

Decoding the Therapeutic Potential of *Agave Americana* Via In Vitro Bioactivity Evaluation and in Silico Molecular Docking Against Oral Pathogens

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Abstract

Objective: The increasing prevalence of antimicrobial-resistant oral pathogens has created an urgent need for alternative therapeutic agents. This study evaluated the antibacterial activity of *Agave americana* leaf extracts against clinically isolated oral pathogens and assessed their potential as a natural antimicrobial for oral healthcare.

Methods: A total of 150 clinical specimens from dental patients were microbiologically analyzed. Bacterial isolates were identified using culture, Gram staining, biochemical tests, and automated confirmation. Representative *Staphylococcus* and *Streptococcus* isolates were selected for further investigation. Antimicrobial susceptibility was determined by the Kirby–Bauer disk diffusion method. Methanol, ethanol, and ethyl acetate leaf extracts (25%, 50%, and 75%) were evaluated using agar diffusion and broth microdilution assays. Synergistic effects with antibiotics, GC–MS phytochemical profiling, cytotoxicity on HEK-293 cells, and molecular docking analyses were also performed.

Results: Ten multidrug-resistant *Staphylococcus* and *Streptococcus* isolates were selected. Carbapenems, particularly imipenem and meropenem, showed the greatest antibacterial activity, whereas resistance to cephalosporins was common. The 75% ethyl acetate extract demonstrated the strongest antibacterial effect, with inhibition zones up to 33 mm and MIC values ranging from 0.125 to 1.000 mg/mL. The extract enhanced the activity of carbapenems and doxycycline, indicating synergistic interactions. GC–MS identified 4H-pyran-4-one (40.30%) and 2H-pyran-2-one (32.26%) as the major compounds. Cell viability exceeded 97%, and molecular docking showed favorable binding of these compounds to sortase A (up to -5.4 kcal/mol).

Conclusion: *Agave americana* leaf extract demonstrated potent antibacterial, synergistic, and biocompatible properties, supporting its potential as a natural therapeutic candidate for preventing and managing multidrug-resistant oral infections.

Keywords: *Agave americana*, Oral pathogens, antimicrobial activity, Dental caries, Medicinal plants, Disk diffusion, MIC.

Introduction

The oral cavity hosts a highly diverse microbial ecosystem, comprising more than 700 naturally occurring bacterial species. Among these, the genera *Streptococcus* and *Staphylococcus* are among the most prominent colonizers. *Streptococcus* species including *S. mitis*, *S. sanguinis*, *S. salivarius*, and *S. anginosus* are dominant members of the oral microbiota, while *Staphylococcus* species, such as *S. aureus*, *S. epidermidis*, and *S. hominis*, are also frequently isolated from oral tissues and associated with opportunistic infections. (Becker et al., 2014; Lowy, 1998; Ryan & Ray, 2004; Tong et al., 2015). Certain *Streptococci* exhibit hemolytic properties (e.g., α -hemolytic and β -hemolytic groups), which contribute to their pathogenic potential, while other oral bacteria demonstrate metabolic capabilities such as xylose fermentation (e.g., *Staphylococcus xylosus*), reflecting their adaptability within oral niches. These microorganisms possess the ability to colonize the gingival crevices and tooth surfaces, forming structured biofilms that contribute to dental plaque development. Their metabolic activity, particularly acid production, leads to the demineralization of enamel, ultimately causing dental caries and other oral infections.

The diagnosis of oral infections is primarily clinical, with antibiotics often prescribed empirically for treatment or prophylaxis. However, the characterization of causative organisms, along with definitive diagnosis and antimicrobial susceptibility testing, is critical for guiding appropriate therapeutic strategies and preventing disease progression. Oral bacterial infections significantly contribute to the global burden of dental diseases, particularly dental caries, which affects approximately 2.5 billion people worldwide and has shown a 14.6% increase over the past decade. Recent literature reviews have reported that the global prevalence of dental caries is 46.2% in primary dentition and 53.8% in permanent dentition, with lower prevalence in European populations and higher prevalence in African regions (Rajasekaran et al., 2024; Spatafora et al., 2024; Kazeminia et al., 2020; Frencken et al., 2017).

