

Evaluation of the antibacterial activity of *Myrtus communis* and glutaraldehyde against *Pantoea agglomerans* recovered from intensive care unit surfaces: A comparative *in vitro* study and molecular docking

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Abstract: Multidrug-resistant (MDR) bacteria in intensive care unit (ICU) environments pose an important infection-control challenge. This study investigated *Myrtus communis* as a potential plant-derived alternative to conventional disinfectants. To evaluate and compare the *in vitro* antibacterial activity of methanolic *M. communis* leaf extract and glutaraldehyde disinfectant (2.5%) against MDR *Pantoea agglomerans* (*P. agglomerans*) isolated from ICU inanimate surfaces and to characterize major phytochemical constituents associated with its activity. This study identified two *P. agglomerans* isolates by 16S rRNA gene sequencing. Methanolic *M. communis* leaf extract was prepared by cold maceration. Antibacterial activity was evaluated using agar well diffusion for the extract and agar disc diffusion for antibiotic references, while minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by microdilution. GC-MS was used for phytochemical profiling, and molecular docking was performed to investigate predicted interactions of selected compounds with GroEL-KMgATP. The extract (100 mg/ml) produced a significantly larger inhibition zone (22.5 ± 0.9 mm) than 2.5% glutaraldehyde (9.3 ± 1.7 mm). The MIC and MBC of the extract were both 12.5 mg/ml. GC-MS identified quinic acid as the predominant constituent (40.02%), followed by 4H-pyran-4-one derivative (2.38%), bicyclo (2.2.1)heptan-2-ol, 1,5,5-trimethyl- (1.91%), and myrtenyl acetate (1.4%). Myrtenyl acetate showed the strongest predicted binding affinity to GroEL-KMgATP (-6.2 kcal/mol). *M. communis* leaf extract demonstrated promising *in vitro* antibacterial activity against the tested MDR *P. agglomerans* and greater inhibition than glutaraldehyde under the conditions examined.

Introduction

In the high-risk environment of intensive care units (ICUs), healthcare-associated infections represent a critical challenge to patient safety, mainly among immunocompromised individuals. The persistence and transmission of pathogens in these settings significantly contribute to increased morbidity, mortality, prolonged hospital stays, and escalating healthcare costs. Of particular concern is the global rise of multidrug-resistant (MDR) microorganisms, which severely limit treatment options and undermine infection control strategies [1-3]. Among emerging opportunistic pathogens, *Pantoea agglomerans* (*P. agglomerans*) has gained increasing clinical

relevance in ICU environments. Originally recognized as a plant-associated bacterium, it is now implicated in a wide spectrum of human infections, ranging from localized wound infections to severe systemic conditions such as septicemia, pneumonia, and urinary tract infections. Its presence in ICUs is largely associated with contaminated medical equipment, fluids, and inanimate surfaces [4], which serve as reservoirs for transmission. The situation is further complicated by the growing prevalence of MDR strains of *P. agglomerans*, making infections more difficult to manage and control [5-7]. Effective surface disinfection remains a cornerstone of infection prevention in ICUs. However, conventional chemical disinfectants, such as glutaraldehyde, face several limitations [8, 9]. These include reduced efficacy against biofilm-forming and resistant bacterial strains [10], potential toxicity to healthcare workers, and environmental concerns. Such drawbacks necessitate the search for safer, sustainable, and equally effective alternatives.

In this context, medicinal plants have emerged as promising sources of antimicrobial agents. *Myrtus communis* (*M. communis*), a Mediterranean shrub with a long history of traditional medicinal use [11], has demonstrated notable antibacterial, antioxidant, and anti-inflammatory properties. These bioactivities are largely attributed to its rich content of phenolic compounds and flavonoids, making it a strong candidate for development as a natural disinfectant [12]. The purpose of this study was to investigate and compare the *in vitro* bactericidal effectiveness of *M. communis* leaf extract with that of the common disinfectant glutaraldehyde, which is used as a disinfectant to protect ICU inanimate surfaces from MDR pathogen contamination. This study hypothesized that *M. communis*' complex phytochemical profile would result in pronounced disinfectant activity. *M. communis*' prospective use as a plant-based disinfectant for contaminated healthcare surfaces has received little attention, even though its antibacterial and other biological properties have been thoroughly studied. Specifically, there is insufficient data to directly evaluate the efficacy of *M. communis* leaf extracts with commonly used chemical disinfectants such as glutaraldehyde against multidrug-resistant bacteria isolated from ICU inanimate surfaces. Further, not enough research has been done on *M. communis*'s antibacterial ability against *P. agglomerans* in the particular context of ICU environmental contamination. Consequently, there is a lack of information about whether an extract from *M. communis* leaves that is sourced locally can have significant antibacterial activity against MDR environmental isolates and possibly function as a supplementary or sustainable disinfectant. Thus, can *M. communis* leaf extract serve as a potential plant-derived alternative or complementary disinfectant against MDR *P. agglomerans* contaminating ICU inanimate surfaces, compared with 2.5% glutaraldehyde? Therefore, this study aimed to assess and compare the antibacterial properties of methanolic *M. communis* leaf extract with those of glutaraldehyde against multidrug-resistant *P. agglomerans* isolated from ICU inanimate surfaces. To find substances that might be connected to the extract's reported antibacterial action, the study also aimed to analyze the extract's main phytochemical components using GC-MS.

