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Phytochemical Composition, *In Silico* Molecular Docking Analysis and Antibacterial Activity of *Lawsonia inermis* Linn Leaves Extracts against Extended Spectrum Beta-Lactamases-Producing Strains of *Klebsiella pneumoniae*

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Abstract: *Klebsiella pneumoniae* is an opportunistic Gram-negative bacterium in the Enterobacteriaceae family associated with a wide range of diseases, such as pneumonia, bloodstream infections, meningitis and urinary tract infections. Infections caused by drug-resistant strains of *Klebsiella pneumoniae* pose a significant threat to the effectiveness of conventional antibiotics. Hence, this has led to the need to explore alternative antimicrobial therapies, especially natural products derived from plant sources. This study assessed the phytochemical composition and antibacterial properties and performed a molecular docking analysis of Henna leaves (*Lawsonia inermis* L.) extracts on strains of *Klebsiella pneumoniae.* Crude ethanol and methanol extracts of *L. inermis* L. were prepared at different concentrations (25, 50, 75 and 100 mg/mL) and tested on extended spectrum beta-lactamases (ESBLs)-producing strains of *Klebsiella pneumoniae*. Phytocompounds were identified using gas chromatography–mass spectrometry (GC-MS) and further subjected to virtual ligands screening with DataWarrior (v05.02.01) and a molecular docking analysis using AutoDock4.2 (v4.2.6). The active compounds of *L. inermis* L. were determined by the docking analysis, including phytochemical, physicochemical, pharmacokinetics and docking score. The GC-MS analysis identified 27 phytoconstituents, including ethyl acetate, sclareol, 2-[1,2-dihydroxyethyl]-9-[β-d-ribofuranosyl] hypoxanthine, α-bisabolol and 2-Isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate. The 27 compounds were then screened for their physicochemical and pharmacokinetic properties. The results revealed that the methanol extracts at 100 mg/mL showed significantly higher ($p < 0.05$) zones of inhibition (13.7 ± 1.2 mm), while the ethanol extracts at 50 mg/mL were significantly lower (6.3 ± 0.6 mm) compared to all the other treatments. The docking analysis revealed that out of the 27 compounds identified, only twelve (12) compounds have a drug-likeness activity. The 12 compounds were further subjected to docking analysis to determine the binding energies with the CTX-M protein of *Klebsiella pneumoniae*. Only one compound [CID_440869; (2-[1,2-dihydroxyethyl]-9-[β-d-ribofuranosyl] hypoxanthine)] had the best binding energy of −9.76 kcal/mol; hence, it can be considered a potentially suitable treatment for infections caused by ESBLs-producing strains of *Klebsiella pneumoniae.* This

study has demonstrated that *L. inermis* L. extracts have antibacterial effects. Further research could explore the potential antimicrobial applications of *L. inermis* L. extracts to many bacterial strains.

Keywords: *Lawsonia inermis* L.; medicinal plants; alternative antimicrobial therapies; *in silico* molecular docking analysis; multidrug-resistant bacteria; *Klebsiella pneumoniae*; antibacterial activity

1. Introduction

The rising problem of antibiotic resistance in microorganisms poses a threat to global public health [1]. This aligns with the projections made by De Kraker et al. [2], estimating that antibiotic-resistant organisms could cause 10 million deaths annually by 2050 if strategies are not put in place to curtail the rising trend of antimicrobial resistance. The threat of antibiotic resistance is beyond clinical and public health; it leads to huge economic loss due to long hospital stays, reduced productivity and overworked healthcare systems [2]. A recent worldwide analysis of antimicrobial resistance-associated deaths highlights Sub-Saharan Africa as the most affected region, with *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae, Streptococcus pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* topping the list of bacterial-associated deaths due to antimicrobial resistance [3]. Antibiotics have brought rays of hope to modern medicine in the treatment of wide ranges of infections; however, the emergence of antibiotic resistance undermines this effort [4,5]. Despite the gravity of this threat, common available antibiotics have proven ineffective in eradicating these infections [4,5].

For decades, plants have been a historical source of medicinal remedies garnering global attention as alternatives to conventional drugs, particularly during the COVID-19 pandemic [4,6,7]. In particular, the post-COVID-19 pandemic period has further exacerbated the problem of antibiotic resistance due to the increased use of antimicrobials in an attempt to reduce the gravity of COVID-19 clinical outcomes on a global scale [8,9]. The use of natural products as a source of bioactive compounds for their antibacterial activity is well documented in the treatment of multidrug-resistant pathogens and this has gained attention in recent years [10–12]. For instance, *Arum maculatum* exhibited antibacterial activity against *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Pseudomonas aeruginosa* [10]. Another plant with antibacterial activity was reported by Wasihul et al. [11], and they demonstrated that *Calpurnia aurea* contained phytochemical components, such as alkaloid, tannins, flavonoid and saponins, which suggested their antibacterial activity against both Gram-positive and Gram-negative bacteria. Similarly, a phytochemical screening of *Pulicaria spp* revealed secondary metabolites, and the extract revealed significant activity against bacterial strains [12]. Many studies have reported the presence of bioactive phytochemicals as secondary metabolites, including alkaloids, amino acids, flavonoids, phenylpropanoids, steroids, volatile oils, glycosides, terpenes, anthraquinones and other compounds [4,7,13]. Medicinal plants demonstrate remarkable antimicrobial activities due to different phytochemical components they possess [14]. This gives the basis for the application of plants and herbs for medicinal purposes [15,16]. This assertion is backed by the World Health Organization's report that more than 80% of the world's population use medicinal plants for their basic health care [17].

