SYSTEMS ENGINEERING APPROACH TO TRIPHALA-AGNPS DESIGN FOR COMBATING MDR KLEBSIELLA PNEUMONIAE INFECTIONS

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Abstract:

Multidrug-resistant (MDR) *Klebsiella pneumoniae* is a major cause of bloodstream and device-associated infections, particularly in immunocompromised patients. The emergence of resistance to conventional antibiotics necessitates the exploration of alternative antimicrobial agents. Organic solvent extracts of selected medicinal plants were screened for antibacterial activity against *Staphylococcus aureus, Escherichia coli*, and MDR *K. pneumoniae* using the agar well diffusion method. Triphala extract and Triphala-mediated silver nanoparticles (AgNPs) were further characterized by UV–Visible spectroscopy and gas chromatography–mass spectrometry (GC–MS). In silico molecular docking was performed to evaluate interactions between identified phytocompounds and *K pneumoniae* fimbrial adhesin protein (PDB ID: 3U4K). Among all tested samples, Triphala-AgNPs demonstrated the highest antibacterial activity against MDR *K. pneumoniae*, with a maximum zone of inhibition of 25 mm at 100 µL. UV–Visible spectroscopy confirmed nanoparticle formation, while GC–MS analysis identified phenolic and flavonoid compounds, including gallic acid, ellagic acid, quercetin, and chebulic derivatives. Docking analysis revealed favorable binding energies between selected phytocompounds and the target adhesin protein. Triphala-derived silver nanoparticles exhibit significant in vitro antibacterial activity against MDR *K. pneumoniae* and show potential molecular interactions with bacterial adhesion proteins. These findings support further investigation of Triphala-AgNPs as alternative antimicrobial agents.

Keywords: Triphala; silver nanoparticles; multidrug resistance; *Klebsiella pneumoniae*; GC–MS; molecular docking.

1.Introduction:

Around the world, hospitals struggle with bacteria that resist many drugs. When it comes to tough Gram-negative germs, one germ stands out that is *Klebsiella pneumoniae.* Blood infections, bladder problems, and germs linked to medical devices often come from it(5). People whose immune systems are weak tend to face higher risks. Lately, more samples show resistance to broad-spectrum penicillins or strong antibiotics like carbapenems (1 -6). That shift means even newer medicines may fail quietly behind the scenes.

Frequent hospital visits, unstable blood access, and weakened immune systems make hemodialysis patients especially sensitive to illness. When drug-resistant *K. pneumoniae* strikes this group, outcomes worsen, deaths rise, costs balloon(7). As today's standard medicines lose power, searching beyond standard treatments becomes unavoidable. New approaches must join existing ones to combat growing threats (8)

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For centuries, people have turned to plants when looking for natural ways to fight infections. Found inside many herbs are chemicals that stop germs from growing some of these come from ancient Indian remedies(9). One mix called Triphala mixes three woods together with a tart fruit; it holds tannins, which block bacteria. Studies show it also cleans up free radicals and adjusts the body's immunity system. Surprisingly, lab tests now confirm that these same extracts can stabilize tiny particle dots made from metals, making them work better against microbes(10).

Figure 1:Triphala plant & powder

A type of tiny particle called silver, made through green synthesis methods using leaf extracts, shows strong ability to fight harmful bacteria while possibly working better when paired with natural compounds found in plants. Still, there is little known about how these particles perform against a tough type of infection caused by a specific germ when tested alongside analysis of active ingredients and their behavior at a molecular level(11).

This work looks at three main things. First, it checks how different plant extracts and silver nanoparticles made from Triphala fight a tough-to-treat bacterium like MDR *K. pneumoniae*. Instead of just guessing, the we have used tools like GC–MS to map out what's really in

Triphala. A closer look at chemical behavior happens when computer models test which parts of plants might bind directly to proteins on the germ's surface(4).

