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In Vitro and *In Silico* Evaluation of *Melissa officinalis* Terpenoids as Potential Antibacterial Agents Against Dental Caries-Associated Bacteria

Gulbahar Fatihollah Karim[1*], Ali Hasan Mohamed[1], Emad Jalel Ibrahem[2,3], Omeed Omar Darweesh[4], Sarah Ahmed Hasan[1]

1. *Basic Science Department, College of Nursing, University of Kirkuk, Kirkuk, Iraq*
2. *Medical Laboratory Analysis Department, College of Health Science, Cihan University, Sulaimanya, Iraq*
3. *Harem Private Hospital, Sulaimanya, Iraq*
4. *College of Pharmacy, Al-Kitab University, Kirkuk, Iraq*

ABSTRACT

Background and Aim: The growing prevalence of antibiotic-resistant oral pathogens has increased interest in medicinal plants as alternative sources of antimicrobial agents. *Melissa (M.) officinalis* L. (lemon balm) is rich in bioactive phytochemicals, particularly terpenoids, known for their antimicrobial potential. This study aimed to evaluate the antibacterial activity of *M. officinalis* extracts and isolated terpenoids against dental plaque–associated bacteria and to investigate their inhibitory mechanisms against penicillin-binding protein (PBP) and β-lactamase using *in vitro* and *in silico* approaches.

Materials and Methods: *M. officinalis* extracts and terpenoids were tested against *Streptococcus viridans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Aggregatibacter actinomycetemcomitans*. Antibacterial efficacy was assessed using inhibition zone assay, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC), with amoxicillin as a reference drug. Molecular docking was conducted to analyze the binding interactions of ursolic acid and amoxicillin with consensus-modeled PBP and β-lactamase proteins.

Results: Both crude extracts and terpenoids exhibited strong antibacterial activity, producing large inhibition zones and low MIC and MBC values as compared to amoxicillin. *S. viridans* displayed greater resistance, with MIC and MBC values approximately twice those of other bacteria. Docking analysis demonstrated strong binding affinities and high specificity for terpenoids toward β-lactamase and PBP, forming stable protein–ligand complexes.

Conclusion: *M. officinalis* terpenoids represented promising natural inhibitors of PBP and β-lactamase and may serve as alternative antimicrobial agents for controlling dental plaque–associated infections.

Keywords: β-lactamase, Dental Caries, *Melissa officinalis*, PBP, Terpenoids

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Corresponding Information: **Gulbahar Fatihollah Karim**, *College of Nursing, University of Kirkuk, Kirkuk, Iraq* & Email: kulbharkarim@uokirkuk.edu.iq

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1. Introduction

Melissa (M.) officinalis L., commonly known as lemon balm, is an aromatic medicinal plant from the *Lamiaceae* family **(1)**. It contains various phytochemical compounds such as phenolic acids, flavonoids, and terpenoids contributing to different biological activities. Previous studies indicated that the extracts of *M. officinalis* had significant inhibitory

effects against bacteria, fungi, and viruses **(2)**. It can be utilized to treat diseases of the respiratory, gastrointestinal, hepatic, and nervous systems **(3)**.

Terpenoids, the secondary plant metabolites, are normally produced by several genera of the *Lamiaceae* family, with ursane-type and oleanane-type triterpenes being abundant in the Melissa genera **(4)**. Terpenoids are natural metabolites that act as plant growth hormones, photosynthetic pigments, and antibacterial agents **(5, 6)**.

Penicillin-binding proteins (PBPs) are transpeptidase or carboxypeptidase proteins that facilitate the catalysis of the glycan chain crosslinking, thus stimulate the polymerization reaction. It is involved in the late stage of bacterial maturation that contributes to peptidoglycan assembly and maintenance **(7)**. PBP is the main target of β-lactam antibiotics, influencing cell wall synthesis and bacterial growth. Through evolution, bacteria developed resistance towards β-lactam antibiotics by generating a β-lactamase enzyme, which hydrolyzes the amide bond of the β-lactam ring. It can be categorized into 2 groups based on the type of nucleophile involved in the hydrolytic reaction, which are serine and metallo β-lactamases **(8)**. PBPs and β-lactamases are enzymes contributing to antibacterial effect and bacterial resistance development **(9)**. Thereafter, they are potent target proteins of drugs.

Dental plaque is defined as a community of diverse microorganisms that are mostly made up of bacteria existing on the teeth's surface. It is embedded in the matrix of salivary origin and bacterial polymers. Dental plaque is a type of biofilm **(10)**. There is a homeostatic balance between the microbial population and the host. The residing microbes are referred to as ecosystem stability that competes with pathogenic microbes and prevents their invasion, involving in the defence system and assisting in normal tissue development **(11)**. The species of microorganisms residing within oral cavity included *Streptococcus (S.) spp.* (e.g., *S. sanguinis, S. mitis, S. mutans, S. salivarius*), *Lactobacillus (L.)* spp., *Actinomyces (A.)* spp., *Veillonella (V.)* spp., *Neisseria (N.)* spp., *Peptostreptococcus (P.)* spp., and others **(12)**. The ecological change in the oral cavity and homeostasis disruption showed alteration in the structure of the microbial community inside the mouth, increased the abundance of pathobionts, and led to dental caries and periodontal disease **(13)**.

Traditional medicine has become increasingly used against microbial infections worldwide. Previous studies stated that the whole plant and leaves of *Melissa (M.) officinalis* L. can be employed to treat dental caries and periodontal diseases by inhibiting the disease-causing bacteria **(14, 15)**. However, the impact of terpenoids from *M. officinalis* L. on removal of dental plaque has not been explored. The current research investigated the antimicrobial activity of terpenoids extracted from *M. officinalis* L. against bacteria embedded in dental plaques. The underlying inhibitory mechanism of terpenoids from *M. officinalis* was studied by *in vitro* and *in silico* methods. For *in-silico* approaches, the conserved regions of the target proteins were firstly predicted from genomic analysis to modelling novel structures with high specificity to the desired phytochemical compounds. The good-quality novel structures predicted through this approach improved the precision of molecular docking analysis.

2. Materials and Methods

2.1 Preparation of *M. officinalis* Aqueous Extract

To prepare the aqueous extract, 50 gr of dried powder obtained from the leaves of *M. officinalis* was steeped in 100 mL of distilled water for 72 hr at room temperature. The solution was then passed through a 0.45 µm Millipore filter paper for homogenization. The filtrate was lyophilized to yield a dry extract. A 30% (w/v) solution was prepared by dissolving 3 gr of the dried extract in 10 mL of distilled water.