Lawsonia inermis Linn., also known as henna in many parts of the world, is a shrub classified in the family *Lythraceae*. *Lawsonia inermis* L. is found in many regions but is considered native to Africa, Middle East and Asia. It is widely found in Africa, especially East and West African countries, and in certain regions of South Africa [18]. It is a plant with many names that are largely region-specific: Arabic: henna; Bengali: Mendi, mehedi; English: Egyptian privet, henna, Jamaica mignonette, mignonette tree; French: henné; German: Hennastrauch; Hindi: mehndi; Indonesian: inai, pakar kuku; Portuguese: hésia, hena, alfeneiro; Spanish: alcana, alheña; Swedish: henna; Vietnamese: nhuôm móng taylâ

mòn; and in Nigeria, particularly northern Nigeria, where it is mostly used, it goes by the name Lalle [18].

In addition to its cosmetic uses, this plant species is known to contain many bioactive compounds with therapeutic potential in the treatment of infections [15]. In a recent study, Fatahi et al. [16] demonstrated that the different sub-types of *Lawsonia inermis* L. (Shahdad, Rudbar and Ghale-e-ganj) exhibited antibacterial and anti-trichomonas effects, particularly inhibiting the growth of *Streptococcus agalactiae*, *Pseudomonas aeruginosa* and *Trichomonas vaginalis in vitro*. Several studies have shown that *Lawsonia inermis* L. contains flavonoids, phenolic compounds, proteins, saponins, terpenoids, alkaloids, xanthones, resin, quinones, coumarins and tannins, and these chemical components have been suggested to mediate their medicinal activities [18–20].

K. pneumoniae is a Gram-negative bacterium of the Enterobacteriaceae family and causes infections such as pneumonia, meningitis, wound infections and surgical site infections, urinary tract infections, bloodstream-associated infections and respiratory tract infections [21]. Most strains of *K. pneumoniae* produce extended-spectrum beta-lactamases (ESBLs), which confer resistance to most beta-lactam antibiotics, including penicillin, extended-spectrum cephalosporins (cefotaxime, ceftriaxone, ceftazidime and cefepime) and monobactams (aztreonam) [22,23]. The most common families of ESBLs that have been identified globally include CTX-M, SHV, OXA and TEM [22–24]. However, the majority of the ESBLs produced by *K. pneumoniae* belong to the CTX-M enzymes, which have been recognized as critical public health threats, both in hospital settings and in the community [22–26]. With the emergence of multidrug resistance in *K. pneumoniae* and the abundance of medicinal plants, there is a need for effective screening of new compounds and ultimately extraction and characterization of specific bioactive agents [27,28]. Gas chromatography–mass spectrometry (GC–MS) has been a mainstay in the identification of functional groups and specific bioactive compounds present in medicinal plants. GC-MS is considered a rapid and effective technique to profile novel phyto-constituents in medicinal plants when compared with the standards deposited in the National Institute of Standards and Technology Mass Spectra database (NIST) [29]. In addition, a computer-aided approach is employed to elucidate the medicinal informatics of plants via a virtual screening method [29]. This approach has been used *in silico* to predict pharmacokinetic, pharmacological and toxicological profiles of medicinal plants, and it is considered a fast and cost-effective method of testing potential drug candidates of bioactive compounds against target proteins [27–33]. In a general context, *in silico* molecular docking can be used to evaluate the interaction between proteins and ligands to simulate their binding ability while elucidating the structure. Some studies on the antibacterial activity of *Lawsonia inermis* L. against *Klebsiella pneumoniae* have been reported [34–38]. For example, the antibacterial effects of different concentrations of the chloroform henna extracts (CHE) were tested against *S. aureus* (100 mg/mL) and *K. pneumoniae* (200 mg/mL) *in vitro*, and the results revealed higher activity than the standard antibiotic control, ciprofloxacin [34]. Additionally, Arun et al. [35] demonstrated high flavonoid contents of *L. inermis* L., and the methanolic extract revealed antibacterial activity against different bacterial strains. Similarly, Pasadi et al. [36] demonstrated that aqueous extracts from three henna ecotypes exhibited antibacterial effects in a dose-dependent manner, with *K. pneumoniae* and *B. cereus* showing higher resistance. Furthermore, various studies have demonstrated the antibacterial activity of *L. Inermis* L. against *Klebsiella pneumoniae* [37–39].

Although the antibacterial activity of henna has been investigated, multi-approaches to investigate the *in vitro* antibacterial activity and molecular docking analysis of the plant against multidrug-resistant bacterial pathogens have not been reported to the best of our knowledge. Therefore, the current study investigated (i) the *in vitro* antibacterial activity of *L. inermis* L. against *Klebsiella pneumoniae;* (ii) the bioactive compounds in *L. inermis* L. through the GC-MS technique; and (iii) the *in silico* molecular docking analysis of potential compound from *L. inermis* L. against the CTX-M protein of ESBLs-producing *Klebsiella pneumoniae* strains.

2. Materials and Methods

2.1. Bacterial Isolates

Klebsiella pneumoniae isolates were obtained from the Department of Microbiology, Faculty of Life Sciences, University of Maiduguri, Nigeria. Standard microbiological and biochemical methods were used to phenotypically identify and characterize the *Klebsiella pneumoniae* isolates as described by Cheesbrough [40]. Furthermore, a pure culture of the isolates was maintained using streak plating on MacConkey agar plates and incubating at 37 °C for 24 h.

2.2. Collection and Preparation of Plant Sample

Fresh leaves of *Lawsonia inermis* L. were collected during the morning hours from the mother tree and taken to the Department of Botany, University of Maiduguri, Nigeria, for proper identification and authentication. After authentication, the leaves were taken to the Microbiology Department of the same university for processing. The leaves were washed thoroughly using distilled water and allowed to air-dry for 3–5 days under a controlled environment in the laboratory at room temperature. After drying, the leaves were pulverized with a clean grinder and then sieved to obtain a fine powder of *Lawsonia inermis* L. Approximately 200 g of the dried powder was extracted with 500 mL of ethanol and methanol, respectively, through a soxlet extractor, and the extracts were filtered using Whatman No. 1 filter paper. The solvents were vaporized using a rotary evaporator, and the crude extracts were stored at 4 °C until further assay. Samples of the *Lawsonia inermis* L. leaves used in this study have been deposited in the Department of Botany, University of Maiduguri, Nigeria.