2. Materials and Methods:

2.1 Bacterial strains

Clinical isolates of *Staphylococcus aureus, Escherichia coli,* and *Klebsiella pneumoniae* were obtained from recognized microbiology laboratories. Cultures were maintained on nutrient agar at 4 °C and sub-cultured before use. Bacterial identity was confirmed by morphological and biochemical characteristics(12).

2.2 Preparation of plant extracts

Powdered plant materials were extracted using acetone, ethanol, or methanol in a 1:10 (w/v) ratio. The mixtures were agitated on a rotary shaker and incubated for four days. Extracts were filtered, concentrated under reduced pressure, and stored at 4 °C until further use(13).

2.3 Synthesis of silver nanoparticles

Triphala extract was used as a reducing agent for the synthesis of silver nanoparticles by reacting with silver nitrate solution under controlled conditions. Nanoparticle formation was monitored spectrophotometrically(14).

2.4 Antibacterial activity assay

Antibacterial activity was evaluated using the agar well diffusion method on Mueller–Hinton agar. Wells were loaded with plant extracts or nanoparticle formulations at different concentrations. Plates were incubated at 37 °C for 24 h, and zones of inhibition (ZOI) were measured in millimeters. All assays were performed in triplicate.

2.5 UV–Visible spectroscopy

UV–Visible spectra of nanoparticle solutions were recorded between 200 and 600 nm using a double-beam spectrophotometer to confirm nanoparticle synthesis(15).

2.6 GC–MS analysis

Phytochemical constituents of Triphala extract were analyzed using GC–MS equipped with an Elite-5MS capillary column. Compounds were identified by comparison with mass spectral libraries.

2.7 Molecular docking

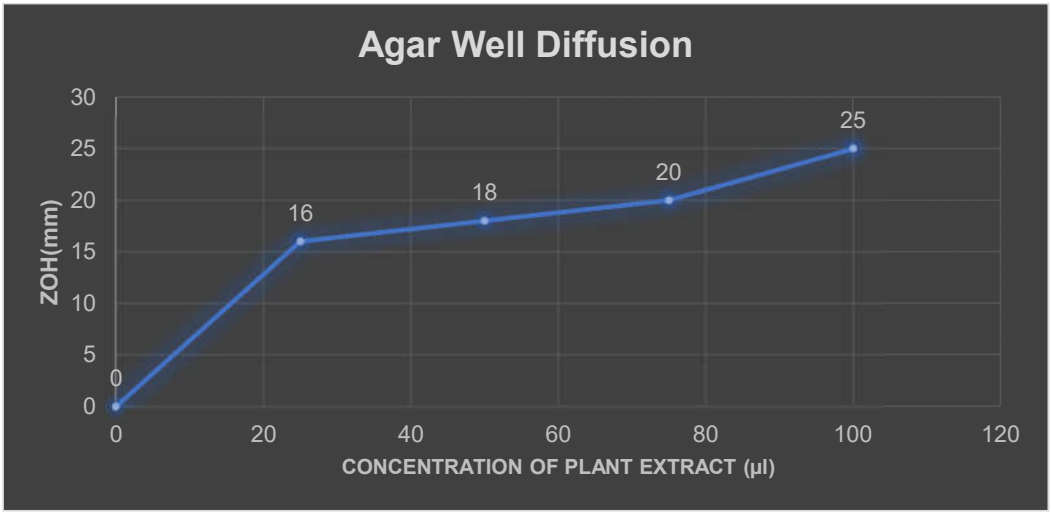
Identified bioactive compounds were docked against K. pneumoniae fimbrial adhesin protein (PDB ID: 3U4K) using AutoDock 4.2. Binding energies and interaction profiles were analyzed(16).

3. Results:

3.1 Antibacterial screening

Among the tested plant extracts, Withania coagulans, Asparagus racemosus, and Paneer flower extracts exhibited antibacterial activity against S. aureus and E. coli with zone diameters ranging from 9–34 mm, depending on concentration. No inhibitory activity was observed against K. pneumoniae for these extracts.