2.2 Terpenoids

The terpenoids were supplied by Nature Science Technologies, and the β-lactam antibiotic amoxicillin was obtained from Selleck Chemicals.

2.3 Isolation and Identification of Bacteria

Bacterial strains (*Streptococcus viridans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Aggregatibacter actinomycetemcomitans*) were obtained from periodontal samples at the Microbiology Laboratory, Harem Private Hospital, Sulaimanya City, Iraq. The strains were diagnosed employing routine microbiological procedures, and confirmed by 16S rRNA gene sequencing. They were reactivated in thioglycolate broth, subcultured on blood agar, and then incubated at 37°C for 24–72 hr, following Bergey's Manual of Determinative Bacteriology **(16)**.

2.4 Antibacterial Susceptibility Test

Amoxicillin served as the positive control, and its antibacterial activity was determined using disc diffusion technique **(17, 18)**. Bacterial isolates were first cultured in nutrient broth at 37°C for 18 hr. The suspensions were then adjusted to 0.5 McFarland standard using normal saline, corresponding to approximately 1.5×10^3 CFU/mL. A sterile cotton swab was used to evenly inoculate Mueller–Hinton agar

plates. Paper discs with a diameter of 6 mm were dipped in the antibacterial solution contained amoxicillin and placed on Mueller-Hinton agar inoculated with bacteria. The agar plates were kept at room temperature for 15-30 min to facilitate diffusion before incubating at 37°C for 24 hr. The antibacterial activity of *M. officinalis* extract and terpenoids were evaluated using the agar well diffusion method. Similarly, standardized bacterial suspensions were spread onto Mueller-Hinton agar, then, wells were created in the agar and filled with 20% (w/v) plant extract solutions. After allowing the surface to dry for 5–15 min at room temperature, plates were incubated overnight at 37°C. The inhibition zones were measured in mm against a dark background. Tests were performed in triplicate and averages were recorded **(19)**.

2.5 Statistical Analysis

Data were analyzed using GraphPad Prism (version 10.4.1) **(20)** with two-way ANOVA to evaluate differences between *M. officinalis* extracts (crude and terpenoids) and amoxicillin. A P-value <0.05 was considered significant.

2.6 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC of the antibiotic and plant extracts (crude and active components) were detected using the two-fold agar dilution method **(21)**. Serial two-fold dilutions of the crude extract, terpenoids, and amoxicillin were prepared, ranging from 2 to 512 µg/mL. As described by Ericsson and Sherris in 1971 **(22)**, *M. officinalis* extracts and amoxicillin were diluted 1:10 in liquid Mueller-Hinton agar equilibrated at 45–50°C. The agar-extract mixture was dropped onto petri dishes and left to harden at room temperature.

Bacterial inocula were prepared from 18-hr cultures, adjusted to a 0.5 McFarland standard, and diluted 1:10 in sterile saline. The agar plates were labeled for proper inoculum placement, and a 1 µL aliquot of each inoculum was spotted onto the agar surface using a standardized loop. The plates were left at room temperature for 30 min to allow for absorption of the inoculum moisture before being inverted and incubated at 37°C for 16–20 hours. The MIC was the lowest possible concentration that suppressed observable growth (ignoring single colonies or slight haze). The MBC was defined as the lowest concentration resulting in ≥99.9% reduction in viable bacterial count compared to the initial inoculum. MBC determination was performed by subculturing samples from spots showing no visible growth onto antimicrobial-free agar plates. The MICs were then evaluated against the breakpoints recommended by the NCCLS in 2002 **(23)**.

2.7 Tertiary Structure Prediction of PBP and β-Lactamase

Penicillin-binding proteins and β-lactamase were chosen as the target proteins in this study. FASTA sequences of PBP2 from *Streptococcus viridans* (A0A3S4PXI0), *Porphyromonas gingivalis* (A0AAE9XBX4), *Prevotella intermedia* (A0A2D3NB84) and *Aggregatibacter actinomycetemcomitans* (G4A9U7) as well as β-lactamase from *Streptococcus viridans* (A0A3S4MT98), *Porphyromonas gingivalis* (A0AAE9XBT4), and *Prevotella intermedia* (A0A3R7XK90) were retrieved from UniProt. Consensus sequences were generated using ClustalW in BioEdit (version 7.7.1) **(24)**.

The tertiary structures of the target proteins were predicted using AlphaFold Colab **(25)**. The predicted models underwent structural validation by SAVES 6.1 webserver, which provided PROCHECK **(26)**, ERRAT **(27)** and Verify3D **(28)** analyses. Then, structural refinement was carried out using GalaxyRefine **(29)** and UCSF Chimera **(30)**.

2.8 Molecular Docking

Docking was conducted using AutoDock Vina (version 1.1.2) **(31)** to determine binding affinities of terpenoid (ursolic acid) and amoxicillin to PBP and β-lactamase. The SMILE files of the ligands, specifically ursolic acid (ID: 64945), and amoxicillin (ID: 33613) were retrieved from the PubChem database. UCSF Chimera version 1.17.3 was employed to build and optimize the structures of the ligands. The PDB files of the target proteins (PBP and β-lactamase) and ligands (ursolic acid and amoxicillin) were converted into PDBQT format using AutoDockTools 1.5.7 for docking preparation **(32)**. Grid boxes were set for PBP (35×35×35 Å, centered at-17.2029, 3.9904, 9.7476) and β-lactamase (40×45×50 Å, centered at 6.59, 1.41, -2.38). The grid box was designed based on the location and size of the target protein active or binding site. Exhaustiveness was set to 8, and 1000 modes were evaluated. The lowest binding energy conformations were visualized using PyMOL (version 3.0) **(33)** and Discovery Studio Visualizer **(34)**.

3. Results

3.1 Antibacterial Susceptibility Test Results

The bacteria found in the patient with dental carris were identified as *Streptococcus viridans, Porphyromonas gingivalis, Prevotella intermedia,* and *Aggregatibacter actinomycetemcomitans*. The results of the antibacterial susceptibility test (Figure 1) revealed

that the extracts and terpenoids of *M. officinalis* significantly inhibited all bacteria compared to amoxicillin, the antibiotic used. Across all tested bacterial strains, *M. officinalis* consistently produced the largest inhibition zones, followed by the terpenoids, while amoxicillin showed comparatively smaller zones. *Streptococcus viridans* was less sensitive to *M. officinalis* extracts, isolated terpenoids, and amoxicillin due to smaller inhibition zones formed. For *S. viridans*, inhibition zones were 29.33 ± 0.58 mm (extract), 27.67 ± 0.58 mm (terpenoid), and 10.00 ± 0.00 mm (amoxicillin). For *P. gingivalis*, the extract demonstrated the highest activity (35.33 ± 0.58 mm), compared with terpenoid (30.67 ± 0.58 mm) and amoxicillin (16.67 ± 0.58 mm). Similarly, for *P. intermedia*, inhibition zones were 36.33 ± 0.58 mm for *M. officinalis* extract, 30.33 ± 0.58 mm for terpenoid, and 15.67 ± 0.58 mm for amoxicillin. In the case of *A. actinomycetemcomitans*, the extract again showed the strongest antibacterial effect (34.67 ± 0.58 mm), followed by terpenoid (30.33 ± 0.58 mm), whereas amoxicillin produced a smaller inhibition zone of 15.00 ± 0.00 mm.

