BIOACTIVE COMPOUNDS IN CURCUMA LONGA EXTRACTS: POTENTIAL INHIBITORS OF MULTIDRUG-RESISTANT KLEBSIELLA SPP.

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Abstract. This study evaluated the inhibitory effects of turmeric (Curcuma longa) extracts against selected multidrug-resistant Klebsiella spp. Freshly ground C. longa was extracted using the maceration method, and the susceptibility of the organisms to the extract was tested using the agar well diffusion technique. Gas chromatography-mass spectrometry (GC-MS) was employed to identify the bioactive compounds in the extract. Molecular docking was performed using the Glide module of Maestro Schrödinger to predict binding affinities and interaction modes of C. longa phytochemicals with SHV-1 (beta-lactamase-1) of K. pneumoniae and NpsA (phosphoribosyltransferase) of K. oxytoca. The inhibition zones of C. longa extract against the antibiotic-resistant Klebsiella spp. ranged from 17±0.88 mm to 18.67±0.88 mm. The minimum inhibitory concentration (MIC) of the extract against the test organisms ranged from 25 mg/ml to 50 mg/ml, while the maximum bactericidal concentration (MBC) was 100 mg/ml. GC-MS analysis identified 31 compounds in the C. longa extract, with the main components being n-Hexadecanoic acid (9.14%), methyl tetradecanoate (7.77%), octadecanamide (7.74%), maltose (7.74%), and phytol (7.45%). Molecular docking analyses identified C. longa phytochemicals with strong inhibitory potential against SHV-1 in Klebsiella pneumoniae and NpsA in K. oxytoca. 1,2-Benzenedicarboxylic acid, diheptyl ester showed the highest binding scores (−8.641 and −9.765 kcal/mol), while other compounds exhibited stable interactions and favorable pharmacokinetic profiles, outperforming standard antibiotics. These findings suggest that C. longa extract could be a promising alternative for combating infections caused by antibiotic-resistant Klebsiella spp.
Keywords: *Klebsiella Pneumoniae, Klebsiella Oxytoca, multidrug-resistance, Curcuma Longa, GC-MS, phytochemicals*

Introduction

Klebsiella spp. are widely distributed in nature, found in both environmental habitats, such as surface water, sewage, and soil, and on the mucosal surfaces of mammals like humans, horses, and swine. These bacteria are capable of causing a broad range of infections, including pneumonia, soft tissue and surgical wound infections, urinary tract infections, bloodstream infections, and sepsis (Holt et al., 2015). Klebsiella spp. are considered one of the most significant causes of both nosocomial (hospital-acquired) and community-acquired infections. It is grouped into cohorts, namely Klebsiella pneumoniae species complex (KpSC), which includes Klebsiella pneumoniae, Klebsiella quasipneumoniae, and Klebsiella variicola; while Klebsiella oxytoca, Klebsiella indica, and Klebsiella terrigena are assigned to another genetically distinct group (Dong et al., 2022). The KpSC group is responsible for most healthcare-associated infections (Dong et al., 2022; Martin and Bachman, 2018; Stojowska-Swędrzyńska and Krawczyk, 2016; Prado et al. 2008). In healthcare settings, Klebsiella infections are a major concern due to their ability to spread easily within hospital environments, often leading to outbreaks. The infections are particularly dangerous for

high-risk individuals, including immunocompromised patients, neonates, and the elderly, as these populations are more vulnerable to severe illness. The presence of Klebsiella in hospitals has led to high morbidity and mortality rates, as these infections are often difficult to treat due to increasing antibiotic resistance (Bengoechea and Pessoa, 2019; Li et al., 2019; Holt et al., 2015; Bergogne-Berezin, 1995). The burden of Klebsiella infections continues to rise, making them a critical focus for both clinical research and infection control strategies.

Plants are a source of diversified repertoire of bioactive compounds representing rich prospects for drug development (Oluyele and Akinyueke, 2025). Medicinal plants have been an essential part of human life for centuries. These plants are recognized for their therapeutic, tonic, and various pharmacological properties (Louis et al., 2018). Curcuma longa (turmeric) is a rhizomatous, herbaceous perennial plant from the ginger family Zingiberaceae, native to tropical South Asia. A total of 133 species of Curcuma have been identified globally, many of which are known by local names and are utilized in various medicinal formulations (Bannuru et al., 2018). Turmeric is nutritionally rich containing carbohydrates, fiber, proteins, vitamin C, pyridoxine, magnesium, phosphorus, potassium, and calcium (Urmila et al., 2020). Its key component, curcumin, acts as a natural oxygen scavenger and nitrogen provider, and has been shown to be effective in alleviating pain caused by arthritis. Chemical compounds present in these plants mediate their effects on human body through processes identical to those already well understood from chemical compounds in conventional drugs (Ann et al., 2018). C. longa and its curcumin constituent have significant antioxidant activity, equivalent to both vitamin C and vitamin E, in both water and fat-soluble extracts. Additionally, they have demonstrated promising antioxidant and anti-inflammatory activities, being considered a valuable complementary therapy to pharmaceuticals in Crohn's, diabetes and cancer between other disorders (Meng et al., 2018; Amalraj et al., 2017; Bengmark et al., 2009). Klebsiella pneumoniae and Klebsiella oxytoca are among the most prevalent Klebsiella species in clinical environments. In particular, the World Health Organization has identified K. pneumoniae as a global priority pathogen requiring next-generation antibiotics due to its high diversity of antimicrobial resistance genes. The increasing prevalence of antimicrobial-resistant Klebsiella spp. presents a major global health threat, emphasizing the need for alternative treatments. This study explores the potential of Curcuma longa extract as a therapeutic option against antibiotic-resistant K. pneumoniae and K. oxytoca.