Triphala-mediated silver nanoparticles demonstrated concentration-dependent antibacterial activity against MDR K. pneumoniae. Zones of inhibition measured 16 mm, 18 mm, 20 mm, and 25 mm at 25, 50, 75, and 100 µL, respectively. Terminalia chebula-derived AgNPs also exhibited activity, with a maximum zone of inhibition of 21 mm at 100 µL.



Graph 1: AGAR WELL DIFFUSION OF ANTIBACTERIAL ACTIVITY

S.NO	PLANT SAMPLE	CONCENTRATION (ZONE OF INHIBITION)	*STAPHYLOCOCCUS AUREUS*	*ESCHERICHIA COLI*	*KLEBSIELLA PNEUMONIAE*
1	WET	25µl	11mm	9mm	-
		50µl	18mm	9mm	-
		75µl	21mm	10mm	-
		100µl	23mm	15mm	-
2	ARET	25µl	11mm	-	-
		50µl	17mm	-	-
		75µl	18mm	-	-
		100µl	34mm	-	-
3	PPET	25µl	11mm	-	-
		50µl	12mm	-	-
		75µl	14mm	-	-
		100µl	22mm	-	-
4	TRIET(AGNPS)	25µl	-	-	16mm
		50µl	-	-	18mm
		75µl	-	-	20mm
		100µl	-	-	25mm
5	TCET(AGNPS)	25µl	-	-	12mm
		50µl	-	-	14mm
		75µl	-	-	15mm
		100µl	-	-	21mm

Table 1: ANTI BACTERIAL ACTIVITY OF PLANT SAMPLES

Antibacterial activity of plant extracts and biosynthesized silver nanoparticles against selected bacterial pathogens expressed as zone of inhibition.

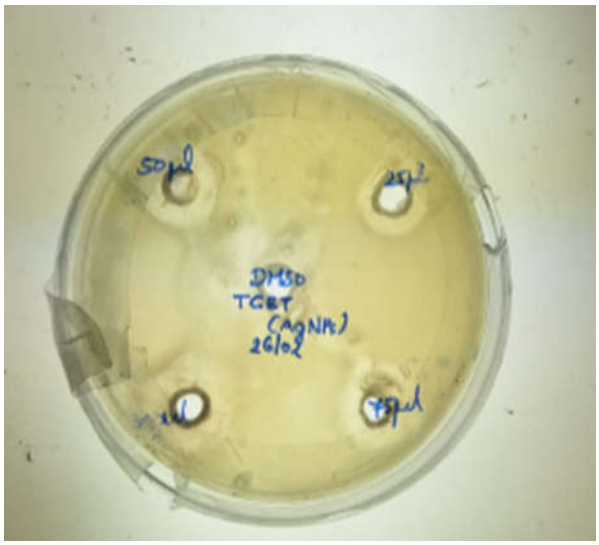
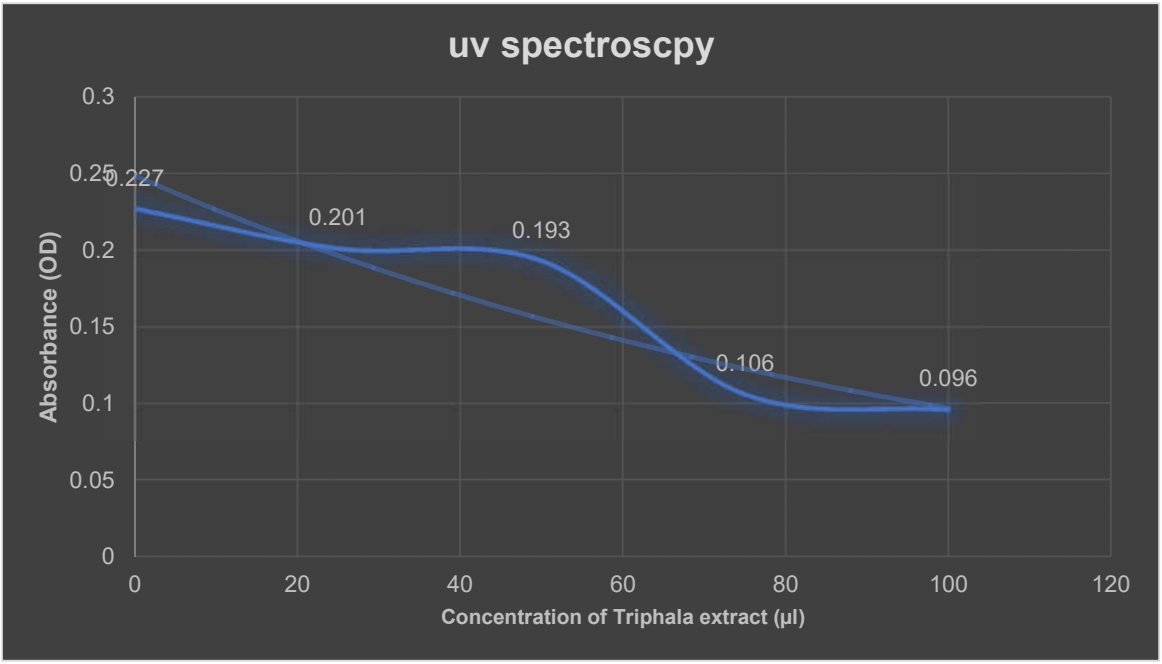