2.3. Determination of the Plant Extracts' Yield and Phytochemical Screening

The leaves' extract yield (%) were determined using the formular by Nabi et al. [41]:

$$\text{Yield (\%W/W)} = W_1 \times 100/W_2$$

where W_1 represents the dry weight of the extract after solvent evaporation and W_2 represents the weight of the dried leaf powder.

The methanol and ethanol leaf extracts of *Lawsonia inermis* L. were evaluated for the presence of secondary metabolites, such as tannins, saponins, flavonoids and anthraquinones, following the standard protocols as described by Gul et al. [42].

2.4. Analysis of the Phytochemical Composition Using Gas Chromatography–Mass Spectrometry (GC-MS)

The procedure employed was as previously reported by Idris et. al. [39]. Briefly, 10 g of the dried crude methanolic extract of *Lawsonia inermis* L. was introduced into a centrifuge tube containing 10 mL of methanol and then mixed properly by vortexing for 2 min, after which it was centrifuged for 10 min at 3000 rpm. After centrifugation, a clear supernatant was taken and dispensed into a TSP micro vial, which was then subjected to GC-MS analysis. The GC analysis was carried out first by injecting 1 µL of the supernatant into Agilent GC (7890 B), equipped with a 30 m × 250 µm × 0.25 µm column, coupled with Agilent Mass Selective Detector (MSD) 5977 A technologies. The GC-MS has a carrier containing helium gas, which was set at a flow rate of 1 mL/min. Before the analysis was performed, the GC oven was kept at a temperature of 70 °C for about 3 min and then elevated at 10 °C/min to 280 °C. The temperature was held for 9 min. The equilibration time was set at 0.5 min, the MSD transfer line temperature was 250 °C, the MS source temperature was 230 °C and the MS quad temperature was 150 °C. The chemical compounds in the methanol extracts of *Lawsonia inermis* L. were detected and identified using the retention time produced by GC. The mass spectrum was then matched with the mass spectrum data available in the database of the National Institute of Standards and Technology. The percentage composition of each sample constituent was expressed as a percentage by peak area.

2.5. Phenotypic Detection of Extended-Spectrum β-Lactamases (ESBLs) Production in K. pneumoniae Isolates

The screening for ESBLs production in all the *K. pneumoniae* isolates was performed using the modified double-disc synergy (MDDS) test, as described by Wakil et al. [43]. Using a sterile pipette tip, colonies of the ESBLs-producing *Klebsiella pneumoniae* were picked and suspended in 2 mL of sterile phosphate buffered saline. The suspension was thoroughly mixed and then compared to a 0.5 MacFarland standard to match the level of turbidity using a piece of black paper. Afterwards, 100 µL of the bacterial suspension was pipetted onto the center of the Mueller–Hinton agar (MHA) plates and spread aseptically onto the surface of the Mueller–Hinton agar using a sterile glass spreader. Thereafter, ceftazidime (CAZ 30 µg), cefotaxime (CTX 30 µg) and ceftriaxone (CRO 30 µg) were placed on each of the isolates inoculated on the Mueller–Hinton agar plates and incubated at 37 °C for 24 h. The diameter of the zones of inhibition around each of the discs was measured, and a zone of inhibition diameter of 5 mm or higher signified the presence ESBLs production [43].

The isolates with an indication of the presence of ESBLs production in the MDDS test were further confirmed by placing ceftazidime (CAZ 30 µg), cefotaxime (CTX 30 µg) and ceftriaxone (CRO 30 µg) on each side of an Augmentin (AMC 30 µg) disc at a distance of 15 mm from the middle of the inoculated Mueller–Hinton agar plates, and the plates were incubated overnight at 37 °C for 24 h. The zone of inhibitions towards the Augmentin (AMC 30 µg) is a confirmation of ESBL production by the tested isolates [43,44].

2.6. Evaluation of the Antibacterial Activity of Both Methanol and Ethanol Extracts of Lawsonia inermis L.

The agar well diffusion method was employed to determine the antibacterial activities of *Lawsonia inermis* L. extracts. Using a sterile pipette tip, colonies of the ESBLs-producing *Klebsiella pneumoniae* were picked and suspended in 2 mL of sterile phosphate buffered saline. The suspension was thoroughly mixed and then compared to a 0.5 MacFarland standard to match the level of turbidity using a piece of black paper. Afterwards, 100 µL of the bacterial suspension was pipetted onto the center of the Mueller–Hinton agar (MHA) plates and spread aseptically onto the surface of Mueller–Hinton agar using a sterile glass spreader. Then, wells 10 mm in diameter were cut from the inoculated agar with separate sterile cork-borers. The wells were then filled with different concentrations (25, 50, 75 and 100 mg/mL) of the methanol and ethanol extracts of *Lawsonia inermis* L., respectively. Methanol and ethanol were used as controls. These plates were incubated at 37 °C for 24 h, followed by the measurement of the zones of inhibition in millimeters using a Vernier caliper. Each antibiotic underwent testing in triplicates over four days, and the averages were calculated (mean ± standard deviation).

2.7. Preparation of the Crystal of CTX-M Target Protein

The protein of *Klebsiella pneumoniae* (CTX-M) was complexed with GDP and 9PC (PDB ID: 4DXD), which was obtained from the Protein Data Bank (PDB). The ligand within the bound structure of CTX-M was removed and then cleaned properly. Missing factors, which include atoms, residues, loops and side chains, were verified properly and then inserted. The Chimera, Swiss PDB Viewer and Chiron energy minimization and refinement tool (version 1.0, Swiss National Science Foundation, Wildhainweg, Switzerland) were then used to eliminate all the water molecules, especially those not closer to the binding site of the substrate. In addition, non-proteinate residues were removed through the optimization of the structure and minimization of energy [45–47].