Materials and Methods

Collection of plant material and extraction of phytochemicals

Rhizomes of Curcuma longa Linn. (Zingiberaceae) sourced from Sabo market, Ikorodu-Latitude -6.6194°N and Longitude -3.5105°E Lagos State, Nigeria was used for the experiment. The Curcuma longa rhizomes were identified and authenticated in Plant Science and Biotechnology Departmental Herbarium (PSBH), Adekunle Ajasin University, Ondo State, Nigeria. Both rhizomes and whole plant voucher specimen designated as PSBH-258 were deposited at the herbarium. The extraction of the plant part was carried using cold maceration method. One hundred grams (100g) of the plant material was weighed and dissolved in 1000ml of the extraction solvent (ethanol) inside a 2 litres conical flask and covered with parafilm. The flasks were shaken vigorously at 30 minutes interval and left to stand for 7 days at room temperature. The resultant

mixture was then filtered with whatman's No.4 filter paper and cotton wool to remove particles of the plant sample. The clear solution obtained was distilled at 65°C under low pressure on a steam bath. The semi-solid concentrations of the extracts were then collected in sterile pre-weighed screw capped bottles and labeled accordingly. The extracts were stored at 4°C until when needed. Serial dilutions of the extracts were prepared to obtain concentrations ranging from 12.5 mg/ml to 100 mg/ml (Ogunjobi et al., 2014).

Antimicrobial susceptibility test

The antimicrobial potential of the extract was evaluated using the agar well diffusion method (Oluyele and Oladunmoye, 2017). A 1 ml aliquot of each standardized test organism suspension was spread evenly on sterilized Mueller-Hinton agar plates. After drying, uniform wells (6mm in diameter) were made in the plates, and 50 µL of the extract (100 mg/mL in 5% dimethyl sulfoxide) was added to the wells, with Ciprofloxacin/Augmentin as a control. The plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract were determined using tube-dilution and plating methods (Oluyele and Oladunmoye, 2017). For MIC, various concentrations of the extract (ranging from 100 to 3.125 mg/ml) were prepared, followed by adding 0.5 ml of the test inoculum into each tube. Negative and positive control tubes were also prepared. The tubes were incubated at 37°C for 24 hours, and the MIC was determined as the lowest concentration with no visible turbidity. To determine the MBC, samples from MIC tubes and other non-turbid tubes were subcultured onto fresh Mueller-Hinton agar, incubated at 37°C, and the MBC was identified as the concentration with no visible growth.

Gas Chromatography Mass Spectroscopic (GCMS) analysis of C. longa extract

The samples were subjected to chromatographic analysis using a Varian 3800/4000 gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column VF-5MS fussed silica capillary column (30.0m x 0.25um, composed of 5% phenyl/95% dimethylpolysiloxane), operating in electron impact mode at 70ev; nitrogen (99.999%) was used as carrier gas at a constant flow of 1. ml/min and an injection volume of 0.5ul was employed (split ratio of 10:1) injector temperature 240°C ion source temperature 200°C. The oven temperature was programmed from 70°C (isothermal for 3 min), with an increase of 10°C /min, to 240°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 40 to 1000Da. The identification of the various components was based on comparison of their mass spectra with those of NIST Library mass Spectra data base and mass spectra from Literature.

Compound library and protein preparation

GC-MS-identified phytochemicals from Curcuma longa and the standard drug compounds were retrieved from the PubChem database in structure data file (SDF) format. These compounds were imported into the Schrödinger Workspace and prepared for docking using the LigPrep tool. Protein structures were obtained from the RCSB Protein Data Bank, specifically SHV-1 (class A β-lactamase from Klebsiella pneumoniae, PDB ID: 2ZD8) and NpsA (nicotinate phosphoribosyltransferase from

Klebsiella oxytoca, PDB ID: 6VHV), both complexed with co-crystallized ligands. Missing residues, loops, and side chain anomalies were corrected using the Protein Preparation Wizard in Schrödinger Suite 2021, followed by energy minimization with the OPLS4 force field. Receptor grid generation was performed at the co-ligand binding sites using the Glide Grid Generator tool.

Structure-based virtual screening, binding energy calculation and ADME/Tox profiling

Prepared C. longa phytochemicals and standard ligands were screened against SHV-1 and NpsA using the Extra Precision (XP) docking protocol in the Glide module of the Maestro Schrödinger Suite (v2021). This method offers high accuracy in distinguishing binding affinities, albeit with increased computational demand (Oluyele et al., 2025). The resulting protein-ligand complexes were further refined using the local optimization feature in Prime, and their binding free energies (ΔG_bind) were calculated using MM/GBSA with the OPLS4 force field. To assess pharmacokinetic properties and toxicity, hit compounds were evaluated using the SwissADME and ProTox-II web servers for ADME/Tox profiling.

Results and Discussion

Susceptibility of selected antibiotics resistant Klebsiella spp to C. longa extract

Table 1 presents the antibacterial activity of C. longa extract against the test organisms. The extract exhibited the largest inhibition zone against Klebsiella oxytoca (18.67 ± 0.88), with the minimum inhibitory concentration ranging from 25 mg/ml to 50 mg/ml.