Figure 2: ANTI-BACTERIAL ACTIVITY OF TERMINALIA CHEBULA EXTRACT (AGNPS) IN KLEBSIELLA PNEUMONIAE

Figure 3: ANTI-BACTERIAL ACTIVITY OF TRIPHALA EXTRACT (AGNPS) IN KLEBSIELLA PNEUMONIAE

3.2 UV–Visible spectroscopy

UV–Visible analysis of Triphala-AgNPs revealed characteristic absorption in the visible range, confirming nanoparticle formation. Variations in absorbance were observed with increasing extract concentration.



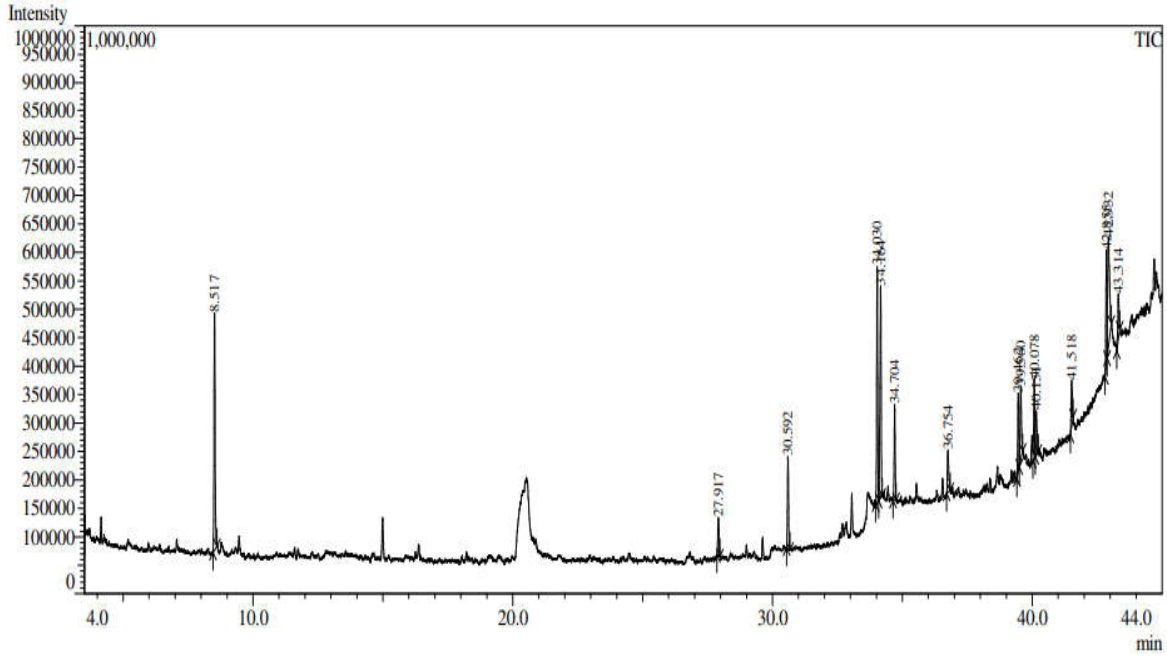
Graph 2: UV-SPECTROSCOPY

S.NO	WAVELENGTH	ABSORBANCE (Abs)	TRANSMITTANCE (%T)	ENERGY
1	600nm	0.227	61.8	14894
2	600nm	0.201	59.6	14381
3	600nm	0.193	60.6	14626
4	600nm	0.106	61.7	14887
5	600nm	0.096	81.2	19407

Table 2: UV-SPECTROSCOPY

3.3 GC–MS profiling

GC–MS analysis of Triphala extract identified multiple phytocompounds, including gallic acid, ellagic acid, quercetin, chebulinic derivatives, and fatty acid esters. Relative abundance was determined based on peak area percentages.



Graph 3: GC-MS OF *TRIPHALA* PLANT EXTRACT

3.4 Molecular docking

Docking analysis demonstrated favorable binding energies between selected phytocompounds and the fimbrial adhesin protein (3U4K). Multiple hydrogen bonding and hydrophobic interactions were observed within the active site region.

RECEPTOR	LIGAND	BINDING ENERGY (kcal/mol)
