7	0	0
n-Hexa-decanoic acid	-4.3	0	0	-4.3	0	0
Squalene	-5.7	0	0	-5.5	0	0
(+/-) α -Tocopherol acetate	-5.8	0	0	-5.9	0	0
Levofloxacin	-6.7	0	0	-7.2	0	0

Diethyl phthalate showed the lowest plasma protein binding (69.28%) and blood-brain barrier penetration (0.115), indicating greater body distribution compared to other compounds. It was also observed that diethyl phthalate was a strong inhibitor of cytochrome p450, and the Cyp450 substrate indicated that it was more metabolised in the body. Clearance values of Diethyl phthalate, squalene, and α -tocopherol were 2.7 mL/min/kg, 2.3 mL/min/kg, and 5.0 mL/min/kg, respectively, indicating efficient removal from the body, and their half-lives were 0.357 hour, 0.005 hour, and 0.018 hour, respectively. These values suggested that squalene cleared more rapidly, while clearance of diethyl phthalate was also significant.

From the data given in Table 5, diethyl phthalate, hexadecenoic acid, and α -tocopherol have lower hepatotoxicity values (0.005, 0.026, and 0.033) and lower DILI values (0.404, 0.043, and 0.287). Carcinogenicity scores of 0.035, 0.064, and 0.034 indicated they are less toxic than the other chemicals. Additionally, diethyl phthalate presented a low bioconcentration factor (0.578) and a higher IGC 50 (3.585 mg/L), indicating that it has less environmental toxicity and higher ecological safety than other chemicals. Overall, both 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone and diethyl phthalate passed all the rules of drug-likeness. Compared with the standard antibiotic levofloxacin, diethyl phthalate and 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone

are better in terms of solubility and clearance, while the metabolism, absorption, and half-life of levofloxacin are superior. Comparing toxicity, diethyl phthalate and 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone are safer compared to levofloxacin, while their druglike properties are the same.

Molecular docking of selected phytoconstituents from *H. suaveolens* leaves

The docking result of 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone, diethyl phthalate, n-hexadecanoic acid, squalene, and (+/-)- α -tocopherol acetate from *H. suaveolens* against two proteins, DNA polymerase III subunit beta from *E. coli* (PDB ID:4K3P) and Intermolecular adhesion protein from *S. aureus* (PDB ID:3GEU) shown in Table 6. Among them, 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone showed the maximum binding affinity of -7.1 kcal/mol (4K3P) and -6.2 kcal/mol (3GEU). Diethyl phthalate showed -5.2 and -4.7 kcal/mol, n-hexadecanoic acid showed -4.3 and -4.3 kcal/mol, squalene showed -5.7 and -5.5 kcal/mol (+/-)- α -tocopherol acetate showed -5.8 and -5.9 kcal/mol, and levofloxacin showed -6.7 and -7.2 kcal/mol, respectively. The above data indicated that 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone performed comparably with the standard antibiotic. A detailed view of ligand-protein interaction, including amino acid contacts and binding conformations, is shown in (Fig. 3A-L).