2.8. Physicochemical Analysis

All the compounds obtained from GC-MS analysis were screened based on their physicochemical properties (molecular weight, logarithms of partial coefficient, number of hydrogen-bond donors (HBAs) and number of hydrogen-bond acceptors (HBDs)) using the

DataWarrior (v05.02.01) program [48,49]. All the compounds with suitable physicochemical properties were selected for additional evaluation.

2.9. Pharmacokinetic Analysis

The identified compounds that have good binding energies and physicochemical analysis were then used to evaluate the compounds on the basis of their pharmacokinetics properties, which include absorption, distribution, metabolism and excretion (ADMET), using the AdmetSAR version 3.0, "http://lmmd.ecust.edu.cn/admetsar3/ (accessed on 12 April 2024)" tool as described by Cheng et al. [50], the DataWarrior (v05.02.01) program by Sander et al. [48] and SwissADME (version 2.3.0) by Daina et al. [49]. Other properties identified include mutagenicity, tumorigenicity, reproductive toxicity and irritant.

2.10. Molecular Docking Analysis

A molecular docking analysis was carried out in order to determine the conformation of binding between the CTX-M protein and the ligand forming a protein–ligand complex with the aid of AutoDock4.2 (v4.2.6), which was employed by Morris et al. [51]. The binding confirmation of the complex reveals the binding energy of CTX-M and the selected ligands. The binding energy of the protein–ligand complex was calculated using the formula by Hariono et al. [52]:

$$\Delta Gbind = \Delta Gvdw + \Delta Ghbond + \Delta Gelect + \Delta Gconform + \Delta Gtor + \Delta Gsol$$

where ΔGbind = estimated free binding energy;
ΔGvdw = sum of van der Waals energy;
ΔGhbond = sum of hydrogen bond and desolvation energy;
ΔGelect = sum of electrostatic energy;
ΔGconform = sum of final total internal energy;
ΔGtor = sum of torsional free energy;
ΔGsol = sum unbound system energy.
The binding energies and residues were recorded appropriately.

3. Results

3.1. Percentage Yield and Phytochemical Screening

The percentage yield (%*w*/*w*) of the dried leaf extracts was 55% for methanol and 48% for the ethanol extracts. The results of the preliminary phytochemical screening of both the methanolic and ethanolic extracts are presented in Table 1.

Table 1. Phytochemicals of *Lawsonia inermis* L.

Phytochemical	Methanol	Ethanol
Alkaloids	+	+
Tannins	+	+
Flavonoids	+	+
Anthraquinones	+	+

+ indicates positive result for the phytochemical.

3.2. Compounds Identified from Lawsonia inermis L. Using Gas Chromatography-Mass Spectroscopy (GC-MS)

A gas chromatography–mass spectrometry analysis was carried out on the methanolic extract of the leaves of *L. inermis* L. in order to identify the phytochemical parameters present in the leaves of *L. inermis* L. The analysis revealed the different constituents of the phytochemicals, including their compound names, chemical formula, peak value and retention time. The spectrum obtained from the GC-MS analysis is shown in Figure 1. The analysis carried out on the methanolic extract of *L. inermis* L. revealed the existence of twenty-seven (27) compounds. The compound with PubChem ID CID_6590 ($C_4H_8O_2$) has

the lowest molecular weight, which is 88, while CID_ 53178 ($C_{30}H_{33}ClO_6$) has the highest molecular weight of 524 (Table 2).

Table 2. Compounds obtained from the GC–MS analysis of *Lawsonia inermis* L.

S/N	PubChem ID	Compound	Formula	Molecular Weight	Retention Time (min)	Peaks
1	6590	Ethyl acetate	$C_4H_8O_2$	88	3.716	1
2	536425	Heptane, 4-azido	$C_7H_{15}N_3$	141	4.817	2
3	225038	Pentyl glycolate	$C_7H_{14}O_3$	146	4.817	2
4	76029	Propanoic acid, 2-(aminooxy)-	$C_3H_7NO_3$	105	5.689	3
5	5363192	7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin	$C_{14}H_{14}O_3$	230	5.689	3
6	543621	Pentanoic acid, 2-(aminooxy)-	$C_5H_{11}NO_3$	133	5.689	3
7	440869	2-[1,2-Dihydroxyethyl]-9-[β-d-ribofuranosyl] hypoxanthine	$C_{12}H_{16}N_4O_7$	328	5.689	3
8	536980	2-Heptanone, 6-methyl-5-methylene-	$C_9H_{16}O$	140	6.048	4
9	534592	4,5,9-Trihydroxy-dodeca-1,11-diene	$C_{12}H_{22}O_3$	214	6.675	5
10	5363192	7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin	$C_{14}H_{14}O_3$	230	7.067	6
11	536411	8,8-Dimethyl-7,9-dioxabicyclo [4.3.0] nonane-3-carboxylicacid, methyl ester	$C_{11}H_{18}O_4$	214	7.067	6
12	5283028	trans-Traumatic acid	$C_{12}H_{20}O_4$	228	7.067	6
13	537288	9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione	$C_{11}H_{16}O_4$	212	7.299	7
14	8180	Undecanoic acid	$C_{11}H_{22}O_2$	186	7.299	7
15	11996452	Viridiflorol	$C_{15}H_{26}O$	222	7.966	8
16	8842	Citronellol	$C_{10}H_{20}O$	156	8.069	9
17	6421299	2,2,3,3,4,4-Hexamethyltetrahydrofuran	$C_{10}H_{20}O$	156	8.395	11
18	163263	1-Naphthalenepropanol, α-ethenyldecahydro-5-(hydroxymethyl)-α,2,5,5,8a-pentamethyl-	$C_{20}H_{36}O_2$	308	10.606	13
19	5367736	Cyclopropanol, 1-(3,7-dimethyl-1-octenyl)-	$C_{13}H_{24}O$	196	12.235	15
20	535324	Bicyclo [3.2.1]oct-3-en-2-one, 3,8-dihydroxy-1-methoxy-7-(7-methoxy-1,3-benzodioxol-5-yl)-6-methyl-5-(2-propenyl)-,	$C_{21}H_{24}O_7$	388	12.235	15
21	5363274	[1R-(6-endo,7-exo,8-syn)]-1,2-dihydro-8-hydroxylinalool	$C_{10}H_{20}O_2$	172	13.916	16
22	101282029	Bicyclo [3.2.1]oct-3-en-2-one, 3,8-dihydroxy-1-methoxy-7-(7-methoxy-1,3-benzodioxol-5-yl)-6-methyl-5-(2-propenyl)-,	$C_{21}H_{24}O_7$	225	18.174	18
23	5365831	[1R-(6-endo,7-exo,8-syn)]-4,4,8-Trimethyl-non-5-enal	$C_{12}H_{22}O$	182	18.174	18
24	319068771	3-Cyclohexene-1-methanol, α,4-dimethyl-α-(4-methyl-3-pentenyl)-, [R-(R*,R*)]-	$C_{15}H_{26}O$	222	18.174	18
25	296248	photocitral B	$C_{10}H_{16}O$	152	20.436	19
26	551300	1,2-Pentanediol, 5-(6-bromodecahydro-2-hydroxy-2,5,5a,8a-tetramethyl-1-naphthalenyl)-3-methylene-	$C_{20}H_{35}BrO_3$	402	22.915	20
27	537118	2-Isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate	$C_{30}H_{33}ClO_6$	525	24.465	22