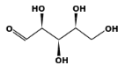
***Table 1**. Susceptibility of Selected Multidrug Resistant Klebsiella spp to C. longa Extract.*

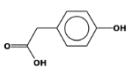
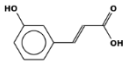
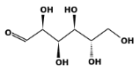
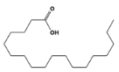
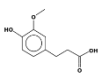
Organism	ZOI	Control	MIC	MBC
Klebsiella pneumoniae	17.67 ± 0.88	33.67 ± 0.58	25 mg/ml	100 mg/ml
Klebsiella oxytoca	18.67 ± 0.88	28.33 ± 0.88	50 mg/ml	100 mg/ml

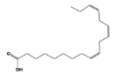
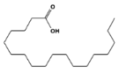
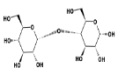
GCMS identified compounds of C. longa extract

GC-MS analysis identified thirty-one compounds in the Curcuma longa extract. The primary components included n-Hexadecanoic acid (9.14%), methyl tetradecanoate (7.77%), octadecanamide (7.74%), maltose (7.74%), and phytol (7.45%). The detailed results are shown in *Table 2* and *Figure 1*.

***Table 2**. GCMS identified compounds in C. longa.*

Peak	RT	CD	MF	MW	PA(%)	C(wt%)	m/z	S
1	3.50	Acetic acid	$C_2H_4O_2$	60	0.45	0.96	43, 45, 60	
2	6.00	Xylose	$C_5H_{10}O_5$	150	0.58	1.01	43, 73, 150	
3	10.02	2-Methoxy-5-methylphenol	$C_8H_{10}O_2$	138	1.37	0.92	57, 123, 138	
4	10.64	2-Hydroxy-5-methylbenz	$C_8H_8O_2$	136	2.52	2.03	50, 118, 158	

5	12.75	aldehyde 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	1.60	1.88	43, 101, 144	
6	13.07	Benzene, 1-methyl-3-propyl-	$C_{10}H_{14}$	134	0.90	0.72	77, 105, 134	
7	16.37	2-Dodecanol	$C_{12}H_{26}O$	186	2.54	2.27	41, 45, 186	
8	15.60	2(3H)-Furanone, 5-heptyldihydro-	$C_{12}H_{24}O_2$	184	0.52	0.28	85, 128, 184	
9	17.58	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	$C_9H_{10}O_4$	182	2.24	2.77	65, 111, 182	
10	19.00	Benzeneacetic acid, 4-hydroxy-	$C_8H_8O_3$	152	0.91	1.54	77, 107, 152	
11	19.72	2-Propenoic acid, 3-(3-hydroxyphenyl)-	$C_9H_8O_3$	164	0.49	0.30	65, 91, 164	
12	21.64	Glucose	$C_6H_{12}O_6$	180	0.24	0.44	43, 60, 180	
13	22.80	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	0.69	1.75	55, 69, 296	
14	24.50	Octadecanoic acid	$C_{18}H_{36}O_2$	284	3.26	2.65	43, 73, 284	
15	25.50	9-Octadecenal, (Z)-	$C_{18}H_{34}O$	266	0.68	1.53	41, 55, 266	
16	25.98	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	0.62	0.41	43, 74, 296	
17	27.00	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.74	0.62	43, 73, 228	
18	27.50	Oleic acid	$C_{18}H_{34}O_2$	282	6.42	7.83	41, 55, 282	
19	28.62	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	3.09	3.00	41, 67, 280	
20	31.78	β-(4-Hydroxy-3-methoxyphenyl)propionic acid	$C_{10}H_{12}O_4$	196	3.11	3.76	77, 133, 196	
21	34.98	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	7.77	7.43	43, 74, 254	

22	35.27	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	9.14	9.00	43, 82, 240	
23	36.48	Phytol	$C_{20}H_{40}O$	296	7.45	7.11	43, 71, 296	
24	36.51	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	3.66	3.32	41, 79, 292	
25	37.50	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	5.55	6.00	43, 79, 278	
26	37.75	Octadecanamide	$C_{18}H_{37}NO$	283	7.74	6.68	43, 59, 283	
27	38.50	Octadecanoic acid	$C_{18}H_{36}O_2$	284	3.50	3.15	43, 73, 284	
28	40.25	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	3.18	3.83	43, 81, 296	
29	42.25	Maltose	$C_{12}H_{22}O_{11}$	342	7.74	6.96	43, 73, 342	
30	43.67	1,2-Benzenedicarboxylic acid, diheptyl ester	$C_{22}H_{34}O_4$	362	7.39	7.00	41, 149, 362	
31	44.50	Squalene	$C_{30}H_{50}$	410	3.84	3.16	41, 69, 410	

Note: CD=Compound Detected; MF=Mol. Formula; PA(%)=Peak Area (%); C(wt%)=Comp(wt%); S=Structure.