Periodontal diseases also represent a significant public health concern. In 2019, Western Sub-Saharan Africa experienced the highest burden of periodontitis, with Gambia reporting the greatest disease prevalence. The burden of periodontitis has been shown to correlate negatively with socioeconomic development. Although the highest prevalence occurs in individuals aged 55–59 years, recent studies indicate a rising incidence among younger populations, suggesting a changing epidemiological trend (Wu et al., 2022).

The oral microbiota consists largely of opportunistic organisms that can become pathogenic when there is a disruption in host–microbe interactions. In the early stages of infection, the microbial profile reflects the normal oral flora; however, as the infection progresses, anaerobic species tend to predominate, leading to more severe tissue destruction and inflammation (Li et al., 2022; Rajasekaran et al., 2024). In addition to causing localized infections in oral soft tissues and bone, these pathogens may contribute to broader health complications.

Given the limitations associated with conventional antibiotic therapy, including resistance, adverse effects, and lack of specificity, there is an increasing need to explore alternative therapeutic options. Natural products, particularly plant-derived compounds, have gained considerable attention due to their safety, accessibility, and reduced side effects. Several studies have investigated the use of natural household remedies, including essential oils and herbal preparations, in the management of oral conditions such as toothache and gingival inflammation (Anwar et al., 2025; Gloria-Garza et al., 2025; Hoja et al., 2025). More recent scientific investigations have demonstrated the antibacterial potential of extracts derived from medicinal plants, highlighting their possible application in clinical settings for the prevention and treatment of oral diseases (Gloria-Garza et al., 2025; Anwar et al., 2025).

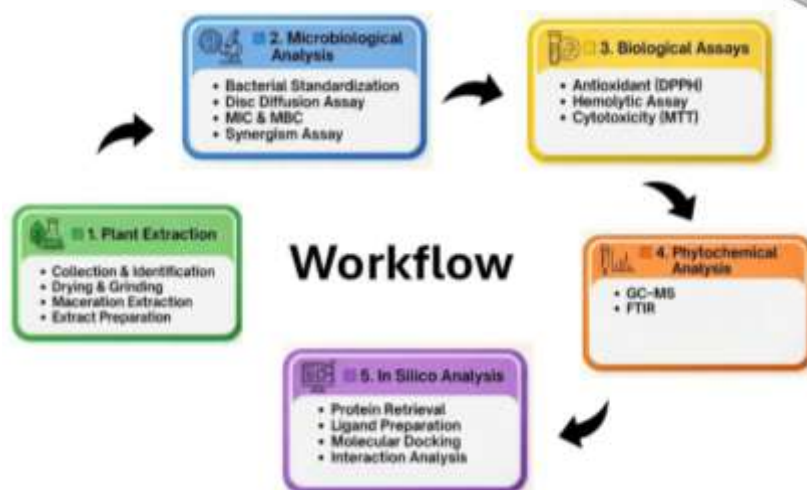
Staphylococcus species, in particular, have become increasingly of interest in the field of oral pathogens due to their capacity to be opportunistic colonizers and to play a role in oral infections. Staphylococcus aureus may promote the complexity of biofilms and their resistance to antimicrobial therapy in the dental biofilm, particularly in dental pockets, and thus is frequently found in saliva and dental plaque (Loberto et al., 2004). Furthermore, oral mucosal infections and implant-associated biofilms have been linked to the presence of *S. epidermidis* and other coagulase-negative staphylococci, which has since shown to be very adaptive in the oral cavity (Olsen et al., 2012). The presence of these species in oral biofilms underscores the clinical relevance of these species as a target for alternative antimicrobial therapies.