Materials and methods

Plant collection and extraction: The collection of *M. communis* leaves was carried out with formal authorization from the Research and Consulting Department, Faculty of Pharmacy, Omar Al-Mukhtar University, Al-Bayda, Libya. Twice, in July 2025 and 2026, fresh leaves of the *M. communis* plant were picked up from AL-Hmama, a region around Al-Bayda City, located in the northeast of Libya at the geographical coordinates of 32°55'39.8"N - 21°40'06.0" E (**Image 1**). The plant is identified and classified by Ensaf H. Dakeel, the taxonomist at the Faculty of Sciences at Omar Al-Mukhtar University, Al-Bayda, Libya, and given the voucher number: 96.1.1.

The leaves were washed with tap water, dried in the air, ground up, and then, with cold maceration, extracted with methanol (1: 10 w/v) for 72 hrs at room temperature. The extract was filtered, and the filtrate was concentrated by use of a rotary evaporator and left to air-dry. The extract was kept in a closed, clean glass

container at - 20 °C, ready for investigation steps. To prepare the stock solution, 0.2 g of dry extract was dissolved in 2.0 mL of 10.0% dimethyl sulfoxide (DMSO) to end with a concentration of 100 mg/ml.

Reference antibiotics: Standard antibiotic disks (Oxoid Ltd.) were bought from the local market. They included amoxicillin 25 µg, amoxicillin-clavulanate 30 µg, cefuroxime 30 µg, ceftriaxone 30 µg, cefepime 30 µg, meropenem 10 µg, azithromycin 15 µg, clarithromycin 15 µg, levofloxacin 5 µg, and gentamicin 10 µg.

Bacterial isolates: Two environmental isolates of *P. agglomerans* were isolated from inanimate surfaces inside the ICU at AL-Wahda Hospital, Derna City, Libya.



Image 1: Geographical coordinates of the *Myrtus communis* collection site

Disinfectant: Prodex 995 (glutaraldehyde 99.0%) was obtained from AL-Wahda Hospital, Derna City, Libya. It is a disinfectant used at a concentration of 2.5% for cleaning inside the same ICU and was tested in this study against *P. agglomerans*.

Agar well diffusion assay: This assay was used to determine the antibacterial activity of the plant extract and disinfectant. The agar well diffusion method was used as described by Eltawaty and others [13]. For the bacterial isolates, overnight cultures grown in broth were adjusted to an inoculum size of 1.5×10^8 colony-forming units (CFU)/mL using a 0.5 McFarland standard, and 100 µL was inoculated onto agar plates. The plates were allowed to stand for five minutes to allow the diffusion of the extracts. Then 50 µl of each extract and Prodex were added to four separate replicate wells (four 7.0 mm diameter holes were cut into the agar gel plates). A 50 µl aliquot of the solvent (DMSO 10.0%) was added to a specified well in each plate as a negative control. The plates were incubated at 37 °C for 24 hrs, and the diameters (mm) of the inhibition zones were measured.

Evaluation of minimum inhibitory (MIC) and bactericidal (MBC) concentration: The micro-dilution method was used to determine the MIC according to the National Committee for Clinical Laboratory Standards guidelines, 1999 [14], with modifications. Twofold serial dilution of crude extract was prepared directly in a microtiter plate containing Mueller-Hinton broth to obtain various concentrations (100 - 0.39 mg/ml). The bacterial inoculum was added to each well. Two controls were used (control A contains media and bacteria, and control B contains only media). The plate was covered with a sterile cover and incubated for 24 hrs at 37 °C. Resazurin (20 µl) was added to each well of the microtiter plate and incubated at 37 °C for 3 - 4 hrs. The wells containing bacterial growth turned pink, whereas the wells without bacterial growth remained blue. The MIC was defined as the lowest sample concentration exhibiting inhibition of growth. The MBC was evaluated by subculturing 10 µL of each well with no growth onto Mueller-Hinton agar (MHA) plates and incubating at 37 °C for 24 hrs. The MBC was determined as the lowest concentration that showed no colony formation on agar plates.