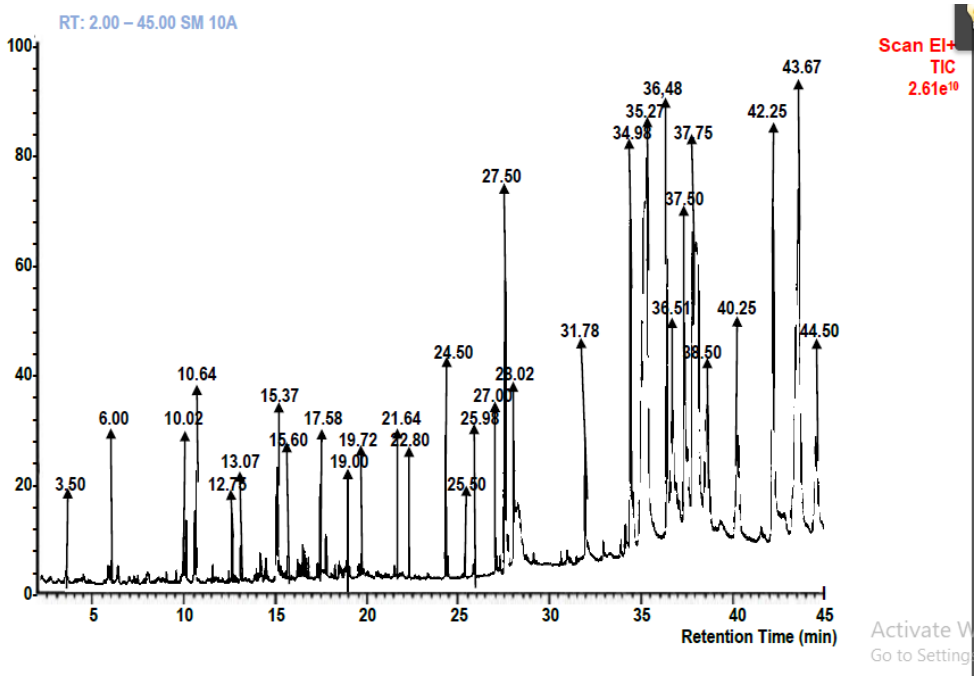


Figure 1. *Chromatograph of C. longa Extract.*

Binding affinity, interaction analysis and drug-likeness results

Molecular docking and MM/GBSA analyses revealed that several phytochemicals from Curcuma longa exhibited strong binding affinities and stable interactions with key therapeutic targets in Klebsiella pneumoniae and Klebsiella oxytoca. Against the β-lactamase SHV-1 of K. pneumoniae, the top compounds demonstrated superior inhibitory potential compared to the reference drug meropenem, with 1,2-benzenedicarboxylic acid, diheptyl ester showing the highest binding affinity (−8.641 kcal/mol) and maltose presenting the most stable binding energy (−56.69 kcal/mol) (*Figure 2*). In the case of K. oxytoca, targeting the NpsA protein, 1,2-benzenedicarboxylic acid, diheptyl ester also recorded the best docking score (−9.765 kcal/mol), while benzeneacetic acid, 4-hydroxy- showed the most favorable binding energy (−44.58 kcal/mol) (*Figure 3*). Two-dimensional interaction analyses (*Table 3* and *Table 4*; *Figure 4* and *Figure 5*) confirmed the involvement of key amino acid residues through hydrogen bonding and hydrophobic interactions, reinforcing the observed binding stability. ADMET profiling (*Table 5* and *Table 6*) indicated that the majority of lead compounds had high gastrointestinal absorption, good solubility, and did not inhibit CYP450 enzymes, suggesting favorable pharmacokinetic properties and low toxicity. Overall, the top C. longa compounds displayed comparable or enhanced performance relative to meropenem and ciprofloxacin

Table 3. *Hydrogen bonds and hydrophobic interactions of the hit phytochemicals of C. longa for K. pneumoniae.*

Compound name	H-bound	Hydrophobic interactions	Other interaction
1,2-Benzenedicarboxylic acid, diheptyl ester	None	ILE 263, VAL 261, ILE 246, ALA 248, LEU 250, ALA 217, LEU 220, ILE 221, VAL 224, LEU 225, ILE 231, ILE 287, ALA 280, ILE 279	None
Maltose	None	VAL 224, ILE 221, LEU 220, ALA 217, ILE 246, ALA 248, LEU 250, ILE 231, LEU 286, ILE 282, ALA 280, ILE 279, VAL 261, ILE 263	None
2-Propenoic acid, 3-(3hydroxyphenyl)-	ILE 279, VAL 224	VAL 261, ILE 263, ILE 246, ALA 248, LEU 250, ILE 231, ILE 221, VAL 224, LEU 225, PRO 226, ILE 279, ALA 280, ILE 282, ALA 284, ILE 287	None
2-Hydroxy-5-methylbenzaldehyde	ILE 279	VAL 261, ILE 263, ILE 246, ALA 248, LEU 249, LEU 250, ILE 231, ILE 221, VAL 224, LEU 225, ILE 287, ILE 279, ALA 280	None
Meropenem	GLN 277	ILE 246, ILE 279, ALA 280, ALA 217, LEU 220, ILE 221, VAL 224, ALA 273	None

Table 4. *Hydrogen bonds and hydrophobic interactions of the hit phytochemicals of C. longa for K. oxytoca.*

Compound name	H-bound	Hydrophobic interactions	Other interaction
1,2-Benzenedicarboxylic acid, diheptyl ester	ASP 388, ARG 403, ASP 387, ARG 370, LYS 167, GLY 296, THR 298, GLU 299, THR 159	LEU 400, TYR 384, TYR 361, CYS 297	None
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	GLY 272, GLY 294, ASN 293	LEU 295, PHE 274, VAL 275, VAL 323, VAL 321. PRO 320, LEU 400, VAL 270	None
Benzeneacetic acid, 4-hydroxy-	LEU 400, VAL 321, GLY 294, GLY 296, GLY 272	VAL 399, LEU 400, VAL 323, PRO 322, VAL 321, PRO 320, VAL 270, LEU 295, PHE 274	None
2-Propenoic acid, 3-(3, 4-hydroxyphenyl)-	GLY 294, GLY 272, VAL 321	LEU 295, PHE 274, VAL 275, VAL 323, VAL 321, PRO320, PHE 280, LEU 400	PI-PI STACK: PHE 274, SALT BRIDGE: VAL 275
Ciprofloxacin	GLY 296, GLY 272, CYS 492, VAL 321	LEU 291,CYS 297, PHE 274, VAL 271, VAL 122, PRO 322, VAL 321, PRO 320, PHE 280, VAL 199, LEU 400	PI-PI STACK: PHE 274