Agave americana (commonly known as the century plant or American aloe) is a medicinal plant valued for its diverse bioactive compounds, which exhibit various physiological and pharmacological effects. It has historically played a significant role in maintaining and improving human health and quality of life (Ponmadasamy et al., 2026; Nigussie, 2024). The plant contains important phytoconstituents, including steroidal saponins, isoflavones, and coumarins, which are associated with multiple therapeutic properties.

Despite the growing interest in plant-based therapeutics, many species remain insufficiently explored. *A. americana* has been traditionally used to treat a wide range of conditions, and its pharmacological potential has been supported by previous experimental studies (Ponmadasamy et al., 2026; Kablan et al., 2024; Shegute et al., 2020; González-Cortazar et al., 2022; Monterrosas-Brisson et al., 2013). Given its rich phytochemical profile and reported medicinal properties, it represents a promising candidate for further investigation.

Therefore, the present study aims to evaluate the antimicrobial effects of Agave americana extract against oral pathogens, with the objective of identifying safe, effective, and naturally derived alternatives for the prevention and management of dental infections as shown in figure (1).

Figure 1: Overview of the multi-stage research methodology: extraction, microbiological/biological assays, phytochemical profiling, and molecular docking studies



Materials and Methods

Sample Collection and bacterial isolation

A total of 150 clinical samples were collected from patients attending private dental clinics. All sample collections were conducted under the supervision of a specialist dentist to ensure accurate identification and verification of oral pathological conditions. The sampling process was additionally overseen by Dr. Jabir Faez Al-Ibrahimi, Specialist in operative and esthetics. The study was approved by the relevant ethical committees in Iraq, University of Kufa, College of Medicine, under letter number 4147 dated 2024, and was conducted under the supervision and approval of Ain Shams University, Cairo, Faculty of Science, under letter number [code: Asu-Sci/MIR/2024/11/2]. Dentistry, College of Dentistry, University of Kufa, Iraq, to guarantee proper diagnostic assessment and adherence to clinical standards. The ethical committee approved the sampling of the isolates.

Sterile dental instruments, including spoon excavators, bond brushes, and periodontal probes, were used for sample acquisition. Each sample was immediately transferred into sterile nutrient broth (HiMedia, India) in a 1 mL Eppendorf tube to allow the growth of the bacterial isolates. The inoculated tubes were then incubated at 37°C for 24 hours. Bacterial isolates were recovered from various clinical conditions of the oral cavity, including gingival infections, dental caries, root canal infections, purulent root canal lesions, and saliva samples.

Bacterial Identification

Cultural Identification

Following incubation in nutrient broth, each sample was streaked onto three selective and differential culture media including Mannitol Salt Agar was used as a selective medium to support the growth of salt-tolerant bacteria and to differentiate mannitol-fermenting organisms based on a visible color change of the medium (Forbes et al., 2016). MacConkey Agar was applied for the selective isolation of Gram-negative bacteria and for distinguishing lactose fermenters from non-lactose fermenters according to colony coloration (Winn et al., 2017). In addition, Mitis Salivarius Agar was utilized for the isolation of oral streptococci, allowing differentiation of species based on their characteristic colony morphology (Forbes et al., 2016; Facklam, 2002). colony morphology. All inoculated media were incubated at 37°C for 24–48 hours, after which colony appearance and growth characteristics were carefully observed and recorded (all from HiMedia, India). The plates were incubated at 37°C for 24 hours.

Samples that failed to grow on Mannitol Salt Agar were subsequently cultured on Blood Agar (Forbes et al., 2016) to enhance bacterial recovery. Additionally, isolates originally grown on Mitis Salivarius Agar were transferred to nutrient agar plates for preservation, as the Mitis medium serves primarily as a diagnostic rather than a maintenance medium.

Microscopic identification

The use of Gram staining for microscopic identification of bacterial isolates according to Bergey's Manual (Bay et al., 1994) is a fundamental and widely accepted method in clinical microbiology. Gram staining allows differentiation of

bacteria based on cell wall structure, morphology, and cellular arrangement. All strains were subjected to Gram staining and were examined under oil immersion (100×) is the standard magnification for assessing bacterial morphology and Gram reaction.