A two-way ANOVA analysis demonstrated that treatment, bacterial species, and their interaction significantly affected the measured response (P<0.0001), which was the inhibition zones. Treatment was the predominant source of variation, accounting for 93.01% of the total variance ($F(2,24) = 4598$), followed by bacterial species (6.03%; $F(3,24) = 198.7$). The significant interaction effect ($F(6,24) = 11.77$, P<0.0001) indicated that treatment efficacy differed among bacterial species. *Post-hoc* analysis using Dunnett's multiple comparisons test revealed that both *M. officinalis* extracts and terpenoids produced significantly greater effects compared to amoxicillin across all tested species, all adjusted P<0.0001. The mean differences ranged from −14.00 to −20.67, with *M. officinalis* extracts consistently showing the largest differences relative to amoxicillin. Collectively, these findings confirmed that treatment type strongly influences the response, with plant-derived treatments demonstrating significantly enhanced activity compared to the control across all bacterial species tested.

The effects of *M. officinalis* extracts, isolated terpenoids, and amoxicillin on the bacterial isolates were further investigated determining the MIC and MBC. Based on Table 1, the MBCs of *M. officinalis* extracts and isolated terpenoids on all identified bacteria were four times the value of their MICs whereas the MBCs of amoxicillin were two times their MIC values. The MIC of *M. officinalis* extract on *Streptococcus viridans* was 32 µg/mL and the MICs of the extracts on other bacteria were 16 µg/mL. The MICs of terpenoids and amoxicillin were two-fold and eight-fold the MICs of *M. officinalis* extract, respectively.

3.2 Phylogenetic Analysis

The conserved region of PBP and β-lactamase were predicted through genomic analyses. To construct the consensus sequence for PBP, sequences from *Streptococcus viridans, Porphyromonas gingivalis, Prevotella intermedia,* and *Aggregatibacter actinomycetemcomitans* were aligned. Similarly, the β-lactamase consensus sequence was derived from alignments of *S. viridans, P. gingivalis,* and *P. intermedia* (Figure 2). The β-lactamase sequence of *A. actinomycetemcomitans* was not found in the UniProt database. Phylogenetic analysis revealed evolutionary relationships among these species. *Porphyromonas gingivalis* and *Prevotella intermedia* were closely related, while *A. actinomycetemcomitans* was more distantly associated with this cluster. *Streptococcus viridans* exhibited the greatest divergence, with branch lengths of 1.25 for PBP and 1.62 for β-lactamase, indicating significant sequence differences.

3.3 Tertiary Structure Prediction

The consensus sequences predicted from genomic analysis for both target proteins were utilized for protein modeling via AlphaFold2. The modeled structures were then validated by PROCHECK and additional analyses (ERRAT and VERIFY3D). The validation results of the refined PBP structure were 92.51% (ERRAT), 79.58% (VERIFY3D) and 94.0% (PROCHECK). The validation results of the predicted β-lactamase structure were 93.71% (ERRAT), 81.60% (VERIFY3D), and 96.1% (PROCHECK). PBP consisted of 15 β-strands and 23 α-helices, and β-lactamase contained 11 β-strands and 13 α-helices. An antiparallel β-sheet surrounded by α-helices was found in the structure of PBP and β-lactamase (Figure 3).

3.4 Molecular Docking

The docking analyses between target proteins (PBP and β-lactamase) and ligands (ursolic acid and amoxicillin) were performed to determine the binding affinities. Figure 4 and Table 2 demonstrated the molecular interactions between proteins and ligands. The results indicated that the interactions and binding affinities of terpenoids (oleanolic and ursolic acids) with both target proteins were better than those of amoxicillin. The docking of PBP and β-lactamase with amoxicillin resulted in -6.7 kcal/mol of binding affinity, with 3 interactions for PBP and 8 interactions for β-lactamase. For PBP, 3 hydrogen bonds were formed between amoxicillin and Asn293, Thr422 and Gly453. For the β-lactamase-amoxicillin complex, a salt bridge and a hydrogen bond were formed with Glu137 and Asn191. A pi-sulfur interaction was generated within amoxicillin structures. There were 5 hydrophobic interactions with Tyr189, Phe348, Leu349, and Trp251 found in the complex of β-lactamase with amoxicillin.

The binding affinities increased significantly when the target proteins were docked with ursolic acid. For PBP,

the ursolic acid was docked at the active site with a binding affinity of -7.9 kcal/mol, involving 3 hydrogen bonds with Ser251, Ser 291 and Thr422. Additionally, 3 hydrophobic interactions were observed between ursolic acid and Ala454 and Phe452. The binding affinity of β-lactamase with ursolic acid was -9.6 kcal/mol. In the docking between β-lactamase and ursolic acid, 2 hydrogen bonds were formed with Asn138 and Asn191. 11 hydrophobic interactions that bound to Phe348, Leu349, Phe147, Tyr189, and Trp251 existed within β-lactamase-ursolic acid complex.

Table 1. Determination of MIC and MBC (µg/mL) for *M. officinalis* extracts, isolated terpenoids, and amoxicillin.

Bacteria	Concentration of MIC (µg/mL)			Concentration of MBC (µg/mL)		
	M. officinalis extract	Terpenoids	Amoxicillin	*M. officinalis* extract	Terpenoids	Amoxicillin
Streptococcus viridans	32	64	256	128	256	512
Porphyromonas gingivalis	16	32	128	64	128	256
Prevotella intermedia	16	32	128	64	128	256
Aggregatibacter actinomycetemcomitans	16	32	128	64	128	256

Table 2. Molecular docking interactions of PBP and β-lactamase with amoxicillin and ursolic acid.