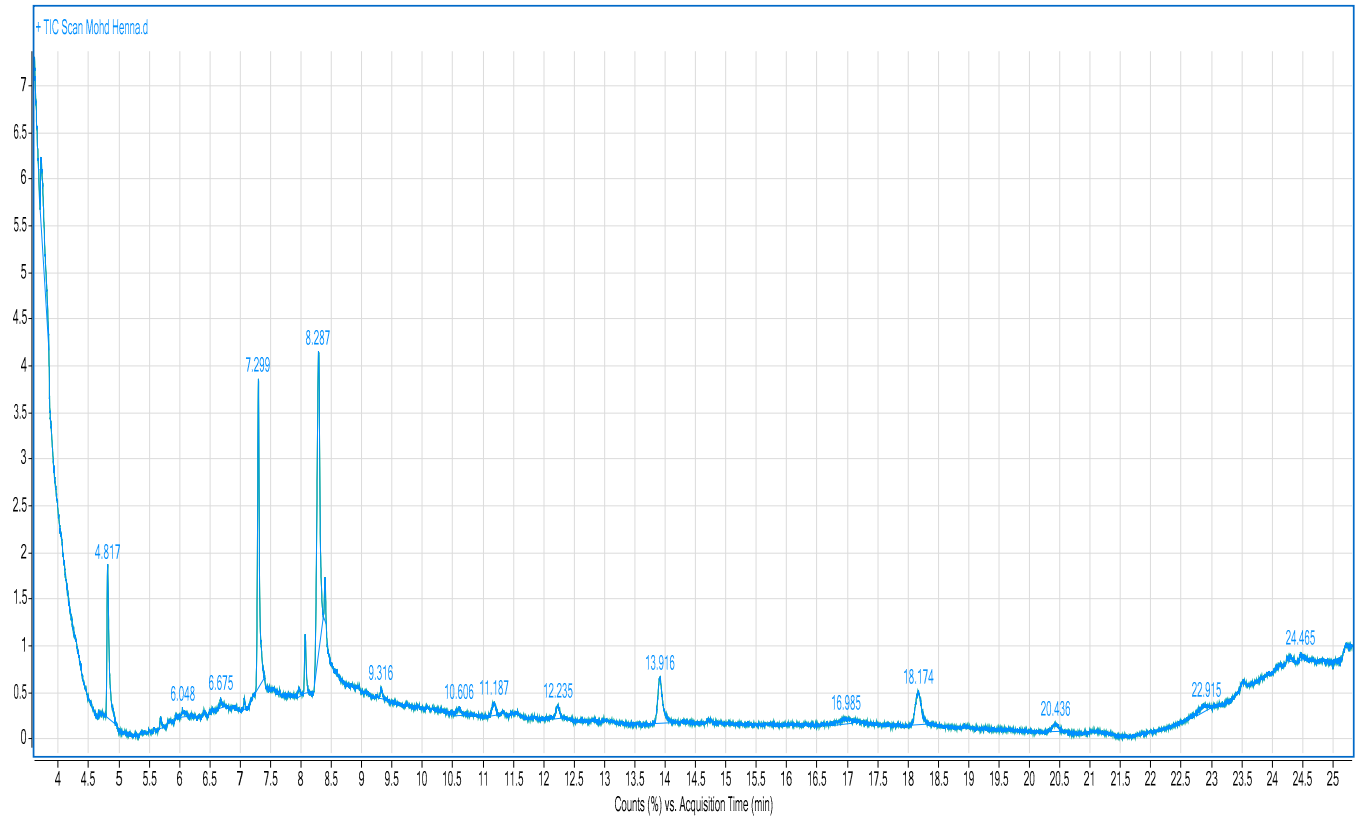

Figure 1. Chromatogram of the gas chromatography–mass spectroscopy analysis performed on the methanolic extract of the *Lawsonia inermis* leaves (GC 7890B, MSD 5977A, Agilent Tech, Santa Clara, CA, USA).