Agar disk diffusion assay: This assay was used to determine the antibacterial activity of antibiotic references. Sterile antibiotic discs were placed on MHA plates freshly seeded with 100 μ L of bacterial suspension. After 24 hours of incubation at 37 °C, the inhibition zones (mm) were measured.

GC-MS analysis: A Shimadzu GC-MS (Model QP2010-Ultra, Japan) fitted with an Rtx-5MS capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m) was used to analyze the tested extract. The chemical components were identified using an electron ionization mass spectrometer with an ionization energy of 70 eV. The mass transfer line and injector temperatures were set at 250 and 300 °C, respectively, and helium was utilized as a carrier gas at a flow rate of 1.61 ml/min. The temperature program began at 60 °C with a rate of 10 °C/min and ended at 300 °C with a 10-min hold. The split mode was used to inject the sample (1.0 μ l) with a split ratio of 120: 1. The entire run time was 29 min, with a 2-min delay. By comparing their retention index and mass fragmentation patterns with those found in the National Institute of Standards and Technology database, the sample's constituents were identified.

Docking procedure: Auto-blind docking was performed using the default CB-Dock2 method, which systematically locates potential binding pores along the entire protein surface. For each cavity detected, the docking box dimensions and center coordinates (x, y, z) were computed automatically. The binding energies (Vina scores) were calculated using kilocalories per mole (kcal/mol). The generated docked poses were ranked according to their binding affinity scores.

Examination of interactions: Protein-ligand interactions were visualized using the BIOVIA Discovery Studio 2025 Client (Dassault Systèmes). Two-dimensional and three-dimensional interaction maps were made to depict hydrogen bonds, hydrophobic (π -alkyl) interactions, van der Waals contacts, and π -cation interactions between each ligand and the surrounding amino acid residues of EGFR.

Statistical analysis: Descriptive statistics (Mean $\pm$ SD) were used to present the data. A parametric independent-samples t-test was used to compare the data (SPSS v28). This was used to compare the extract and glutaraldehyde-revealed inhibition zones.

Results

The bacterial isolates obtained from the Medicine ICU were identified using 16S rRNA gene sequencing. Sequence analysis and the BLAST comparison revealed that the isolates were the same and belonged to *P. agglomerans* strains. The isolate was given the accession number (PZ269366) and registered as *P. agglomerans* strain 36 16S ribosomal RNA gene, partial sequence.

The antibacterial activity of *M. communis* leaf extract at a concentration of 0.1 g/ml was evaluated against the isolated multidrug-resistant *P. agglomerans* isolates. The plant extract demonstrated strong inhibitory activity against the bacterium, producing a large mean inhibition zone of 22.5 ± 0.9 with equal MIC and MBC of 12.5 mg/ml. Also, Prodex 2.5% (glutaraldehyde) disinfectant was tested for its effectiveness against the bacterium, and it revealed a mean inhibition zone of 9.3 ± 1.7 . The t-test analysis showed highly significant differences ($p = 0.001$) between each extract and disinfectant in terms of potential against the tested bacteria. In addition, the sensitivity of the tested bacterium was evaluated toward the included antibiotic references of varied classes, and the sensitivity varied. The results output clarified that *P. agglomerans* isolate showed 50.0% resistance against amoxicillin 25 μ g, augmentin 30 μ g, cefuroxime 30 μ g, and clarithromycin 15 μ g, while appeared 100% sensitive to ceftriaxone 30 μ g, cefepime 30 μ g, meropenem 10 μ g, azithromycin 15 μ g, levofloxacin 5 μ g, and gentamicin 10 μ g (**Table 1**).

Table 1: Antibigram profile of tested antibiotic references against *Pantoea agglomerans*

No.	Antibiotic discs (μg)	Resistance (%)	Sensitivity (%)
1	Amoxicillin 25	50	50
2	Augmentin 30	50	50
3	Cefuroxime 30	50	50
4	Ceftriaxone 30	0	100
5	Cefepime 30	0	100
6	Meropenem 10	0	100
7	Azithromycin 15	0	100
8	Clarithromycin 15	50	50
9	Levofloxacin 5	0	100
10	Gentamicin 10	0	100

GC-MS analysis: **Table 2** clarified that, among the constituents, quinic acid was the dominant one among those reported to have antibacterial and antibiofilm activities, accounting for the largest portion of the extract at 40.02% (Retention Time: 13.49). Also, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP) accounts for 2.38%; Bicyclo (2.2.1) heptan-2-ol, 1,5,5-trimethyl- Comprises (1.91%), and myrtenyl acetate makes up (1.4%) of the area. In addition, several compounds are present in smaller concentrations less than 1.0%; 2-furan-carboxaldehyde, 5-methyl- (0.80%), phytol (0.19%); alpha-terpineol (0.33%); linoleic acid ethyl ester (0.33%); (-)-myrtenol (0.18%); oleic acid (0.16%); B-myrcene (0.04%); 2,4-dihydroxy-2,5 dimethyl-3(2H)-furanone (0.67%); and eucalyptol 1,8-cineol (0.20%).