Protein	Ligand	Binding Affinity (kcal/mol)	Bond Types	From	To	Distance (Å)
PBP	Amoxicillin	-6.7	Hydrogen Bond	Asn293	Amoxicillin	2.59
			Hydrogen Bond	Thr422	Amoxicillin	2.72
			Hydrogen Bond	Gly453	Amoxicillin	2.18
PBP	Ursolic acid	-7.9	Hydrogen Bond	Ser251	Ursolic acid	2.08
			Hydrogen Bond	Ser291	Ursolic acid	2.44
			Hydrogen Bond	Thr422	Ursolic acid	2.36
			Alkyl Hydrophobic	Ala454	Ursolic acid	3.51
			Pi-Alkyl Hydrophobic	Phe452	Ursolic acid	5.33
			Pi-Alkyl Hydrophobic	Phe452	Ursolic acid	4.59
β-lactamase	Amoxicillin	-6.7	Salt Bridge	Amoxicillin	Glu137	2.26
			Hydrogen Bond	Asn191	Amoxicillin	2.68
			Pi-Sulfur	Amoxicillin	Amoxicillin	5.38
			Pi-Pi Stacked Hydrophobic	Amoxicillin	Tyr189	4.30
			Pi-Pi Stacked Hydrophobic	Amoxicillin	Phe348	4.99
			Alkyl Hydrophobic	Amoxicillin	Leu349	4.47
			Pi-Alkyl Hydrophobic	Trp251	Amoxicillin	5.08
			Pi-Alkyl Hydrophobic	Phe348	Amoxicillin	5.28
β-lactamase	Ursolic acid	-9.6	Hydrogen Bond	Asn138	Ursolic acid	2.18

Protein	Ligand	Binding Affinity (kcal/mol)	Bond Types	From	To	Distance (Å)
			Hydrogen Bond	Asn191	Ursolic acid	2.45
			Pi-Sigma Hydrophobic	Ursolic acid	Phe348	3.99
			Alkyl Hydrophobic	Ursolic acid	Leu349	4.30
			Alkyl Hydrophobic	Ursolic acid	Leu349	3.82
			Pi-Alkyl Hydrophobic	Phe147	Ursolic acid	5.04
			Pi-Alkyl Hydrophobic	Tyr189	Ursolic acid	4.55
			Pi-Alkyl Hydrophobic	Tyr189	Ursolic acid	4.19
			Pi-Alkyl Hydrophobic	Trp251	Ursolic acid	5.20
			Pi-Alkyl Hydrophobic	Phe348	Ursolic acid	5.17
			Pi-Alkyl Hydrophobic	Phe348	Ursolic acid	4.89
			Pi-Alkyl Hydrophobic	Phe348	Ursolic acid	4.82
			Pi-Alkyl Hydrophobic	Phe348	Ursolic acid	5.18

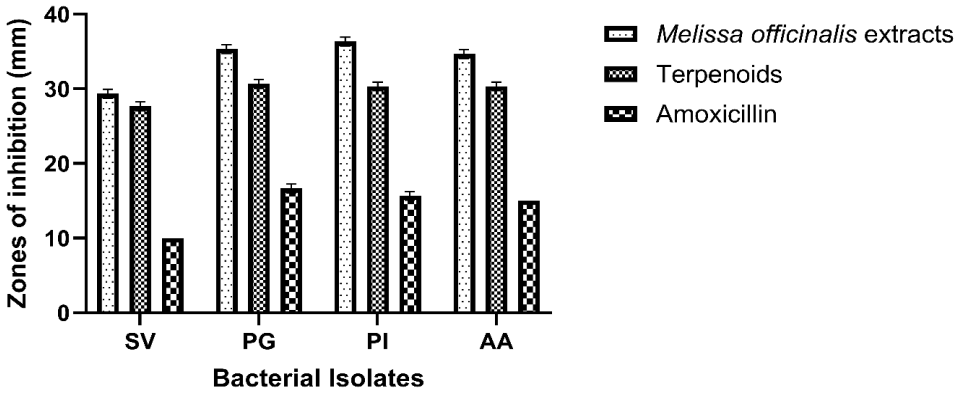
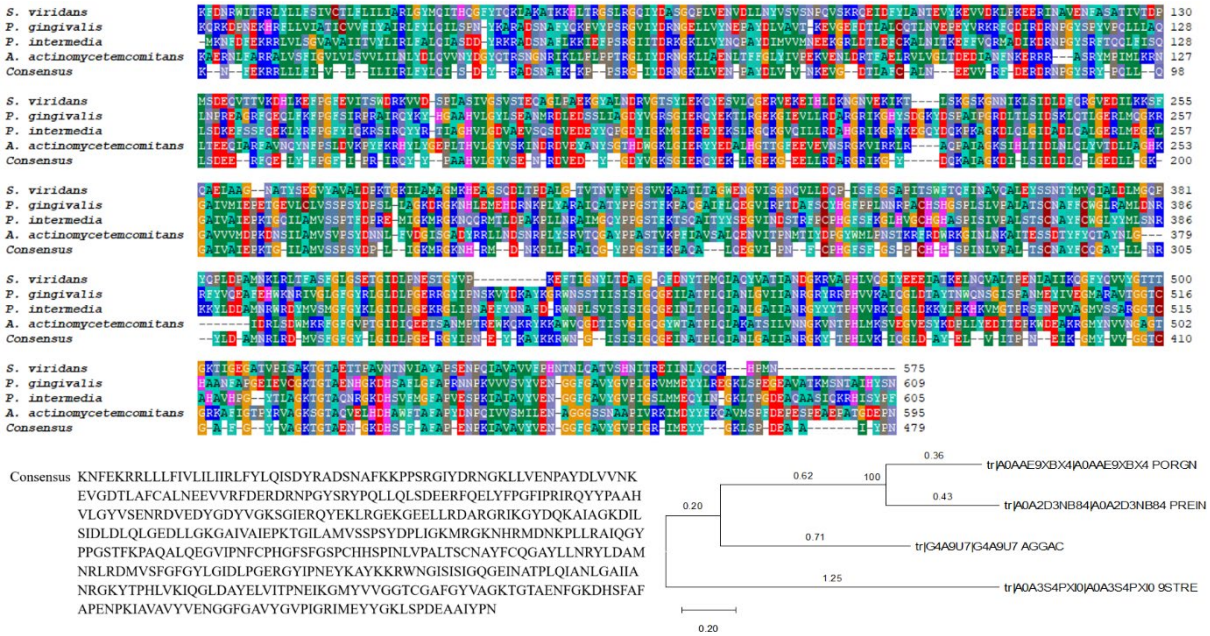


Figure 1. Antibacterial activity results of *M. officinalis* extracts, isolated terpenoids, and amoxicillin against bacteria. (SV: *Streptococcus viridans*, PG: *Porphyromonas gingivalis*, PI: *Prevotella intermedia* and AA: *Aggregatibacter actinomycetemcomitans*) **(Prepared by Authors, 2025)**.