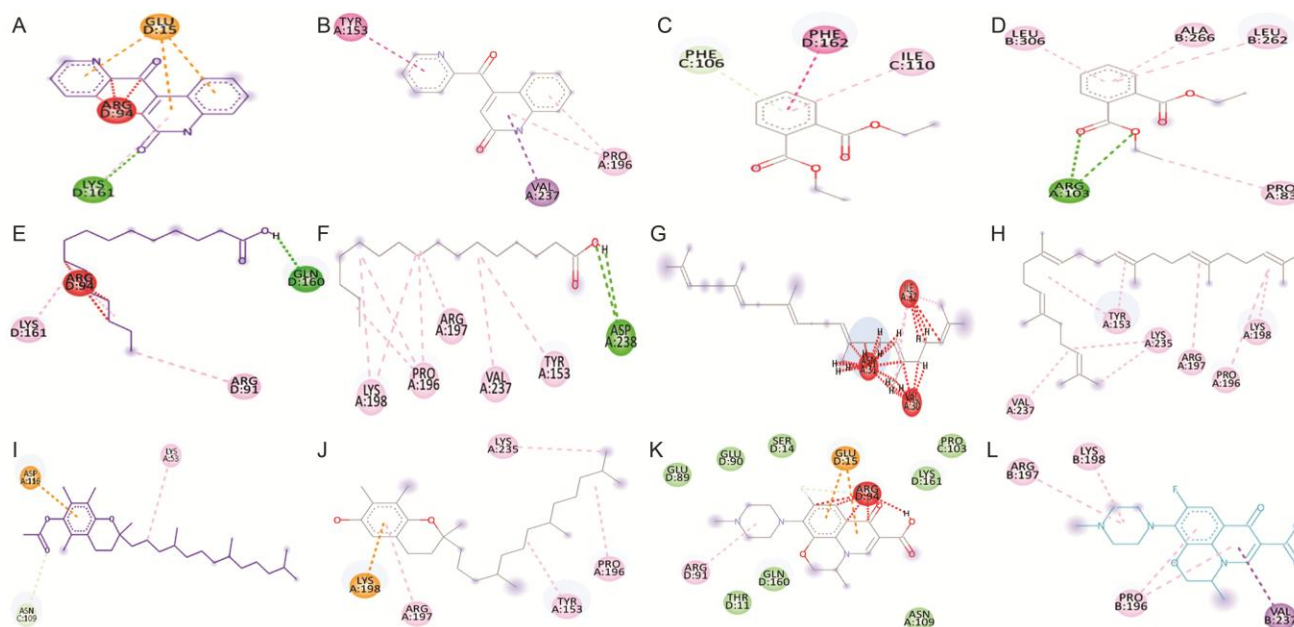


Fig. 3 — Molecular docking of selected phytochemicals from *H. suaveolens* and levofloxacin against target proteins. (A) 1,2-Dihydro-2-oxo-4-quinolyl-2-pyridyl ketone against intermolecular adhesion protein (3geu); (B) 1,2-Dihydro-2-oxo-4-quinolyl-2-pyridyl ketone against DNA polymerase III subunit beta (4k3p); (C) Diethyl phthalate against intermolecular adhesion protein (3geu); (D) Diethyl phthalate against DNA polymerase III subunit beta (4k3p); (E) n-Hexa-decanoic acid against intermolecular adhesion protein (3geu); (F) n-Hexa-decanoic acid against DNA polymerase III subunit beta (4k3p); (G) Squalene against intermolecular adhesion protein (3geu); (H) Squalene against DNA polymerase III subunit beta (4k3p); (I) (+/-) α -tocopherol acetate against intermolecular adhesion protein (3geu); (J) (+/-) α -tocopherol acetate against DNA polymerase III subunit beta (4k3p); (K) Levofloxacin against intermolecular adhesion protein (3geu); (L) Levofloxacin against DNA polymerase III subunit beta (4k3p).

Discussion

Multidrug resistance is a global issue that renders regular medications less effective and complicates the treatment of infectious diseases¹. Pathogens like *S. aureus* and *E. coli* have developed resistance mechanisms as a result of the irresponsible use of conventional antibiotics¹⁰. In addition to increasing death and suffering, this makes treating diseases brought on by resistant bacteria more difficult. Therefore, the current need is to find new drug molecules with unique qualities to combat resistant diseases³⁹. One of the limitations of traditional antibiotics is that phytochemicals found in medicinal plants such as *H. suaveolens* possess antibacterial qualities that can kill germs through a variety of multimodal mechanisms^{5,40}. Therefore, finding bioactive compounds from plants such as *H. suaveolens* yields multi-targeted compounds with antibacterial action against resistant diseases⁴¹.