Table 2: Main antibacterial and anti-biofilm compounds identified in the methanol extract of *Myrtus communis* leaves

Compound	Retenti on time	Area (%)	Molecular weight (g/mol)	Chemical formula	Bioactivity (References)
Quinic acid	13.49	40.02	192	$\text{C}_7\text{H}_{12}\text{O}_6$	Antibiofilm [15]
4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP)	6.67	2.38	144	$\text{C}_6\text{H}_8\text{O}_4$	Antibiofilm [16]
Bicyclo(2.2.1)heptan-2-ol, 1,5,5-trimethyl-	9.79	1.91	154	$\text{C}_{10}\text{H}_{18}\text{O}$	Antibacterial [17]
Myrtenyl acetate	9.25	1.40	194	$\text{C}_{12}\text{H}_{18}\text{O}$	Antibiofilm [18]
2,4-Dihydroxy-2,5 dimethyl-3(2H)-furanone	4.23	0.67	144	$\text{C}_6\text{H}_8\text{O}_4$	Antibiofilm [16]
alpha-Terpineol	7.37	0.33	154	$\text{C}_{10}\text{H}_{18}\text{O}$	Antibiofilm [19]
Linoleic acid ethyl ester	18.43	0.19	296	$\text{C}_{20}\text{H}_{40}\text{O}$	Antibiofilm [20]
Eucalyptol (1,8-cineol)	4.96	0.20	154	$\text{C}_{10}\text{H}_{18}\text{O}$	Antibiofilm [21]
Phytol	18.43	0.19	296	$\text{C}_{20}\text{H}_{40}\text{O}$	Anti-QS [22]
(-)-Myrtenol	7.44	0.18	152	$\text{C}_{10}\text{H}_{16}\text{O}$	Antibiofilm [23]
Oleic acid	18.69	0.16	282	$\text{C}_{18}\text{H}_{34}\text{O}_2$	Antibiofilm [24]
Beta-Myrcene	4.33	0.04	136	$\text{C}_{10}\text{H}_{16}$	Antibiofilm [25]
2-Furancarboxaldehyde,5-methyl-	4.07	0.80	110	$\text{C}_6\text{H}_6\text{O}_2$	Antibacterial [26] Antibiofilm [27]

Molecular docking (protein-ligand interaction): With molecular docking, Quinic acid, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, bicyclo(2.2.1)heptan-2-ol, 1,5,5-trimethyl-, and myrtenyl acetate were the four main substances that were assessed. With a Vina score of - 6.0 kcal/mol, quinic acid was predicted to bind inside cavity C5, which had a volume of 1107 Å³. The matching cavity center had a docking box dimension of 17 × 17 × 26 Å and was situated at coordinates (59, - 21, - 30), as shown in **Table 3** and **Figure 1**. Cavity C2 contained the molecule 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, which had a volume of 1875 Å³ and a Vina score of - 5.0 kcal/mol. The docking box measured 27 × 24 × 16 Å, and the cavity center was located at 123, 43,

- 25 (Table 3, Figure 2). Also, using the same cavity dimensions and docking coordinates, bicyclo(2.2.1)heptan-2-ol, 1,5,5-trimethyl- was predicted to bind within the same C2 cavity and showed a Vina score of - 5.3 kcal/mol (Table 3, Figure 3).

With a Vina score of - 6.2 kcal/mol, myrtenyl acetate showed the greatest projected binding affinity among the substances under investigation (Table 3, Figure 4). Additionally, it was anticipated that this molecule would interact with cavity C2, which had a volume of 1875 Å³, a docking box dimension of 27 × 24 × 18 Å, and a cavity center at (123, 43, - 25). Overall, the docking results showed that myrtenyl acetate had the best predicted binding affinity toward GroEL-KMgATP, followed by quinic acid (- 6.0 kcal/mol), bicyclo(2.2.1)heptan-2-ol, 1,5,5-trimethyl- (- 5.3 kcal/mol), and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (- 5.0 kcal/mol).