***Table 5**. Druglikeness and ADMET profile of C. longa top compounds and Meropenem.*

A	B	C	D	E	F	G	H	I	J	K	L
1,2-Benzenedicarboxylic acid, diheptyl ester	362.5	4	0	52.6	5.02	1	-4.86	High	No	No	0.55
Maltose	342.3	11	8	189.53	0.54	2	0.55	Low	No	No	0.17
2-Propenoic acid, 3-(3hydroxyphenyl)-	180.16	4	3	77.76	0.97	0	-1.89	High	No	No	0.56
2-Hydroxy-5-methylbenzaldehyde	136.15	2	1	37.3	1.34	0	-2.16	High	No	No	0.55
Meropenem	383.46	6	3	135.48	1.82	0	-0.33	Low	No	No	0.55

Note: A=Compound; B=Molecular Weight; C=Hydrogen Bond Acceptor; D=Hydrogen Bond Donor; E=TPSA; F=iLOGP; G=Rule of Five; H=ESOL Log S; I= Gastrointestinal Absorption; J=CYP2CL9 inhibitor; K=CYP2C9 inhibitor; L=Bioavailability.

***Table 6**. Druglikeness and ADMET profile of C. longa top compounds and Ciprofloxacin.*

A	B	C	D	E	F	G	H	I	J	K	L
1,2-Benzenedicarboxylic acid, diheptyl ester	362.5	4	0	52.6	5.02	1	-4.86	High	No	No	0.55
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144.13	4	2	66.76	1.19	0	-0.5	High	No	No	0.85
Benzeneacetic acid, 4-hydroxy-	152.15	3	2	57.53	0.88	0	-1.53	High	No	No	0.85
2-Propenoic acid, 3-(3, 4-hydroxyphenyl)-	180.16	4	3	77.76	0.97	0	-1.89	High	No	No	0.56
Ciprofloxacin	331.34	5	2	74.57	2.24	0	-1.32	High	No	No	0.55

Note: A=Compound; B=Molecular Weight; C=Hydrogen Bond Acceptor; D=Hydrogen Bond Donor; E=TPSA; F=iLOGP; G=Rule of Five; H=ESOL Log S; I= Gastrointestinal Absorption; J=CYP2CL9 inhibitor; K=CYP2C9 inhibitor; L=Bioavailability.

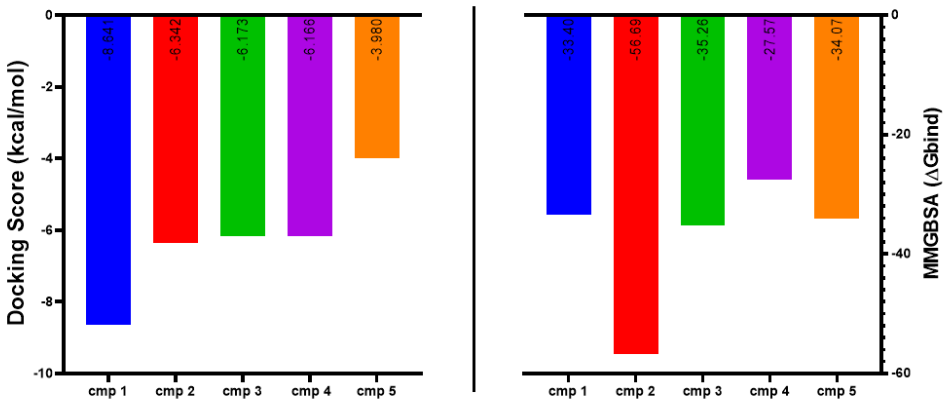


***Figure 2.** Graphical representation of the binding affinity and binding free energy of bioactive compounds of C. longa against K. pneumoniae Beta-lactamase Shv-1.*

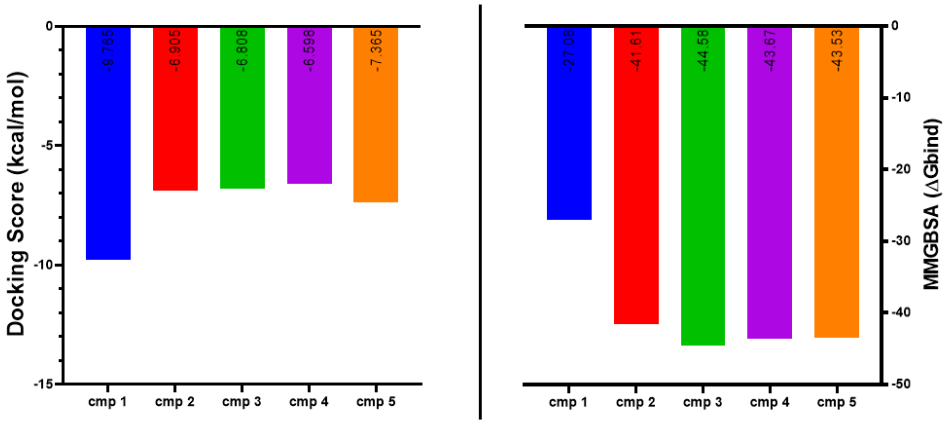


Figure 3. *Graphical representation of the binding affinity and binding free energy of bioactive compounds of C. longa against K. oxytoca NpsA.*