Biochemical Identification

Identification of all strains was performed using the VITEK 2 automated identification system. Additionally, the catalase test was carried out to determine the ability of bacterial isolates to produce the enzyme catalase, which plays a protective role by breaking down hydrogen peroxide into water and oxygen. This test is particularly useful for differentiating catalase-positive staphylococci from catalase-negative streptococci according (Cheesbrough (2006) and Tille (2017). Fresh bacterial colonies grown on solid culture media for 18–24 hours were used to ensure enzyme activity. A small portion of a single colony was transferred onto a clean, dry glass slide using a sterile wooden applicator stick. Subsequently, one drop of freshly prepared 3% hydrogen peroxide was added directly to the bacterial smear. The reaction was observed immediately to produce oxygen bubbles. The appearance of rapid and vigorous bubble formation was considered a positive catalase reaction, whereas the absence of bubbles indicated a negative result in catalase activity, where *Staphylococcus* species are catalase-positive and *Streptococcus* species are catalase-negative (Cheesbrough, 2006; Tille, 2017)

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the disk diffusion method (CLSI, 2018; Forbes et al., 2016) to evaluate the sensitivity patterns of the bacterial isolates against selected antimicrobial agents. Isolated colonies from fresh cultures were suspended in sterile normal saline, and the turbidity of the suspension was adjusted to match the 0.5 McFarland standard, (CLSI, 2018; Forbes et al., 2016) ensuring a uniform bacterial concentration. The standardized inoculum was evenly spread over the surface of Mueller–Hinton agar plates using a sterile cotton swab to obtain a confluent lawn of bacterial growth. After allowing the surface to dry at room temperature, antibiotic-impregnated discs were aseptically placed on the agar surface with adequate spacing. The inoculated plates were incubated at 37°C for 18–24 hours. Following incubation, the diameters of inhibition zones surrounding each antibiotic disc were measured in millimetres, and the isolates were categorized as susceptible, intermediate, or resistant based on standard interpretive criteria

Preparation of *Agave americana* extract

Fresh leaves of *Agave americana* (century plant or American agave), a perennial succulent characterized by thick, fibrous, lance-shaped leaves arranged in a basal rosette, were used in this study (Misra, Varma, & Kumar, 2018; Forbes et al., 2016). The collected leaves From Al-Safeer Plant Nursery, Kufa, near the University of Kufa, as shown in figure (2). It was thoroughly washed with tap water to remove surface contaminants and allowed to air-dry completely under ambient conditions. The fresh leaves were then cut into small pieces and ground into a fine powder using an electric blender.

The powdered plant material (250 g, 500 g, and 750 g) was subjected to maceration following a standard extraction protocol is modification on (Sasidharan et al., 2011). An extraction of ratio of 1:4 was used to prepare the 25% extract, a ratio of 1:2 was used to prepare the 50% extract, and a ratio of 1:1 was used to prepare the 75% extract.

Each batch was extracted separately using 1000 mL of one of the following solvents: 80% methanol, 70% ethanol, or ethyl acetate. The mixtures were left at room temperature for 5 days with occasional agitation to facilitate the extraction of bioactive compounds. Following maceration, the extracts were filtered and concentrated to obtain crude dried extracts.