Table 3: Binding interactions of the most abundant compounds (in *Myrtus communis*) with GroEL-KMgATP (Important protein for *Pantoea agglomerans* survival) at 2.00 Å resolution

Compound	CurPocket ID	Vina score	Cavity volume (Å ³)	Center (x, y, z)	Docking size (x, y, z)
Quinic acid	C5	-6.0	1107	59, - 21, - 30	17, 17, 26
4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C2	-5.0	1875	123, 43, - 25	27, 24, 16
Bicyclo(2.2.1)heptan-2-ol, 1,5,5-trimethyl-	C2	-5.3	1875	123, 43, - 25	27, 24, 16
Myrtenyl acetate	C2	-6.2	1875	123, 43, - 25	27, 24, 18

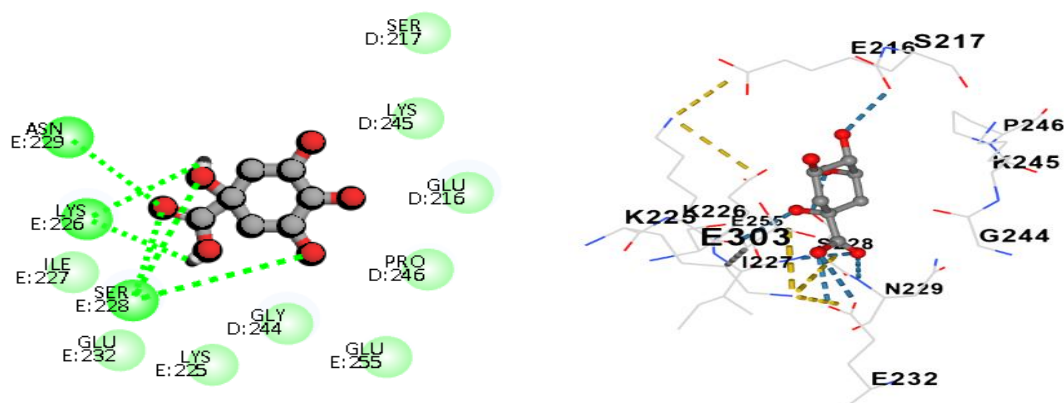


Figure 1: Interaction of protein (GroEL-KMgATP) and ligand (Quinic acid)

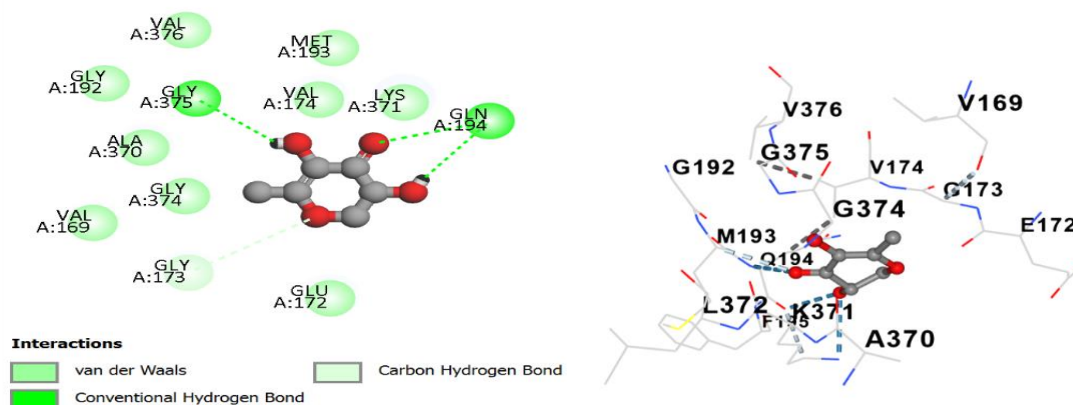


Figure 2: Interaction of protein (GroEL-KMgATP) and ligand (4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-)

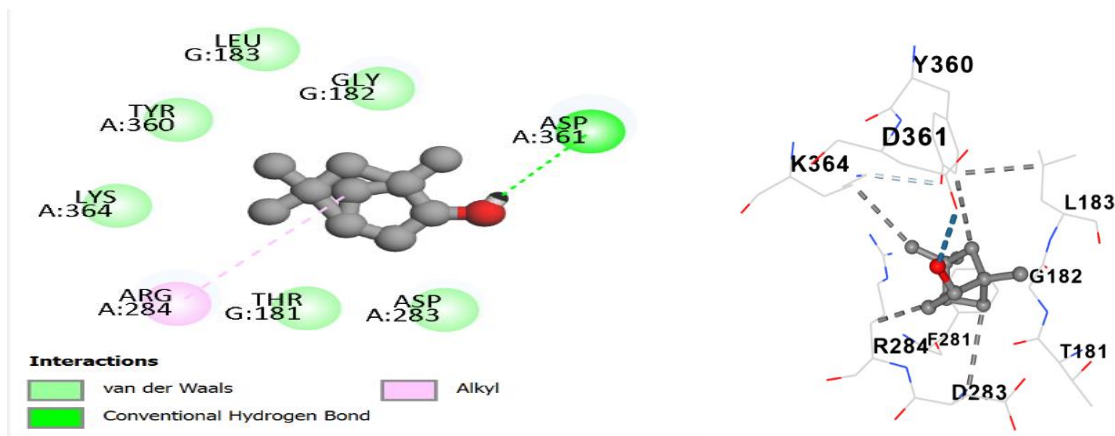


Figure 3: Interaction of protein (GroEL-KMgATP) and ligand (Bicyclo(2.2.1)heptan-2-ol, 1,5,5-trimethyl-)