(a)



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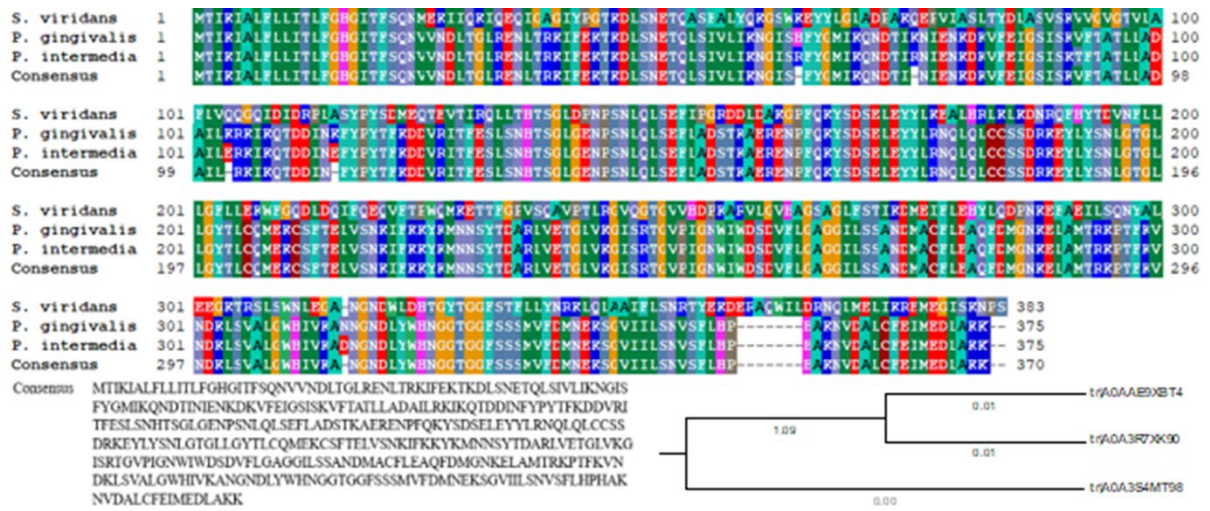


Figure 2. Phylogenetic trees and predicted consensus sequences of target proteins across the bacteria isolates. (a) PBP, and (b) β-lactamase **(Prepared by Authors, 2025)**.

(a) (b)

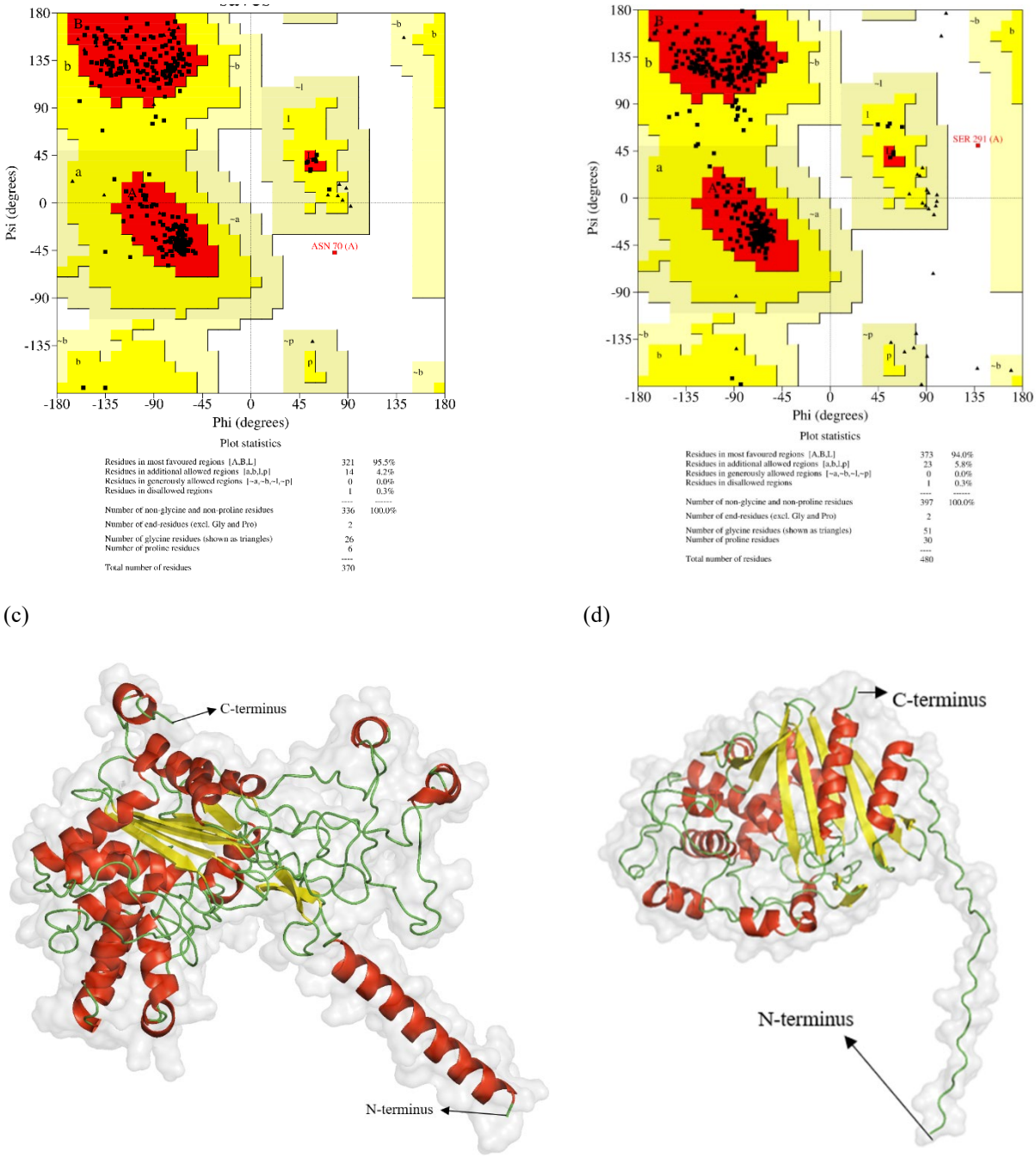
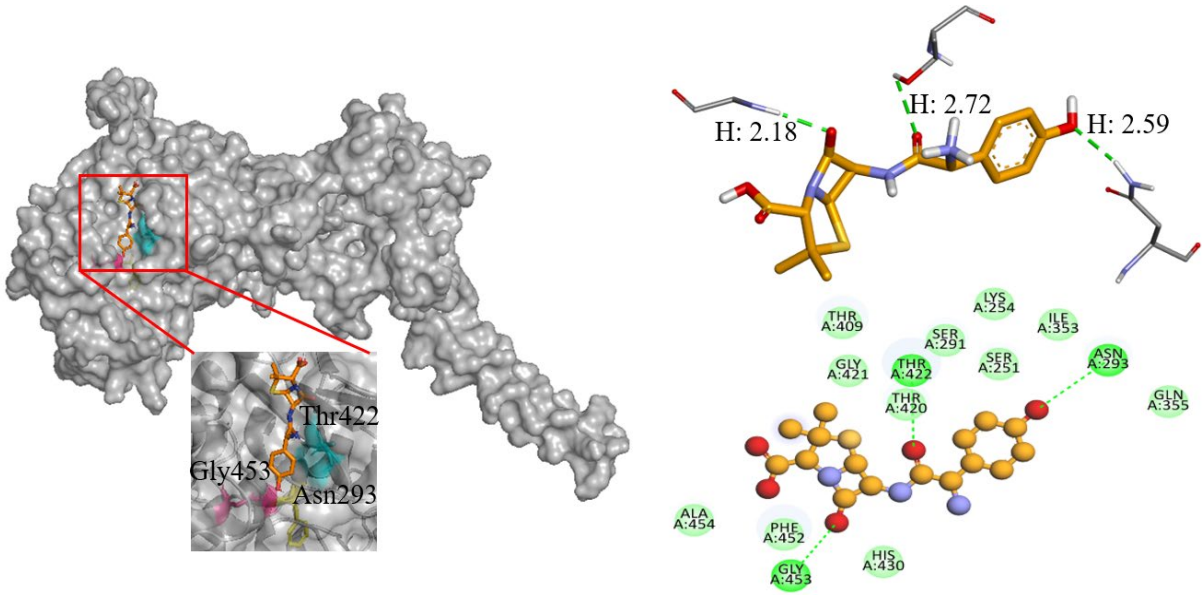