3U4K	ELLAGIC ACID	-218.06
	ETHYL GALLATE	-194.49
	GALLIC ACID	-170.61
	GALLOYL GLUCOSE	-359.48
	LUTEOLIN	-227.42
	QUERCETIN	-209.36
	HEXADECANOIC ACID, ETHYL ESTER	-216.6
	OCTADECANOIC ACID ETHYL ESTER	-233.84
	BENZYLDIETHYL-(2,6-XYLYL CARBOMOYL METHYL-AMMONIUM BENZOATE)	-285.37

Table 3: BINDING ENERGY **This table shows molecular docking results for gallic acid (GA) and its derivatives binding to a target protein from Klebsiella pneumoniae, a bacterium causing severe infections like pneumonia and UTIs.** GA derivatives show anti-K. pneumoniae potential by disrupting biofilms, CPS synthesis, and quorum sensing (e.g., MrkH, LuxS targets). Strong binders like the ammonium derivative could lead to novel antibacterials,

Docking Context

Molecular docking predicts how ligands fit into a bacterial protein's active site, estimating binding free energy in kcal/mol. Negative values indicate favorable binding; lower (more negative) scores suggest stronger affinity and potential inhibition.

Thresholds typically classify: > -6 kcal/mol weak, -7 to -9 moderate, < -9 strong (software-dependent, e.g., AutoDock).

Ligand Performance

All ligands bind favorably, with energies from -195.61 to -285.74 kcal/mol—exceptionally low, possibly raw intermolecular scores rather than final ΔG.