Preparation of *Agave americana* extracts solutions

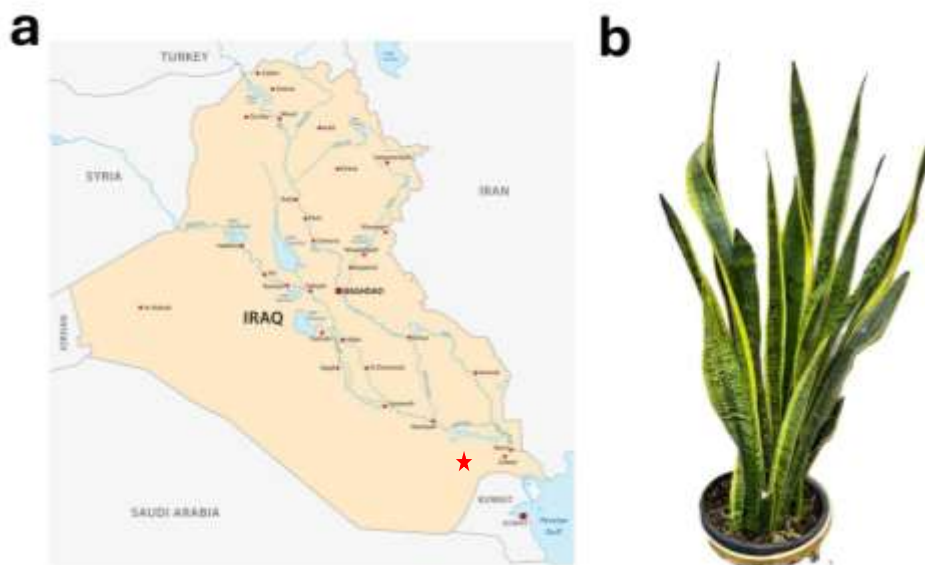
The dried extracts were reconstituted to prepare working solutions at concentrations of 25%, 50%, and 75% (w/v). Extracts obtained using 80% methanol and 70% ethanol and ethyl acetate were dissolved in distilled water. Stock solutions were prepared by dissolving 0.5 g of dried extract in 500 µL of the appropriate solvent, yielding a highly concentrated solution, which was further diluted as required to obtain the desired working concentrations

Antibacterial Assay of *Agave americana* extract

The antibacterial activity of *Agave americana* extracts was evaluated using the disc diffusion method in accordance with standard microbiological guidelines (CLSI, 2020; Baloui et al., 2016). Briefly, pure cultures of selected oral

bacterial isolates were prepared and adjusted to a 0.5 McFarland standard ($\sim 1 \times 10^8$ CFU/mL), then uniformly spread onto Mueller–Hinton agar plates using sterile cotton swabs to obtain a confluent bacterial lawn. Sterile Whatman No. 1 filter paper discs (6 mm diameter) were impregnated with defined concentrations of the plant extracts (25%, 50%, and 75% w/v), allowed to dry under aseptic conditions, and subsequently placed on the inoculated agar surface using sterile forceps. Distilled water (for methanol, ethanol and ethyl acetate extract) served as negative controls, while standard antibiotic discs were included as positive controls where applicable. The plates were incubated at 37 °C for 18–24 hours, after which the antibacterial activity was assessed by measuring the diameter of the zones of inhibition (in mm) surrounding each disc. All assays were performed in triplicate, and mean inhibition zone values were calculated, with larger zones indicating greater antibacterial efficacy of the extracts (Balouiri et al., 2016; CLSI, 2020).

Figure 2: Geographical origin of *Agave americana* and morphological appearance of the plant.



(a) Map of Iraq showing the geographical region from which *Agave americana* was collected for the present study. The highlighted area indicates the sampling location within Iraq.

(b) Photograph of *Agave americana* plant showing its characteristic elongated, thick, and fibrous leaves arranged in a rosette pattern.

MIC Determination of *Agave americana* extract

The minimum inhibitory concentration (MIC) of *Agave americana* extracts was determined using the broth microdilution method. A stock solution of the plant extract (1000 mg/mL) was subjected to two-fold serial dilution by transferring 1 mL of the extract into 1 mL of sterile Mueller–Hinton broth to obtain a concentration of 500 mg/mL. This procedure was repeated sequentially to produce additional concentrations of 250, 125, 62.5, and 31.25 mg/mL (Ibekwe et al., 2001). Each dilution was inoculated with an appropriate volume of standardized bacterial suspension (adjusted to $\sim 1 \times 10^8$ CFU/mL) and incubated at 37 °C for 18–24 h. Bacterial growth was assessed visually by the presence of turbidity, and the MIC was defined as the lowest concentration of extract that completely inhibited visible growth. A negative control consisting of Mueller–Hinton broth with the extract (without bacterial inoculation) was included to ensure sterility.