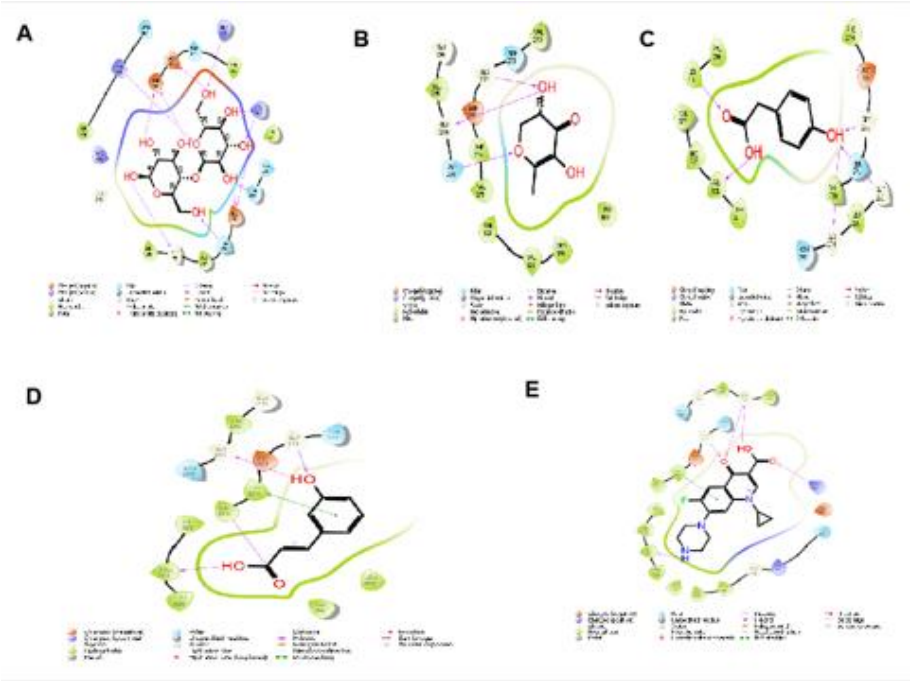


Figure 4. *2D interaction between C. longa compounds and K. pneumoniae Beta-lactamase Shv-1.*

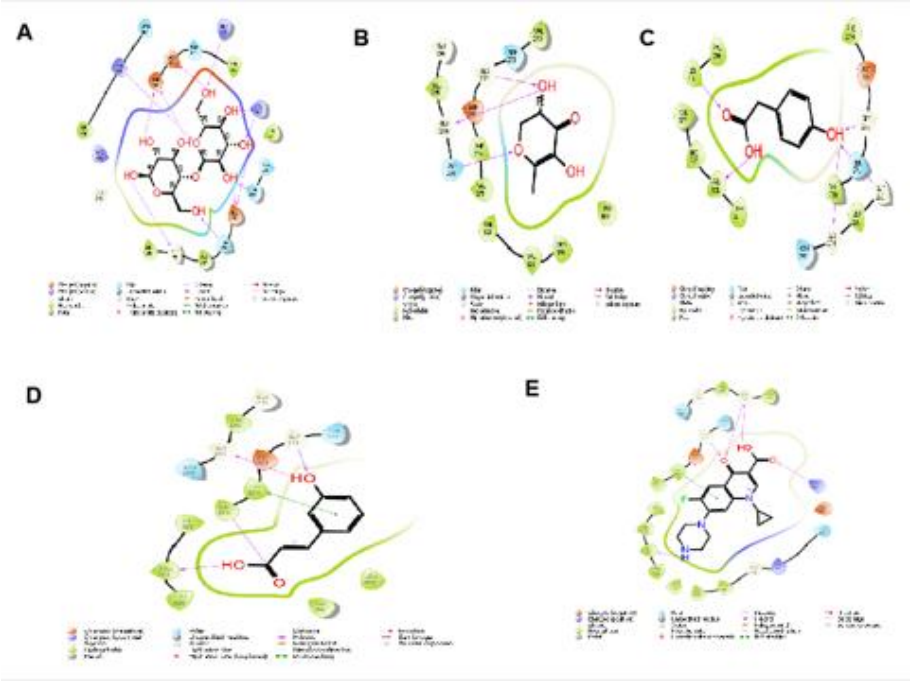


Figure 5. *2D interaction between C. longa compounds and K. oxytoca NpsA.*

Klebsiella species are major contributors to hospital-acquired infections and are recognized as critical priority pathogens in healthcare settings due to their high transmissibility and frequent involvement in nosocomial outbreaks (Bergogne-Berezin, 1995). The escalating threat posed by multidrug-resistant Klebsiella strains highlights the urgent need for novel antimicrobial agents, particularly those derived from medicinal plants with well-documented bioactive properties. In this study, extract of Curcuma longa was tested against MDR pathogenic strains of Klebsiella spp. From our results, Curcuma longa exhibited antimicrobial activity against Klebsiella pneumoniae and Klebsiella oxytoca, with zones of inhibition measuring 17.57 ± 0.88 and 18.67 ± 0.88, respectively. Buttressing our findings, C. longa has been noted to demonstrate strong antimicrobial properties against various bacteria, including Escherichia coli, K. Pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis (Momoh et al., 2022; Chakraborty et al., 2011; Chandrana et al., 2005; Kim et al., 2005). Our study identified several key components of Curcuma longa extract using GC-MS, including n-Hexadecanoic acid, Octadecanamide, Maltose, and Phytol-which are recognized for their pharmacological properties. For example, n-Hexadecanoic acid has antioxidant, hypocholesterolemic, nematicidal, and pesticidal effects (Ravi and Kannabiran, 2017), while Maltose helps inhibit biofilm formation (Bao et al., 2015). Phytol is noted for its broad biological activities, such as antimicrobial, anxiolytic, cytotoxic, immune-modulating, antioxidant, and anti-inflammatory effects (Pejin et al., 2014).