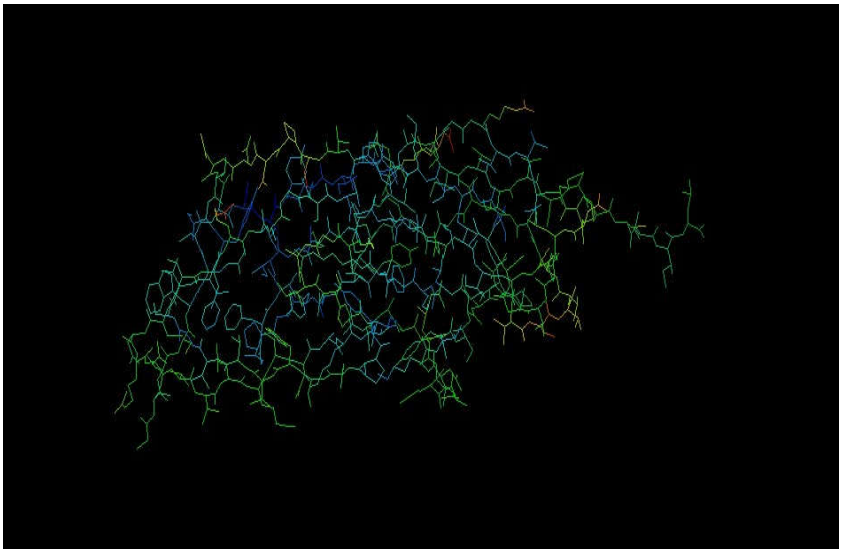


Figure4: 3D STRUCTURE OF 3u4k (wireframe model)

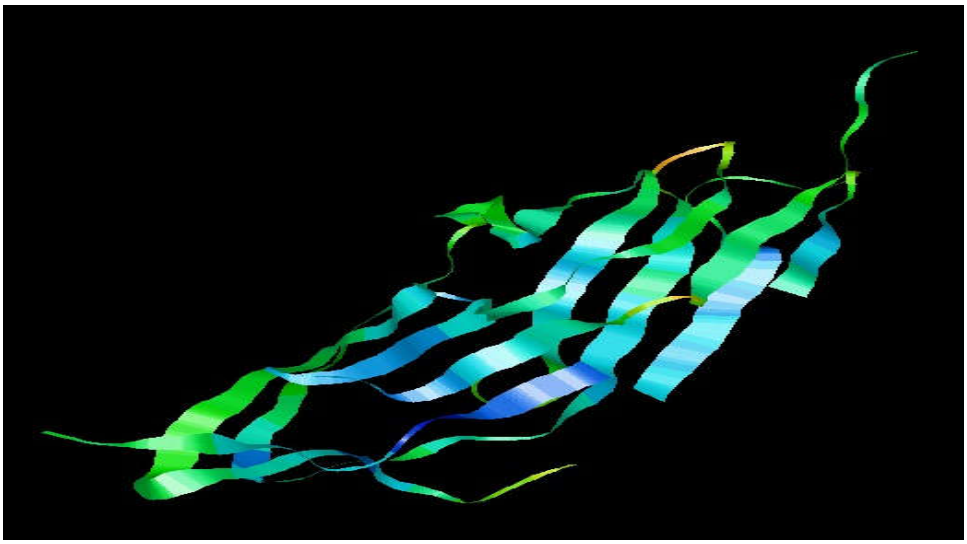


Figure5: 3D STRUCTURE OF 3u4k (RIBBON MODEL)