The 24th fraction of methanolic leaf extract of *H. suaveolens* exhibited moderate but significant antibacterial activity, in agreement with the findings of Bonilla-Landa *et al.*³⁹. The extract inhibited both gram-positive and gram-negative bacteria, consistent

with the results reported by Nigussie *et al.*⁴². However, some studies showed that activity is restricted only to gram-positive bacteria⁴³. These results demonstrate that *H. suaveolens* extract showed noteworthy broad-spectrum antibacterial activity despite structural differences in its cell wall.

The 24th fraction of methanolic leaf extract exhibited an MIC and MBC of 100 $\mu\text{g/mL}$ against the selected bacteria. Since the MBC values were equal to the MIC values and the MBC/MIC ratio was less than 4, the extract can be classified as bactericidal³⁹. Although the standard antibiotic levofloxacin exhibited better antibacterial activity (MIC: 25 $\mu\text{g/mL}$), the methanolic extract showed a comparable MIC response against both bacteria at higher concentrations. This implies that the antibacterial potential of the extract may be due to synergistic or multi-target interactions of phytochemicals, which is an advantage over single-target synthetic drugs that could quickly lead to the development of resistance⁴⁰.

With the increasing global demand for efficient drug discovery systems, Computer-aided drug discovery (CADD) has gained importance in modern

pharmaceutical research⁴⁴. CADD integrates cheminformatics, bioinformatics, molecular modelling, and data mining with artificial intelligence (AI) approaches, including machine learning and deep learning, to help lead identification and optimization⁴⁵. In this study, CADD tools were used to support experimental results and identify active components responsible for antibacterial activity.

The study of the physicochemical, ADMET, and drug-likeness properties of five selected phytochemicals from the 24th fraction of methanolic leaf extract of *H. suaveolens* indicated that 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone and diethyl phthalate possess favourable drug-like characteristics satisfying essential oral drug standards⁴⁶. However, diethyl phthalate, often a model aromatic pollutant, showed low toxicity according to ADMET analysis⁴⁷. Diethyl phthalate and 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone showed better solubility and clearance than levofloxacin, although levofloxacin showed better metabolism, absorption, and half-life properties. Toxicity studies revealed that diethyl phthalate and 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone were less toxic than levofloxacin, indicating their potential safety for therapeutic applications requires further *in vivo* authentication⁴⁸.

Molecular docking studies with selected target proteins, DNA polymerase III subunit beta, and intercellular adhesion protein (required for bacterial pathogenesis) revealed that 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone exhibited strong affinity and minimum binding energy towards both proteins. The strong affinity suggests that these compounds could inhibit bacterial growth, preventing bacterial biofilm formation and DNA replication. The selected compounds showed more binding efficacy to levofloxacin. Along with notable antibacterial potential, the selected compounds may exhibit antiviral properties due to possible multi-target inhibitory activities⁴⁹.

Therefore, the present study validates the antibacterial potential of *H. suaveolens* methanolic fraction and identifies promising lead phytochemicals displaying bactericidal activity, strongly supported by CADD-based predictions. These findings offer a strong basis for future isolation, structural optimization, mechanistic evaluation, and *in vivo* verification to transform these naturally derived compounds into potent therapeutic agents against drug-resistant pathogens.

Conclusion

The present study recommended that the 24th fraction of methanolic leaf extract of *H. suaveolens* has significant antibacterial activity against selected bacterial strains. GC-MS analysis revealed numerous compounds with documented antibacterial activity. Molecular docking and ADMET analysis further supported that selected phytochemicals are safe, possess drug-like properties, and show low toxicity for humans and the environment. Among all the phytochemicals, two – diethyl phthalate and 1,2-dihydro-2-oxo-4-quinolyl-2-pyridyl ketone appeared as promising lead molecules with stronger binding affinity, favourable ADME profile and lower toxicity compared with standard antibiotic levofloxacin. Nevertheless, *in vivo* studies are suggested to validate their therapeutic applicability. These findings suggest that *H. suaveolens* possesses antibacterial potential and should be further explored for natural product-based drug discovery.

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Conflict of interest

The authors declare no conflict of interest.

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