In comparison with our results, Anekwe et al. (2023) identified Oleic acid and n-Hexadecanoic acid among 11 components from C. longa. Yang et al. (2020) found a different set of compounds, including gallic acid, curcumin, and myricetin, in C. longa extract (Akbar et al., 2016). Similarly, Arivoli et al. (2019) identified 10 compounds from methanolic C. longa rhizomes via GC-MS, including Bicyclo [4.1.0]-3-heptene and 6-octen-1-yn-3-ol. Furthermore, top phytochemicals from Curcuma longa were investigated for their therapeutic potential against Klebsiella pneumoniae and Klebsiella

oxytoca. Their efficacy was evaluated through molecular docking, MM/GBSA binding free energy calculations, and ADMET profiling. Ciprofloxacin and meropenem were included as reference antibiotics for K. oxytoca and K. pneumoniae, respectively, to benchmark the comparative performance of the phytochemicals. To elucidate the inhibitory potential of top Curcuma longa phytochemicals against K. pneumoniae, molecular docking studies were conducted targeting the NpsA enzyme-a critical protein involved in bacterial virulence and survival. The results revealed diverse binding affinities and interaction profiles among the compounds. 1,2-Benzenedicarboxylic acid, diheptyl ester emerged as the top-performing ligand, achieving the strongest docking score (−8.641 kcal/mol). Its high binding affinity was primarily driven by hydrophobic interactions within the active site, notably with residues such as ILE 263, VAL 261, and ILE 246, despite the absence of hydrogen bonding. This underscores the crucial role of hydrophobic forces in ligand binding stability for this target. Maltose, while displaying a moderate docking score (−6.342 kcal/mol), achieved the most favorable MM/GBSA value (−56.69 kcal/mol), suggesting robust binding stability that may result from entropic and solvation effects. Similarly, compounds such as 2-Propenoic acid, 3-(3,4-hydroxyphenyl)-and 2-Hydroxy-5-methylbenzaldehyde exhibited moderate docking (−6.173 and −6.166 kcal/mol, respectively) and MM/GBSA scores (−35.26 and −27.57 kcal/mol), indicating respectable binding affinities facilitated by a combination of hydrophobic contacts and hydrogen bonding.

Interestingly, meropenem-the reference β-lactam antibiotic, showed the weakest docking score (−3.980 kcal/mol), yet maintained a moderately stable MM/GBSA value (−34.07 kcal/mol). This apparent discrepancy between binding affinity and complex stability may reflect its known pharmacodynamic limitations against resistant strains. Notably, this aligns with findings by Gülen et al. (2021), who observed synergistic antibacterial effects when meropenem was co-administered with curcumin against carbapenem-resistant K. pneumoniae. Such observations support the potential utility of phytochemical adjuncts to enhance the efficacy of conventional antibiotics. The limited binding performance of meropenem may also reflect broader β-lactam resistance mechanisms prevalent in Klebsiella spp., driven largely by the production of extended-spectrum β-lactamases (ESBLs) and AmpC enzymes (Rubin and Pitout, 2014; Müller-Schulte et al., 2020). Additionally, the excessive clinical and agricultural use of β-lactams has intensified resistance selection pressure, further compromising these cornerstone therapies (Guardabassi et al., 2004). 2D interaction profiling further highlighted the binding nuances of each compound. While 1,2-Benzenedicarboxylic acid, diheptyl ester formed extensive hydrophobic contacts without hydrogen bonds, maltose engaged in broad hydrophobic interactions as well. 2-Propenoic acid, 3-(3,4-hydroxyphenyl)- demonstrated a hybrid interaction pattern, involving both hydrogen bonding (ILE 279, VAL 224) and hydrophobic forces. Meanwhile, 2-Hydroxy-5-methylbenzaldehyde formed a single hydrogen bond (ILE 279) in conjunction with hydrophobic contacts, which may account for its relatively weaker binding. Meropenem, consistent with its known mechanism, formed a hydrogen bond with GLN 277 alongside hydrophobic interactions.

To assess the pharmacokinetic viability and safety of the top Curcuma longa phytochemicals against Klebsiella pneumoniae, in silico ADMET profiling was conducted using SwissADME. These analyses revealed favorable drug-likeness properties for most compounds, with key parameters evaluated including gastrointestinal absorption (GIA), bioavailability (BA), solubility, lipophilicity, and