Figure 3. PROCHECK results and structures of the target proteins. (a) Ramachandran plot of PBP, (b) Ramachandran plot of β-lactamase, (c) Tertiary structure of PBP, and (d) Tertiary structure of β-lactamase **(Prepared by Authors, 2025)**.

(a)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Gly453
Asn293
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H: 2.72
H: 2.59
THR A:409
LYS A:254
ILE A:353
SER A:291
GLY A:421
THR A:422
SER A:251
ASN A:293
GLN A:355
THR A:420
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PHE A:452
HIS A:430
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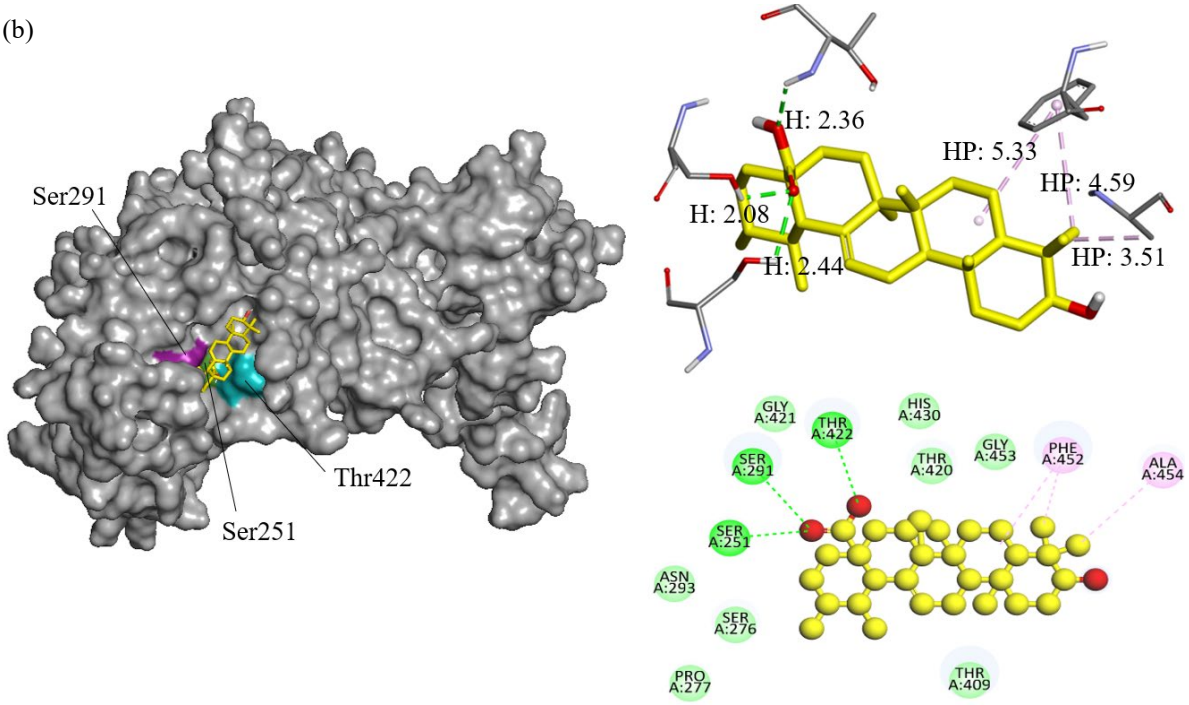
(b)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Ser251
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HP: 4.59
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H: 2.44
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THR A:422
HIS A:430
SER A:291
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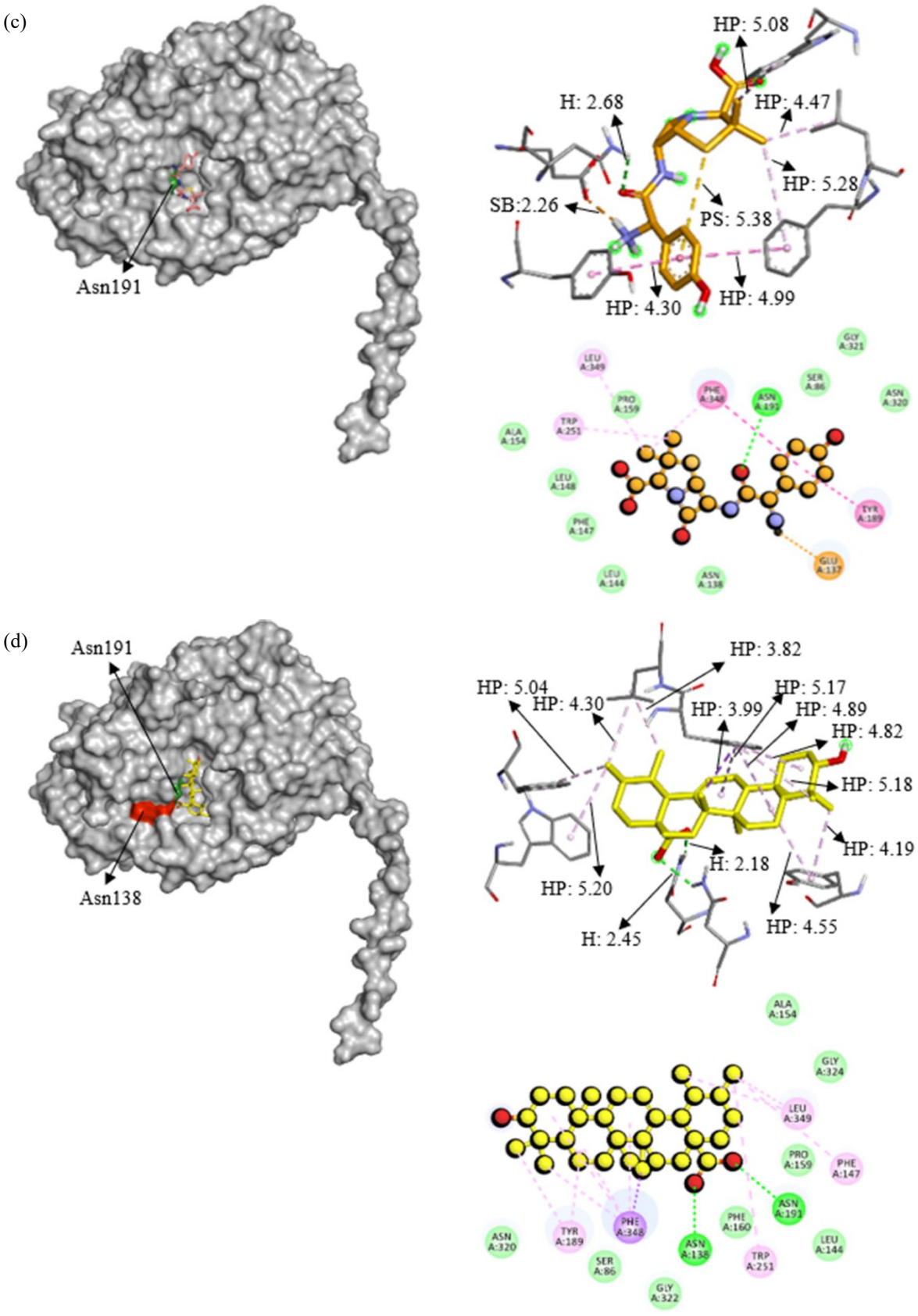
(c)
Asn191
HP: 5.08
H: 2.68
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SB:2.26
PS: 5.38
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HP: 4.99
(d)
Asn191
Asn138
HP: 3.82
HP: 5.04
HP: 4.30
HP: 5.17
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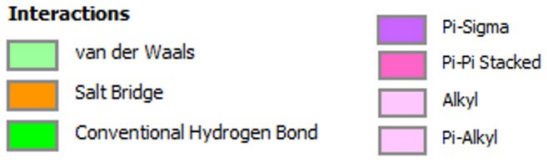