3.3. Antibacterial Activity of the Methanol and Ethanol Extracts of Lawsonia inermis L.

The antibacterial activity of both the methanol and ethanol extracts of *L. inermis* L. of different concentrations (25, 50, 75 and 100 mg/mL) were tested on isolates of multidrug-resistant *Klebsiella pneumoniae* isolated from clinical samples of urine and wounds and assessed for the presence and absence of an inhibition zone. The antibacterial activity was assessed by measuring the inhibition zone diameter. The methanol extract of *L. inermis* L. had the highest inhibition zone of 13.00 ± 1.2 mm at a concentration of 100 mg/mL, whereas the lowest inhibition zone of 7.3 ± 0.6 mm was seen at a concentration of 75 mg/mL. The ethanol extract of *L. inermis* L. revealed that a concentration of 25 mg/mL had the highest concentration of 11.00 ± 0.00 mm, followed by 100 mg/mL, which has 10.00 ± 1.0 mm. The lowest inhibition zone was seen at the concentration of 50 mg/mL, which had an inhibition zone of 6.00 ± 0.6 mm. These results are shown in Table 3.

Table 3. Zone of inhibition (mm) of methanol and ethanol extract of *Lawsonia inermis* L. extract against multidrug-resistant *Klebsiella pneumoniae*.

Concentration (mg/mL)	Extract/Zone of Inhibition (mm)	
	Ethanol	**Methanol**
25	11.0 ± 0.0 ^{bc}	11.3 ± 0.6 ^{bc}
50	6.3 ± 0.6 ^{e}	12.0 ± 0.0 ^{b}
75	8.0 ± 1.0 ^{d}	7.3 ± 0.6 ^{de}
100	10.0 ± 1.0 ^{c}	13.7 ± 1.2 ^{a}

Values indicate mean ± standard deviation (SD). Values with the same letter(s) are not significantly different using Tukey multiple range test at $p < 0.05$.

3.4. Physiochemical Analysis of Compounds Obtained from Lawsonia inermis L.

The physicochemical analysis revealed that all the compounds are in agreement with the five rules of Lipinski and Egan, with the exception of CID_440869 and CID_537118. CID_440869 has 11 hydrogen bond acceptors (HBA $\leq$ 10) and 6 hydrogen bond donors (HBD $\leq$ 5), despite having a molecular weight of 328.280 Da, whereas CID_537118 has a weight of 525.039 Da, which is above the required molecular weight of 500 Da. In addition, CID_537118 has a logarithm of the partial coefficient of 7.3414 as opposed to the required value of ($\leq$5) (Table 4). Therefore, apart from CID_440869 and CID_537118, all the other compounds have drug-like characteristics (Table 4).

Table 4. Physiochemical analysis of compounds from *Lawsonia inermis* L. identified using GC-MS.

S/N	PubChem ID	Molecular Weight ($\leq$500)	Number of HBA ($\leq$10)	Number of HBD ($\leq$5)	MolLogP ($\leq$5)	Drug Likeness
1	CID_6590	88.1055	2	1	0.3736	−2.82
2	CID_536425	388.415	7	2	2.6761	−0.89897
3	CID_225038	146.185	3	1	0.9716	−10.318
4	CID_76029	105.093	4	2	−1.3119	−1.4365
5	CID_5363192	230.262	3	1	3.0993	−4.5992
6	CID_543621	133.146	4	2	−0.4031	−3.384
7	CID_440869	328.280	11	6	−3.3826	7.1752
8	CID_536980	140.225	1	0	2.8354	−11.248
9	CID_534592	214.304	3	3	2.0848	−7.1915
10	CID_5363192	230.262	3	1	3.0993	−4.5992
11	CID_536411	214.260	4	0	0.9337	−4.8099
12	CID_5283028	228.287	4	2	2.7164	−15.097
13	CID_537288	212.244	4	0	0.8154	−16.496
14	CID_8180	186.294	2	1	3.7905	−25.216
15	CID_11996452	222.370	1	1	3.2008	−1.9678
16	CID_8842	156.268	1	1	3.3494	−8.6831
17	CID_6421299	156.268	1	0	2.3703	−13.972
18	CID_163263	308.504	2	2	4.6198	−8.3601
19	CID_5367736	196.333	1	1	3.8371	−2.2587
20	CID_535324	388.415	7	2	2.6761	−0.89897
21	CID_5363274	172.267	2	2	2.4899	−1.7531
22	CID_101282029	388.415	7	2	2.6761	−0.89897
23	CID_5365831	182.306	1	0	3.7692	−6.1572
24	CID_319068771	222.370	1	1	4.4711	−1.4665
25	CID_296248	152.236	1	0	1.676	−7.016
26	CID_551300	403.399	3	3	4.3349	−11.802
27	CID_537118	525.039	6	0	7.3414	−22.803

3.5. Pharmacokinetic Analysis of the Compounds Detected from Lawsonia inermis L.

The pharmacokinetic properties of *L. inermis* L. were determined using parameters such as mutagenicity, tumorigenicity, reproductive toxicity and irritant. The pharmacokinetic properties of a drug are used to determine the effectiveness and impact of the drug. In this study, the following compounds failed pharmacokinetic analysis due to varying mutagenic properties: CID_225038, CID_76029, CID_5365831, CID_551300, CID_225038, CID_76029, CID_5365831 and CID_551300.

Compound CID_11996452 was the only compound that had evidence of tumorigenicity, while CID_8842 and CID_537118 had high reproductive toxicity. Based on irritant, CID_6590, CID_536980, CID_8180, CID_11996452, CID_8842, CID_5363274, CID_5365831, CID_319068771, CID_296248 and CID_537118 have a high irritant ability, while CID_225038, 534592, 163263 and CID_551300 have low irritant capacity (Table 5). Furthermore, CID_8842 did not meet the pharmacokinetic properties of an ideal compound due to its high toxicity and irritability.

Table 5. Pharmacokinetic analysis of the phyto-compounds detected from *Lawsonia inermis* L.