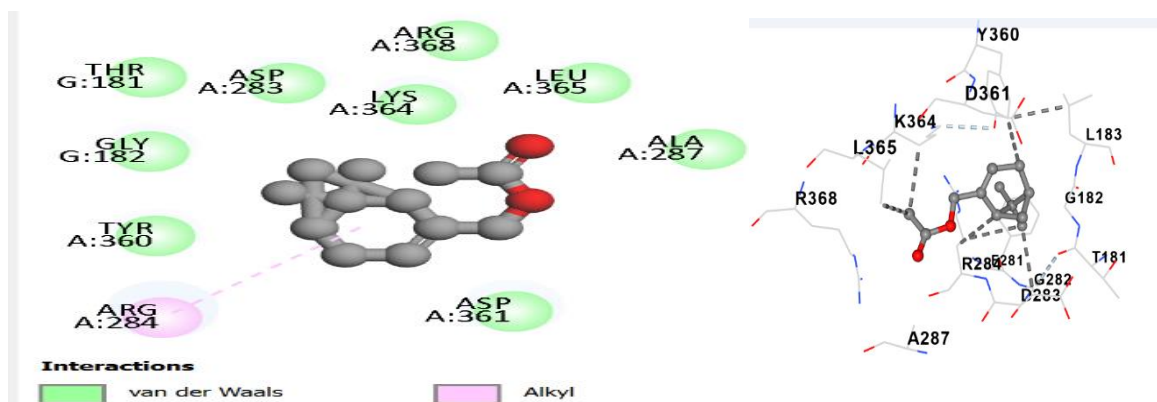


Figure 4: Interaction of protein (GroEL-KMgATP) and ligand (Myrtenyl acetate)

Discussion

The most significant finding of this study is that *M. communis* leaf extract demonstrated markedly superior antibacterial activity compared to glutaraldehyde against the tested isolate, as evidenced by a substantially larger inhibition zone and equal MIC and MBC values. This result highlights the potential of plant-derived bioactive compounds as effective alternatives to conventional chemical disinfectants in clinical settings. The identified *P. agglomerans* strain 36 represents a clinically relevant opportunistic pathogen within the ICU environment. Although traditionally considered an environmental bacterium, its presence on inanimate ICU surfaces signals a potential reservoir for healthcare-associated infections, mainly in immunocompromised patients [28, 29]. The organism's ability to cause diverse infections, including septicemia, respiratory tract infections, and urinary tract infections, furthermore underscores its clinical importance. The antimicrobial susceptibility profile revealed a concerning MDR pattern, with resistance observed against commonly used β -lactam antibiotics such as amoxicillin, amoxicillin/clavulanate, and cefuroxime. A previous study reported that *P. agglomerans* is almost universally resistant to ampicillin [30]. This resistance pattern suggests the likely presence of β -lactamase enzymes, consistent with previous reports. At the same time, full sensitivity to higher-generation cephalosporins (3rd-generation ceftriaxone and 4th-generation cefepime) and meropenem indicates that resistance mechanisms may still be limited to narrower-spectrum pathways. However, the persistence of such MDR organisms in ICU environments highlights the limitations of current disinfection strategies and remains a critical concern, particularly given their ability to form biofilms, which enhance survival and resistance to disinfectants.

In general, biocides, including glutaraldehyde, interact with a variety of bacterial cell targets. An irreversible bactericidal effect or a reversible bacteriostatic one will depend on how many targets are impacted and how much