Synergism Assay of *Agave americana* extract

A synergism assay was conducted on ten bacterial isolates to evaluate the combined antibacterial effect of *Agave americana* extract and selected antibiotics. Sterile filter paper discs impregnated with the plant extract alone (20 μ L per disc) were used as controls. For the synergistic assessment, standard antibiotic discs were supplemented with the plant extract by adding 20 μ L of the extract solution per disc and then placed onto Mueller–Hinton agar plates previously inoculated with bacterial suspensions standardized to a 0.5 McFarland turbidity. The plates were

incubated at 37 °C for 18–24 h. Following incubation, the diameters of the zones of inhibition were measured in millimetres. The interaction between the plant extract and antibiotics was determined by comparing the inhibition zones of the combination discs with those of antibiotic discs alone and interpreted as synergistic, antagonistic, or indifferent based on the increase or decrease in inhibition zone size (CLSI, 2018; Cheesbrough, 2006; Odds, 2003).

Determination of Antioxidant Activity of Agave americana extract using the DPPH Radical Scavenging Assay and IC₅₀ Analysis

The antioxidant activity of Agave americana extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, and the results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) to allow comparison with a standard antioxidant (Gülçin, 2012). Trolox standard solutions at different concentrations were prepared and their radical scavenging activity (RSA%) was measured to construct a calibration curve (µM/L), from which the antioxidant capacity of the plant extracts was calculated using a linear regression equation within the linear range (0.01–0.05 µM/L). The RSA of the extracts was determined by measuring the decrease in absorbance at 517 nm after reaction with DPPH radicals, and calculated using the equation $RSA (\%) = [(A_n - A_s)/A_n] \times 100$ or $RSA (\%) = [1 - (A_s/A_n)] \times 100$, where A_n is the absorbance of the control (DPPH without extract) and A_s is the absorbance of the sample (DPPH with extract); a reduction in absorbance indicates higher radical scavenging activity (Gülçin & Alwasel, 2022). The antioxidant strength was further quantified by determining the IC₅₀ value, defined as the concentration of the extract required to scavenge 50% of the initial DPPH radicals, which was obtained by plotting RSA (%) against extract concentration (µg/mL). Lower IC₅₀ values indicate stronger antioxidant activity, making this parameter a reliable and widely used indicator for comparing the radical scavenging efficiency of different compounds and plant extracts (Koca et al., 2023).

Haemolytic activity of Agave americana extract

The haemolytic activity of Agave americana extracts was evaluated using a standard in vitro spectrophotometric method (Yang et al., 2005). Briefly, 5 mL of fresh human blood obtained from a healthy individual was centrifuged at 1500 rpm for 3 min to separate red blood cells (RBCs), which were then washed three times with sterile phosphate-buffered saline (PBS; pH 7.2 ± 0.2) and resuspended in normal saline to prepare a 0.5% (v/v) erythrocyte suspension. Equal volumes (0.5 mL) of RBC suspension and plant extract at concentrations of 125, 250, 500, and 1000 µg/mL were mixed and incubated at 37 °C for 30 min, followed by centrifugation at 1500 rpm for 10 min. The release of hemoglobin into the supernatant was measured at 540 nm using a UV–Vis spectrophotometer. Phosphate-buffered saline and distilled water served as negative (0% hemolysis) and positive (100% hemolysis) controls, respectively. All experiments were performed in triplicate, and the percentage hemolysis was calculated using the formula: $Hemolysis (\%) = [(A_t - A_n) / (A_c - A_n)] \times 100$, where A_t is the absorbance of the test sample, A_n is the absorbance of the negative control, and A_c is the absorbance of the positive control.