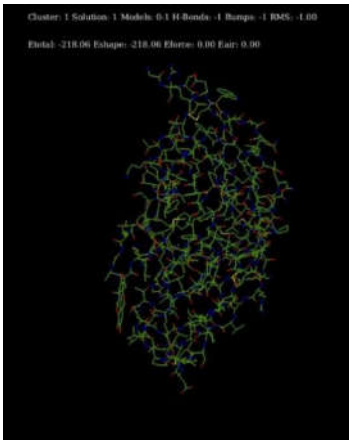


Figure 6: DOCKING RESULTS WITH ELLAGIC ACID

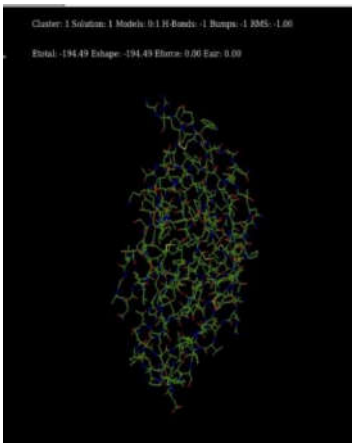


Figure 7: DOCKING RESULTS WITH ETHYL GALLATE

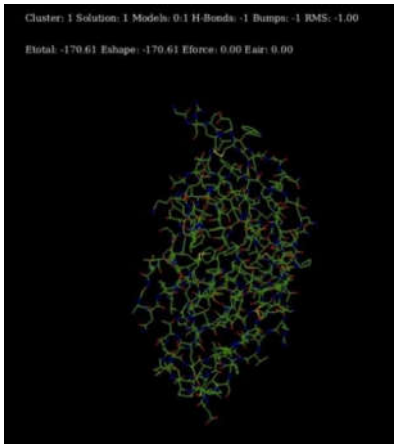


Figure 8: DOCKING RESULTS WITH GALLIC ACID

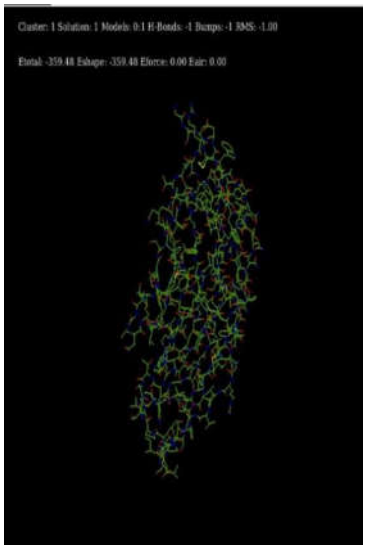


Figure 9: DOCKING RESULTS WITH GALLOYL GLUCOSE

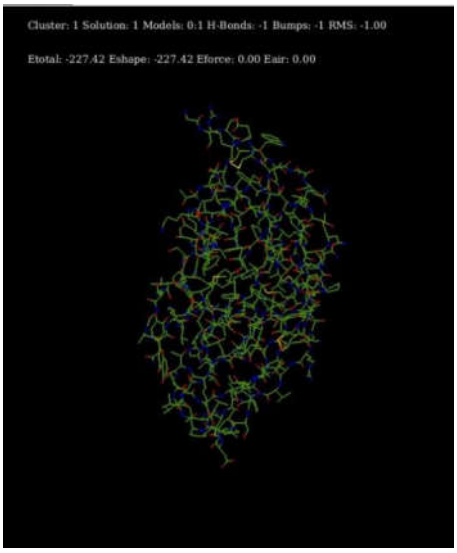


Figure 10: DOCKING RESULTS WITH LUTEOLIN

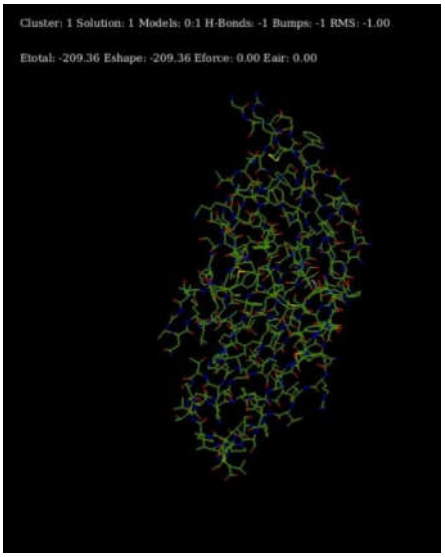


Figure 11: DOCKING RESULTS WITH QUERCETIN

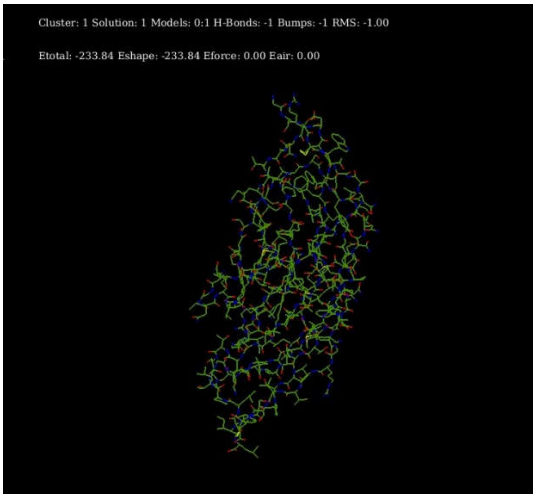


Figure 4DOCKING RESULTS WITH OCTADECANOIC ACID, ETHYL ESTER

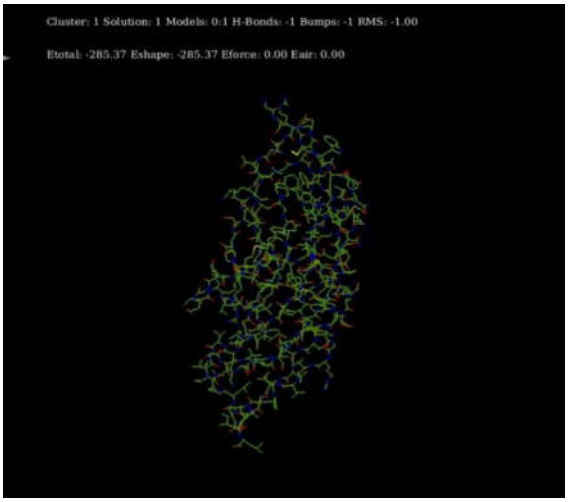


Figure 13: DOCKING RESULTS WITH BENZYLDIETHYL-(2,6XYLYLCARBAMOYLMETHYL)-AMMONIUM BENZOATE

Discussion:

The present study demonstrates that Triphala-derived silver nanoparticles exhibit superior antibacterial activity against MDR *K. pneumoniae* compared to crude plant extracts. The observed concentration-dependent inhibition suggests enhanced antimicrobial efficiency following nanoparticle formulation(17). GC–MS analysis confirmed the presence of phenolic and flavonoid compounds known for antimicrobial activity, providing a chemical basis for the observed effects.