damage is done. The cytoplasmic membrane and enzymes are the main targets of most biocides, though different biocide chemistries have different bactericidal mechanisms. Antimicrobial resistance may result from improper use or low concentrations of a biocide, which can stress bacterial pathogens without eliminating them [8, 10]. Aldehydes like glutaraldehyde are examples of biocides. They work by reacting with functional groups in proteins, especially amino (-NH₂), sulfhydryl (-SH), and hydroxyl (-OH) groups. This results in protein cross-linking, which causes enzymes and structural proteins to lose their biological activity, which stops vital metabolic processes and killing bacteria [31]. Additionally, glutaraldehyde can interact with lipids and membrane proteins to cause intracellular content leakage, increased permeability, loss of membrane integrity, and ultimately cell lysis or irreversible cell damage [32]. In such high-risk environments, the combination of antimicrobial pressure and frequent use of invasive devices creates ideal conditions for biofilm formation. Biofilms significantly reduce the effectiveness of conventional disinfectants such as glutaraldehyde by limiting penetration and enhancing bacterial survival. The isolation of MDR *P. agglomerans* from ICU inanimate surfaces emphasizes the ongoing issue of environmental contamination in critical care settings. Inanimate surfaces such as hospital bed rails, ventilator equipment, and countertops act as reservoirs for opportunistic pathogens, allowing cross-transmission to vulnerable patients. The capacity of *P. agglomerans* to produce biofilms hinders eradication efforts since biofilm-encased bacteria are up to 1000 times more resistant to disinfectants than planktonic cells. The above findings indicate that *M. communis* extract, with its several biofilm-targeting ingredients, may have a strategic advantage over conventional disinfectants in eradicating surface-associated bacterial colonies. Thus, the reduced inhibition observed with glutaraldehyde in this study represents an important finding that may indicate decreased susceptibility or protective biofilm-associated tolerance, warranting further investigation. The P value of less than 0.001 calculated in this study indicates that the difference in efficacy between the *M. communis* extract and the glutaraldehyde disinfectant is highly significant and not due to random chance. The superior antibacterial performance of *M. communis* extract, especially with low MIC and MBC concentrations, can be attributed to its complex phytochemical composition. Many previous studies reported that *M. communis* contains high concentrations of phenolic compounds, flavonoids, and essential oils such as 1,8-cineole [33, 34], α -pinene [35, 36], and myrtucommulones [11, 37] that are known to inhibit protein synthesis and disrupt the cell membrane integrity of Gram-negative bacteria like *Pantoea*.

The superior activity of *M. communis* extract is likely due to its intricate phytochemical composition, which has the potential to exert multiple, synergistic antibacterial mechanisms. Quinic acid, the major compound identified (40.02%), has been previously reported to interfere with the production of extracellular polymeric substances, thereby disrupting bacterial adhesion and inhibiting biofilm formation [38]. In addition, it is recognized that 4H-pyran-4-one derivatives and terpenoids, including myrtenyl acetate and α -terpineol, can compromise the integrity of bacterial membranes, resulting in increased permeability and cellular leakage [39]. The anti-quorum sensing activity of phytol [40], despite its lower concentration of 0.19%, is noteworthy due to its potential to disrupt bacterial cell-to-cell communication and virulence regulation. Furthermore, the analysis highlighted the presence of bicyclo (2.2.1) heptan-2-ol, 1,5,5-trimethyl- and 2-Furancarboxaldehyde, 5-methyl-compounds that provide direct synergistic bactericidal action [17, 29]. This multi-targeted action is in contrast to the singular primary mechanism of glutaraldehyde (protein cross-linking) and may account for the extract's more potent efficacy against MDR isolates. Rather than confirming a definitive mechanism, the observed antibacterial effect of *M. communis* extract is best interpreted as a likely result of synergistic, multi-target interactions among its phytochemical constituents. This multi-component activity may provide an advantage over single-target disinfectants like glutaraldehyde, particularly against MDR organisms and biofilm-forming bacteria. In contrast, glutaraldehyde acts primarily through protein cross-linking and enzyme inactivation. Although it is widely used as a high-level disinfectant, its comparatively lower antibacterial activity in this study suggests a possible reduced

susceptibility of the tested isolate. This finding does not confirm resistance but indicates that further investigation into the efficacy of glutaraldehyde against MDR environmental isolates is warranted, especially in the context of biofilm-associated tolerance.

Further computational evidence for the possible interaction between components of *M. communis* and the GroEL-KMgATP protein of *P. agglomerans* was revealed by the molecular docking analysis. GroEL is a biologically important target when examining substances that can impede bacterial survival because it is a crucial molecular chaperone involved in protein folding and cellular stress responses. GroEL is a highly conserved molecular chaperone that is a member of the heat shock protein 60 (HSP60) family and functions with its co-chaperonin GroES. It is in charge of refolding denatured proteins and enabling the correct folding of crucial nascent polypeptides. Many freshly produced proteins will misfold and create hazardous intracellular aggregates if it is inhibited. Additionally, inhibiting this protein prevents *P. agglomerans* from being able to withstand stressful changes in the host or plant. This protein aids pathogens in adapting to environmental challenges [41, 42]. All four of the compounds under investigation in this work had negative Vina scores, indicating energetically favorable anticipated interactions with the GroEL-KMgATP binding cavities. However, the chemicals' expected affinities differed. Myrtenyl acetate showed the best projected binding affinity (- 6.2 kcal/mol) among the investigated chemicals, followed by quinic acid (- 6.0 kcal/mol). Myrtenyl acetate's higher negative score indicates that, in comparison to the other components under investigation, this chemical may form a relatively more stable contact within the projected binding cavity.