cytochrome P450 (CYP) inhibition potential. 1,2-Benzenedicarboxylic acid, diheptyl ester exhibited high GIA and a BA score of 0.55, supporting its oral bioavailability potential. However, its high lipophilicity (iLOGP = 5.02) and poor solubility (Log S = −4.86) suggest that formulation strategies may be necessary to optimize its delivery. In contrast, maltose showed low GIA and a poor BA (0.17), likely due to its large topological polar surface area (TPSA = 189.53 Åo) and excessive hydrogen bonding capacity, which hinder membrane permeability. Compounds such as 2-Propenoic acid, 3-(3,4-hydroxyphenyl)- and 2-Hydroxy-5-methylbenzaldehyde demonstrated optimal oral pharmacokinetics, with high GIA and BA scores (0.55–0.56), moderate lipophilicity, and favorable solubility profiles. These characteristics indicate strong potential for systemic bioavailability without the need for complex formulations. Meropenem, the reference antibiotic, was predicted to have low GIA due to its high TPSA (135.48 Åo), reflecting its limited passive diffusion-a known pharmacokinetic limitation for β-lactam antibiotics. Importantly, none of the tested compounds were predicted to inhibit major CYP enzymes (e.g., CYP2C9, CYP2C19), suggesting a low likelihood of adverse metabolic interactions. These ADMET predictions support earlier docking and MM/GBSA results, further reinforcing the therapeutic promise of C. longa constituents. Notably, these computational findings align with the in vitro results reported by Rattanasuk et al. (2023), who confirmed the antibacterial activity of C. longa rhizome extracts against K. pneumoniae.

To evaluate the binding potential of Curcuma longa phytochemicals against Klebsiella oxytoca, molecular docking studies were conducted alongside MM/GBSA binding free energy calculations. These analyses revealed diverse and promising interaction profiles between the top compounds and the target protein. Among the phytochemicals, 1,2-Benzenedicarboxylic acid, diheptyl ester recorded the highest docking score (−9.765 kcal/mol), forming an extensive hydrogen bond network with residues such as ASP 388, ARG 403, and ARG 370. However, its MM/GBSA score (−27.08 kcal/mol) was relatively moderate, implying that while the initial binding is strong, the overall complex may exhibit lower stability in physiological conditions. In contrast, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl had a lower docking score (−6.905 kcal/mol) but demonstrated a more favorable MM/GBSA score (−41.61 kcal/mol), indicating greater binding stability. This compound interacted with both polar and hydrophobic residues, supporting a flexible binding mode. Benzeneacetic acid, 4-hydroxy- showed the most stable binding complex, with the best MM/GBSA score (−44.58 kcal/mol), despite a moderate docking score (−6.808 kcal/mol). This stability is likely due to its extensive hydrogen bonding and hydrophobic contacts. Similarly, 2-Propenoic acid, 3-(3,4-hydroxyphenyl)-achieved robust docking (−6.598 kcal/mol) and MM/GBSA (−43.67 kcal/mol) values, enhanced by π–π stacking and salt bridge interactions. Ciprofloxacin, the standard reference antibiotic, produced comparable docking (−7.365 kcal/mol) and MM/GBSA (−43.53 kcal/mol) scores. The fact that several phytochemicals matched or exceeded these values suggests their potential to serve as effective alternatives or adjuncts in combating K. oxytoca infections. To complement the molecular docking and binding energy analyses, the ADMET evaluation provided critical insights into the pharmacokinetic behavior and drug-likeness of the top phytochemicals targeting K. oxytoca. All tested compounds demonstrated favorable ADMET properties, supporting their potential as orally administered therapeutics. Benzeneacetic acid, 4-hydroxy- and 4H-pyran-4-one showed high gastrointestinal absorption (GIA), optimal molecular weights (<160 g/mol), and

high solubility (Log S = −1.53 and −0.5, respectively), indicating strong oral bioavailability and pharmacokinetic profiles. In contrast, 1,2-Benzenedicarboxylic acid, diheptyl ester, while exhibiting excellent docking affinity, displayed high lipophilicity (iLOGP = 5.02) and poor solubility, potentially necessitating advanced formulation strategies to enhance its bioavailability. Meanwhile, 2-Propenoic acid, 3-(3,4-hydroxyphenyl)- presented a well-balanced ADMET profile, with moderate solubility, high GIA, and a bioavailability score (BA) of 0.56, making it a promising candidate for further optimization. Importantly, none of the compounds were predicted to inhibit cytochrome P450 (CYP) enzymes, minimizing concerns over metabolic drug–drug interactions and reinforcing their suitability for therapeutic development.

Conclusion

This study demonstrates the promising antimicrobial potential of Curcuma longa-derived phytochemicals against multidrug-resistant Klebsiella pneumoniae and Klebsiella oxytoca. Through molecular docking and MM/GBSA free energy analyses, several compounds, such as 1,2-Benzenedicarboxylic acid, diheptyl ester; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; benzeneacetic acid, 4-hydroxy-; and 2-Propenoic acid, 3-(3,4-hydroxyphenyl)-exhibited strong binding affinities and stable interactions with bacterial target proteins. Notably, some of these phytochemicals outperformed standard antibiotics (ciprofloxacin and meropenem) in terms of binding energy and interaction profiles. ADMET profiling further supported the therapeutic promise of these compounds, revealing favorable pharmacokinetic properties such as high gastrointestinal absorption, good solubility, and minimal risk of cytochrome P450 inhibition, indicating a low likelihood of adverse drug–drug interactions. However, certain compounds, like 1,2-Benzenedicarboxylic acid, diheptyl ester, may require formulation strategies to address solubility limitations and improve bioavailability. Overall, the combined computational evidence of strong molecular interactions, drug-likeness, and safety profiles underscores the potential of C. longa-derived compounds as candidates for novel antibacterial drug development. Further experimental validation, including in vitro and in vivo studies, as well as isolation and structural characterization of the active constituents, is essential to advance these findings toward clinical application in combating resistant Klebsiella infections.

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Conflict of interest

The authors confirm that there is no conflict of interest involve with any parties in this research study.

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