Figure 4. Molecular surface view of protein-ligand dockings. (a) PBP-Amoxicillin, (b) PBP-Ursolic acid, (c) β-lactamase-Amoxicillin, and (d) β-lactamase-Ursolic acid. (Left panel) (The surfaces of PBP and β-lactamase are shown in grey colour, while the amoxicillin and ursolic acid are shown in orange and yellow stick forms, respectively). 3D and 2D interactions with hydrogen bonds and other non-bonded interactions. (Right panel) (H: hydrogen bond; HP: hydrophobic interaction; SB: salt bridge; PS: Pi-sulfur) **(Prepared by Authors, 2025)**.

4. Discussion

Recent studies have highlighted the widespread of multidrug resistant bacteria **(35)**, and misuse of antibiotics, underscoring the necessity for alternative antimicrobial strategies **(36, 37)**. The antibacterial capacity of a neodymium-doped yttrium aluminum (Nd:YAG) has been evaluated **(38)**. The antibacterial potential of *M. officinalis* L. extract was investigated against dental plaque-causing bacteria. Based on the findings, the crude extract exhibited the strongest inhibitory effect, indicated by larger inhibition zones and lower MIC and MBC values, followed by terpenoids and amoxicillin. However, all treatments exhibited only moderate effectiveness against *Streptococcus viridans*. While typically harmless in the oral cavity, these bacteria can cause severe infections like tonsillitis **(39)**, bacteremia, and infective endocarditis (IE) when they enter sterile sites such as the bloodstream or dental pulp. Amoxicillin-resistant oral streptococci have been detected in 21.3% of children's dental plaque samples, with *S. mitis* and *S. sanguinis* exhibiting MIC values up to 128 µg/mL, particularly concerning for patients at risk for IE **(40)**. These resistant strains often show cross-resistance to other antibiotics like clindamycin, erythromycin, and azithromycin.

Although aqueous extraction of *M. officinalis* primarily yields polar phytochemicals such as phenolic acids (e.g., rosmarinic acid), flavonoid glycosides, and water-soluble triterpene glycoside **(40, 41)**, our mechanistic focus centered on terpenoid-derived bioactivity for several strategic reasons. Terpenoids exhibit antibacterial mechanisms distinct from phenolics, targeting bacterial cell wall synthesis through peptidoglycan inhibition and membrane disruption rather than exerting antioxidant effects **(4, 42)**. Importantly, pentacyclic triterpenes and their glycosides demonstrate strong anti-biofilm activity **(43)**, which is particularly relevant for dental plaque control, as biofilm-associated bacteria are more resistant to conventional antimicrobials.

Our molecular docking studies therefore targeted penicillin-binding protein (PBP) and β-lactamase, enzymes known to interact with terpenoid structures such as ursolic acid (UA) and oleanolic acid (OA). UA and OA are the principal triterpenes present in *M. officinalis* extracts and are well-known for their antibacterial properties **(44)**. UA was selected as the ligand for molecular docking based on comparative studies showing that it equals or outperforms structurally related triterpenes, including oleanolic and betulinic acids, against oral pathogens **(45)**. UA demonstrates low MIC values and consistent activity against cariogenic bacteria and has proven efficacy against key periodontopathogens, including *P. gingivalis* and *P. intermedia*, as well as multiple biofilm-forming streptococci, highlighting its relevance to dental plaque control **(46)**.