Notably, cavity C2 was anticipated to be occupied by myrtenyl acetate, bicyclo(2.2.1)heptan-2-ol, 1,5,5-trimethyl-, and the 4H-pyran-4-one derivative, while cavity C5 was linked to quinic acid. Differences in the chemical structures and physical properties of the compounds under investigation may have an impact on their accommodation within a protein cavity and their capacity to form advantageous molecular interactions, which could explain the variation in expected binding affinity. Myrtenyl acetate is therefore a possible choice for additional research against GroEL-KMgATP due to its exceptionally high score. Nevertheless, the docking score should not be taken as concrete proof of GroEL function inhibition or antibacterial action. While experimental validation is necessary to validate biological activity, docking predicts the viability and relative strength of ligand-protein interactions under a computer model. Examining the anticipated interactions between the ligands and the amino acid residues surrounding the binding sites was made possible by the visualization of the docked complexes. Van der Waals contacts, hydrogen bonding, hydrophobic π -alkyl interactions, and π -cation interactions were all taken into consideration. These interactions may help explain variations in the compounds' expected binding scores and can aid in ligand stability inside a protein-binding pocket. Although this association should be presented cautiously, the docking results may also be related to the observed antibacterial activity of *M. communis*. A crude plant extract's antibacterial action is likely to entail several components and possibly several cellular targets [43]. Therefore, rather than proving the mechanism of antibacterial action, the current docking results should be seen as supporting mechanistic data [44]. Myrtenyl acetate was found to have the lowest Vina score, which gives future research utilizing purified myrtenyl acetate, recombinant GroEL-KMgATP, and suitable functional assays a logical foundation to ascertain whether the anticipated interaction results in real inhibition of bacterial growth and protein function. From an applied perspective, the presence of MDR *P. agglomerans* on ICU surfaces highlights the ongoing challenge of environmental contamination in critical care settings. The previous literature does not seem to include a direct assessment of *M. communis* leaf extract against *P. agglomerans* in comparison with glutaraldehyde, even though the antibacterial potential of *M. communis* extracts and essential oils has been thoroughly studied against a variety of Gram-positive and Gram-negative bacteria. Previous studies have shown that the antibacterial activity of *M. communis* preparations varies according

to the bacterial species examined, chemical composition, and extraction technique. Thus, by assessing the antibacterial ability of *M. communis* leaf extract against *P. agglomerans* and directly comparing its activity with 2.5% glutaraldehyde, the current study demonstrated the efficacy of *M. communis* extract and suggests that plant-based disinfectants could serve as complementary or alternative strategies in infection control protocols. Additionally, such natural products may offer advantages in terms of reduced toxicity and environmental impact compared to conventional chemical disinfectants.

Study limitation: The primary constraint of this investigation was the limited quantity of *P. agglomerans* isolates examined, which restricts the applicability of the results. Furthermore, the antibacterial assessment was carried out *in vitro* and did not replicate real-world ICU surface-disinfection circumstances. Toxicity and biofilm-associated activities were not assessed directly. Lastly, although the active chemicals and the suggested GroEL-KMgATP pathway were not experimentally confirmed, GC-MS and molecular docking offered encouraging chemical and computational evidence.

Recommendations: Standardized surface-disinfection models should be used in future studies to assess *M. communis* activity against more bacterial isolates and formed biofilms. Functional assays should be used to validate the suggested GroEL-KMgATP interaction, and the main phytochemicals, especially quinic acid and myrtenyl acetate, should be experimentally assessed separately and in combination. Before thinking about a healthcare use, it is also important to look into toxicity, stability, contact time, formulation, material compatibility, and environmental safety.

Conclusion: The results answered the research question and reported that under the experimental conditions, methanolic *M. communis* leaf extract exhibited substantially more inhibition than 2.5% glutaraldehyde and showed potential antibacterial activity against the tested *P. agglomerans* isolates. The results of molecular docking and the phytochemical profile support the idea that bioactive components may play a role in this activity. These results, however, are still preliminary *in vitro* evidence; thus, *M. communis* extract shouldn't be used in place of traditional disinfectants just yet. Its effectiveness, safety, stability, and practical usefulness must be confirmed by additional research employing more isolates and standardized surface-disinfection models.

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