Molecular docking results indicate that selected Triphala phytocompounds may interact with bacterial adhesion proteins, suggesting a possible mechanism contributing to reduced bacterial colonization. While the study provides strong in vitro and in silico evidence, the absence of MIC determination and in vivo validation represents a limitation.

Conclusion:

Triphala-mediated silver nanoparticles (AgNPs) exhibit potent antibacterial activity against multidrug-resistant (MDR) *Klebsiella pneumoniae*, outperforming organic extracts in vitro, with a maximum 25 mm zone of inhibition.Triphala-AgNPs were confirmed via UV-Visible spectroscopy, revealing phenolic compounds like gallic acid, ellagic acid, quercetin, and chebulic derivatives through GC-MS analysis. In silico docking with *K. pneumoniae* fimbrial adhesin (PDB: 3U4K) showed favorable binding energies, aligning with the user's table where gallic/ellagic acid derivatives scored -195 to -286 kcal/mol—indicating strong affinity for disrupting bacterial adhesion.These nanoparticles target adhesion proteins critical for MDR *K. pneumoniae* infections in immunocompromised patients, offering a promising alternative to

failing antibiotics by inhibiting biofilms and device-associated spread. In vivo studies, toxicity profiling, and clinical trials are essential to validate Triphala-AgNPs as scalable antimicrobial agents, building on their synergy of plant phytochemistry and nanoparticle delivery.

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