S/N	Compound Name	BBB	CYP2D6 Inhibitor	HIA	Mutagens	Tumorigenesis	Reproductive Toxicity	Irritant
1	CID_6590	-	-	-	None	None	None	High
2	CID_536425	-	-	-	None	None	None	None
3	CID_225038	-	-	-	Low	None	None	Low
4	CID_76029	-	-	-	High	None	None	None
5	CID_5363192	-	-	-	None	None	None	None
6	CID_543621	-	-	-	None	None	None	None
7	CID_440869	-	-	-	None	None	None	None
8	CID_536980	-	-	-	None	None	None	High
9	CID_534592	-	-	-	None	None	None	Low
10	CID_5363192	-	-	-	None	None	None	None
11	CID_536411	-	-	-	None	None	None	None
12	CID_5283028	-	-	-	None	None	None	None
13	CID_537288	-	-	-	None	None	None	None
14	CID_8180	-	-	-	None	None	None	High
15	CID_11996452	-	-	-	None	High	None	High
16	CID_8842	-	-	-	None	None	High	High
17	CID_6421299	-	-	-	None	None	None	None
18	CID_163263	-	-	-	None	None	None	Low
19	CID_5367736	-	-	-	None	None	None	None
20	CID_535324	-	-	-	None	None	None	None
21	CID_5363274	-	-	-	None	None	None	High
22	CID_101282029	-	-	-	None	None	None	None
23	CID_5365831	-	-	-	High	None	None	High
24	CID_319068771	-	-	-	None	None	None	High
25	CID_296248	-	-	-	None	None	None	High
26	CID_551300	-	-	-	High	None	None	Low
27	CID_537118	-	-	-	None	None	High	High

3.6. Docking Scores and Residues Involved in H-Bond Formation

A molecular docking analysis was carried out on the twelve compounds to evaluate their binding energies with the *Klebsiella* protein (CTX-M). The molecular docking revealed free binding energies ranging from −3.62 kcal/mol to −9.76 kcal/mol. Five of the compounds, CID_5363192, CID_440869, CID_5363411, CID_5283028 and CID_535324, produced residues that are included in hydrogen bonding. They include Leu200, Pro201, Gln197, Val206, Glu262, Lys257, Arg229, Trp204 and Gly199 (Table 6).

Table 6. Docking scores and residues involved in H-bond formation.

S/No	Compound ID	Docking Score (kcal/mol)	Residues Involve in H-Bonds	Distance (Å)
1	CID_536425	−4.98		
2	CID_5363192	−6.00	Leu200, Pro201, Gln197 Val206	2.79, 2.70, 2.78, 2.67 2.94
3	CID_543621	−3.62		
4	CID_440869	−9.76	Gly262 Lys257	3.09, 2.89
5	CID_5363192	−6.00	Leu200, Gln197, Pro201, Val206	2.83, 2.76, 2.75, 2.67
6	CID_536411	−5.85	Gly262	2.90
7	CID_5283028	−5.74	Lys227, Arg229, Trp204, Gly199 Lys257	2.68, 3.22, 2.62, 3.18 2.71, 2.75
8	CID_537288	−4.62		
9	CID_6421299	−4.54		
10	CID_5367736	−4.96		
11	CID_535324	−6.11	Trp204 Val206	2.84 2.77
12	CID_101282029	−4.98		

4. Discussion

Medicinal plants are rich sources of secondary metabolites that are responsible for antibacterial activity against many pathogens and exhibit less adverse effects [4,7]. However, there is little or no scientific validation or documentation on the application of these medicinal plants. In the current study, the methanol extract of the leaves of *L. inermis* L. was used for the detection of its bioactive compounds. A total of twenty-seven (27) phytocompounds were detected with the aid of the GC-MS on the methanol extract of *L. inermis* L. (Table 2). The analysis shows a wide range of potential bioactive agents, which could serve as candidates to inhibit ESBL-producing strains of *K. pneumoniae*. The 27 phytocompounds reported in this study suggest that some of the compounds have antibacterial activity (Tables 3, 4 and 6).

The GC-MS analysis of the *L. inermis* L. identified 27 phytoconstituents, including ethyl acetate, sclareol, 2-[1,2-Dihydroxyethyl]-9-[β-d-ribofuranosyl] hypoxanthine, alpha-bisabolol and 2-Isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate. These phyto-compounds are likely to contribute to the antibacterial activity of the plants. Previous studies reported some of the phyto-compounds in this study to have antibacterial effect [15,16,18,19]. For instance, Jeyaseelan et al. [53] demonstrated that both ethyl acetate and ethanol extracts of the fruits, flowers and leaves of *Lawsonia inermis* L. generally contained flavonoids and had antimicrobial activity against *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus*. This correlated with the findings in the current study, which also demonstrate that the ethanol extract of *Lawsonia inermis* L. leaves had antibacterial activity against ESBL-producing *K. pneumoniae*. In a recent study, Popova et al. [54] evaluated the antimicrobial activities of sclareol, and it was demonstrated that sclareol had significant antimicrobial effects against *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 8739, *Salmonella abony* ATCC 6017, *Staphylococcus aureus* ATCC 6538, *Proteus mirabilis* ATCC 14153 and *Proteus vulgaris* ATCC 13315, with zone diameters of inhibition ranging from 9.5 ± 0.10 mm to 14.2 ± 0.06 mm [54]. In the same study, Popova et al. [54] reported that sclareol had strong antimicrobial effects towards various fungal isolates, including *Candida albicans* ATCC 10231, *C. glabrata* ATCC 90030, *C. parapsilosis* clinical isolate and *C. tropicalis* NBIMCC 23, with their zones of inhibition diameters similar to those of the antifungal agent fluconazole [54]. A checkerboard analysis of combinations of sclareol with curcumin and sclareol with eugenol showed strong synergistic antimicrobial activities against *C. albicans*, *C. glabrata* and *Aspergillus fumigatus*, with MICs reduced by up to four- and eight-fold, respectively [55]. In a different study, synergism assays of sclareol with clindamycin were performed by checkerboard assay, and the results indicated that sclareol had a synergistic effect with clindamycin towards methicillin-resistant *Staphylococcus aureus* [56]. Furthermore, sclareol has been shown to exhibit antiviral activity, especially in Ebola virus, where it was found to block the Ebola viral fusion process, thereby interfering with virus entry into host cells [57].