The active site of PBP contains three highly conserved motifs that are necessary for the catalytic reaction of the enzyme. The SXXK motif includes a key serine residue that acts as a nucleophile, enabling covalent interaction with β-lactam antibiotics and inhibiting PBP activity. The (S/Y)XN and (K/H)(S/T)G motifs help stabilize the transition state and substrate binding **(47)**. These motifs are structurally conserved across PBPs, with minimal spatial variation (<1 Å), underscoring their functional importance **(48)**. Notably, similar conserved motifs of PBP were shared by class A and C β-lactamase **(49)**. The SXXK and (K/H)(S/T)G motifs were observed in the β-lactamase sequence, revealing the predicted beta-lactamase belonged to serine β-lactamase that contained 1 α subdomain and 1 α/β subdomain. The active site of the serine β-lactamase was located at the cleft between two subdomains, consistent with the structural organization observed in evolutionarily related PBPs **(50, 51)**. In this study, consensus sequences for PBP and β-lactamase were constructed using a 50% identity threshold from four bacterial species, leveraging evolutionary data to predict stable, functional proteins **(52, 53)**.

The docking analysis revealed that UA and amoxicillin all bound effectively to the PBP active site. UA demonstrated the strongest binding affinity with PBP (-7.9 kcal/mol), forming hydrogen bonds with key residues like Ser251 (motif 1), Ser291 and Asn293

(motif 2), and Thr420 (motif 3). In β-lactamase docking, the ligands bound to the active site but only formed Van der Waals interactions with the catalytic serine (Ser86). UA had the lowest binding affinity (−9.6 kcal/mol) with β-lactamase. Both dockings of UA with target proteins outperformed amoxicillin. UA also formed a higher number of interactions including hydrogen bonds and hydrophobic interactions, contributing to the stability of the ligand-protein complexes **(54)**. Strong interactions formed by terpenoids with target proteins reinforced the protein stability and enhanced the inhibitory effect of terpenoids on the target proteins **(55)**. The favorable protein-ligand interactions suggest the potential antibacterial effect of terpenoids by targeting β-lactamase and PBP.

Comparable findings have been reported in previous *in silico* studies. For example, Nwokebu et al **(56)** conducted a virtual screening of phytochemicals against β-lactamase, determining their potent inhibitory effect on the protein. It demonstrated that the most promising inhibitor, quercetin-3-O-rutinoside, had -9.4 kcal/mol binding affinity with β-lactamase. Another study discovered a natural antibacterial agent, the propolis Neoflavanoide 1 which had -7.0 kcal/mol binding affinity with PBP1. The binding affinities described above were greater than the results obtained in the current work, supporting the potential of terpenoids as the inhibitors of PBP and β-lactamase. In terms of specificity, β-lactamase was the main target of terpenoids and PBP was the subsequent target. When terpenoids bound to the PBP, the transpeptidation reaction involved in the terminal step of cell wall formation was suppressed, forming a weakened cell wall. Finally, the bacterial cell was lysed due to greater osmotic pressure **(57)**. The terpenoids from *M. officinalis* L. bound to the active site of β-lactamase, preventing hydrolysis of the amide bond of the β-lactam ring and decreasing the bacterial resistance towards β-lactam antibiotics **(58)**.

The terpenoids also synergize with β-lactam antibiotics like ampicillin, and oxacillin, reducing their MIC values. UA and OA exhibited synergistic activity with ampicillin against MRSA by disrupting membrane integrity and reducing β-lactamase activity **(59)**. Apart from that, UA combat oral biofilms by preventing their formation and exhibiting bactericidal activity against existing colonies **(44)**. UA effectively inhibits biofilm formation of cariogenic microorganisms such as *S. mutans* and *S. gordonii* by targeting glucosyltransferases and reducing biofilm viability **(60)**. Importantly, UA showed little toxicity to human buccal epithelial cells, suggesting their potential as therapeutic agents in dental care **(47)**.

This study has several limitations that should be acknowledged. First, comprehensive phytochemical characterization (e.g., HPLC or LC-MS) of the aqueous extract was not performed. Second, molecular docking was conducted using free ursolic acid as a representative terpenoid, whereas the aqueous extract likely contains predominantly glycosylated derivatives. Further investigations are recommended to explore the precise mechanisms through which terpenoids or triterpene glycosides inhibit penicillin-binding proteins (PBP) and β-lactamase, utilizing molecular dynamics simulations. It is also recommended to conduct enzyme inhibition assay to confirm the results of molecular docking. Further investigations should also assess the pharmacokinetics and safety profiles of terpenoids like UA when used at therapeutic levels. Understanding their ADMET (absorption, distribution, metabolism, and excretion) in the body will be crucial for their development as therapeutic agents. Third, we tested bacterial isolates growing as free-floating, individual cells, which do not fully represent the complexity of multi-species oral biofilms. Future studies should assess anti-biofilm efficacy using relevant biofilm models to improve clinical relevance.

Additionally, although the crude extract demonstrated greater activity than the purified terpenoid fraction, potential synergistic interactions were not systematically investigated. Future studies employing bioassay-guided fractionation and combination testing could elucidate the specific synergistic interactions and inform development of optimized formulations. Additionally, expanding research to test antimicrobial effects of terpenoids against a broader range of bacterial strains may uncover their potential as broad-spectrum agents. Another important direction is optimizing terpenoid formulations for oral care products to enhance stability, bioavailability, and patient compliance, facilitating their transition from lab research to practical applications.

5. Conclusion

This study evaluated the antibacterial properties of terpenoids extracted from *M. officinalis* L. and their efficacy towards dental-plaque causing bacteria. The results indicated that natural terpenoids like ursolic acid are promising antimicrobial agents, primarily by inhibiting PBP and β-lactamase activity. These natural compounds could play a key role in developing novel antimicrobial agents sourced from plants. There should be focus on clinical trials, pharmacokinetic optimization, and eco-friendly synthesis of terpenoid derivatives to harness their full potential in combating pathogenic dental plaque bacteria.

6. Declarations

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6.2 Ethical Considerations

This article contains no experiments involving human beings or animals conducted by any of the contributors.

6.3 Authors' Contributions

Gulbahar F. Karim: Conceptualization, study design, experiment execution, data analysis, manuscript writing, and final approval. Ali Hasan Mohamed: Experiment execution, data analysis, manuscript writing, and final approval. Emad J. Ibrahem: Experiment execution, and final approval. Omeed Darweesh: Data analysis, and the final approval. All authors reviewed, edited, and approved the final version of the manuscript.

6.4 Conflict of Interests

The authors declare no conflict of interest in the content of this article.

6.5 Financial Support and Sponsorship

This study received no particular grants from public, commercial, or non-profit funding entities.

6.6 Using Artificial Intelligence Tools (AI Tools)

All authors declare that there is no use of AI Tools in this study, including the writing of this manuscript.

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