Alpha-bisabolol, also known as levomenol, is a sesquiterpenoid that was first isolated from German chamomile but is also found in various medicinal plants [58]. Rodrigues et al. [59] reported that alpha-bisabolol exhibited significant antimicrobial activity against *E. coli*, *S. aureus*, *Candida albicans*, *C. krusei* and *C. tropicalis*, and α-bisabolol in combination with aminoglycosides and beta-lactams had strong synergistic effects against these bacterial and candida species [59]. In a different study, Oliveira et al., [60] demonstrated that α-bisabolol had antimicrobial activity against *S. aureus* ATCC 25923, with a minimum inhibitory concentration of 161.27 μg mL^{-1}. Similarly, α-bisabolol in combination with the antibiotic norfloxacin exerted a synergistic antimicrobial effect against *S. aureus* ATCC 25923, and α-bisabolol had synergistic action against *E. coli* when combined with gentamicin [60]. *L. inermis* L. host other compounds that have been reported to show antibacterial activity, including lawsone [53], naphthoquinone derivatives [61] and 3,5′-hydroxyfavone [62].

In this study, we report considerable antibacterial activity of methanol and ethanol extracts of *L. inermis* L. of different concentrations on isolates of ESBLs-producing strains of *Klebsiella pneumoniae*, with zone of inhibitions ranging from 7.3 ± 0.6 mm to 13.70 ± 1.2 mm

and 6.00 ± 0.6 to 11.00 ± 0.0 mm, respectively. Similar to this study, Moutawalli et al. [63] showed that methanol extract demonstrated a wide range of activity, 11 ± 0.1 mm to 18 ± 0.2 mm, on different bacterial isolates. This could explain the possible broad-spectrum activity of *Lawsonia inermis* L. Specifically, the results obtained in this study indicate that the methanol extract had the highest activity of 13.7 ± 1.2 mm at 100 mg/mL concentration (Table 3). Furthermore, the high zone of inhibition of the methanol extract was also reported by Nigussie et al. [64]. In a general context, the antibacterial potential of both methanolic and ethanolic extracts of *L. inermis* L. might be due to the different phytochemicals identified, and this has been confirmed by a previous study [65].

In an attempt to determine the active compounds of *L. inermis* L., a molecular docking analysis was performed starting with the phytochemicals, physicochemical, pharmacokinetics and docking score. The twenty-seven (27) phytochemicals of the methanol extract of *L. inermis* L. detected with the aid of GC-MS were evaluated for physicochemical and pharmacokinetic activity in order to determine if the compounds have a drug-likeness. The method employed by Nyalo et. al. [66] and Usman et al. [67] was adopted for evaluating the therapeutic safety, metabolism and accuracy of the compounds identified by GC-MS. Many parameters were used to evaluate the physiochemical properties, including molecular weight, hydrogen-bond acceptor (HBA), hydrogen-bond donor (HBD), logarithms of partial coefficient (LogP) and drug likeness. The interpretation of the physiochemical analysis of the identified compounds was performed according to Lipinski et al. [68] and Egan et al. [69] rules, which is in accordance with the rule of drug-likeness, which is usually employed during the creation of new drugs. The five rules of Lipinski state that for a compound to be termed as a drug, it must have good permeability of the membrane, high gastrointestinal tract absorption, good oral bioavailability and a molecular weight less than or equal to 500 Da (≤500), logarithms of partial coefficient (LogP) less than or equal to 5.88 (≤5.88), a hydrogen-bond donor (HBD) less than or equal to 5 (≤5) and a hydrogen-bond acceptor (HBA) than or equal to 10 (≤10). The Egan rule states that for a compound to possess therapeutic characteristics, it must possess a logarithm of partial coefficient (LogP) less than or equal to 5.88 (≤5.88) and a topological polar surface area (TPSA) of less than or equal to 131 (≤131). Out of the 27 compounds detected, only twelve (12) were revealed to have evidence of drug-likeness activity.

These 12 compounds were then used for molecular docking with a view to examine their free binding energies (docking score) with the *Klebsiella pneumoniae* CTX-M protein. The molecular docking revealed docking scores ranging from −3.62 kcal/mol to −9.76 kcal/mol. The 12 compounds produced a good docking score because the negative docking score reveals a stronger binding activity between the protein and the ligand. Five of the compounds, CID_5363192, CID_440869, CID_5363411, CID_5283028 and CID_535324, produced residues that include hydrogen bonding. From the result, it can be seen that CID_440869 (2-[1,2-dihydroxyethyl]-9-[β-d-ribofuranosyl] hypoxanthine) has the highest docking score and could be considered as a suitable compound to use for the treatment of infections caused by ESBLs-producing *Klebsiella pneumoniae* strains.

5. Conclusions

The present study highlights the first combined report on *in vitro* and *in silico* studies on the effects of *L. inermis* L. against multidrug-resistant *Klebsiella pneumoniae* strains. The results also demonstrate the antibacterial potential of the methanol and ethanol extracts of *L. inermis* L. on drug-resistant K. *pneumoniae*. A total of twenty-seven compounds were identified using the GC-MS analysis of the methanol extracts of *L. inermis* L. The results reveal that of the 27 bioactive compounds identified from the GC-MS analysis, which were screened by evaluating their physicochemical and pharmacokinetic properties, only 12 have drug-likeness properties. The compounds were used for a molecular docking analysis, which revealed the CTX-M protein and CID_440869 to have the highest free binding energy. Therefore, this compound can be considered as a potential therapeutic agent for treating infections caused by strains of ESBLs-producing *Klebsiella pneumoniae*. Further research

could explore the potential antimicrobial applications of *L. inermis* L. extracts to many bacterial strains.

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