Cytotoxicity of Agave americana extract

The cytotoxicity of Agave americana extracts was evaluated using the human embryonic kidney cell line (HEK-293), obtained from the KUFA Centre for Cell Line, El Kufa- El Iraq, to assess the safety of the extract on normal cells. The cells were cultured in Minimum Essential Medium–Eagle (MEM–Eagle) supplemented with 10% fetal bovine serum (FBS) and maintained under standard conditions (37 °C, 5% CO₂, 95% air, and 100% humidity). For the assay, 100 µL of cell suspension at a density of 1×10^4 cells per well was seeded into 96-well plates and incubated for 24 h to allow cell attachment. The culture medium was then replaced with fresh medium containing varying concentrations of the plant extract (1.9–1000 µL/mL), followed by incubation for 48 h. After treatment, cell viability was assessed using the MTT assay according to the method described by Mosmann (1983), which measures metabolic activity as an indicator of viable cells. The percentage of cell inhibition was calculated using the formula: $\% \text{ cell inhibition} = 100 - (\text{Absorbance of test} / \text{Absorbance of control} \times 100)$. Cell viability data were plotted against the logarithm of extract concentration, and the IC₅₀ value (the concentration causing 50% inhibition of cell viability) was determined using GraphPad Prism software (Version 6.01) following the method of Monks et al. (1991).

Fourier Transform Infrared (FTIR) Spectroscopy analysis of Agave americana extract

Fourier transform infrared (FTIR) spectroscopy was used to identify the functional groups present in Agave americana extract based on their characteristic vibrational frequencies. This technique measures molecular vibrations and

rotations upon exposure to infrared radiation at specific wavelengths (Eid, 2022, as cited in Toka Khairy Abo Alanwar, 2025). The analysis was conducted at the College of Pharmacy, University of Kufa, Iraq, using an FTIR-ATR Bruker Vertex 80 V instrument. Samples were scanned at a resolution of 4 cm⁻¹ over the spectral range of 4000–400 cm⁻¹ at room temperature to obtain infrared spectra for functional group identification.

Gas Chromatography–Mass Spectrometry analysis of *Agave americana* extract

Phytochemical profiling of *Agave americana* was carried out using gas chromatography–mass spectrometry (GC–MS) analysis of the ethyl acetate extract (Bermúdez-Bazán et al., 2021). In this technique, a carrier gas is used to transport vaporized analytes through a capillary column at a controlled flow rate, enabling the separation of compounds based on their physicochemical properties and retention times. Helium was employed as the carrier gas due to its stable flow and high efficiency in chromatographic separation (Guzmán et al., 2014). As the separated compounds eluted from the column, they were detected by the mass spectrometer, which generated characteristic mass spectra for identification. The obtained spectra were compared with reference databases, including the National Institute of Standards and Technology (NIST) and Wiley libraries, to identify the phytochemical constituents present in the extract (Guzmán et al., 2014; Leide et al., 2020).

In Silico Molecular Docking and ADMET Analysis

An integrated in silico approach was employed to evaluate the binding potential of selected phytochemicals against key staphylococcal protein targets. Three receptor proteins include sortase A (srtA), DNA gyrase subunit A (gyrA), and DNA gyrase subunit B (gyrB), were retrieved from the UniProt database using standardized identifiers (The UniProt Consortium, 2025). These targets were selected due to their critical roles in bacterial virulence and survival, where srtA is involved in anchoring surface proteins to the cell wall, while gyrA and gyrB regulate DNA topology and replication (Mazmanian et al., 2001; Marraffini et al., 2006; Collin et al., 2011; Schneewind & Missiakas, 2019). Binding sites were defined based on representative residue coordinates, excluding catalytic sites from this study.

Five ligand compounds identified from GC–MS analysis were obtained from the PubChem database and prepared for docking (Kim et al., 2023). The ligands were converted into optimized three-dimensional structures and subjected to energy minimization using Avogadro software with the MMFF94 force field to reduce structural strain and improve docking accuracy (Halgren, 1996; Hanwell et al., 2012).

Molecular docking simulations were conducted using a QVina2/AutoDock Vina-based protocol. For each ligand–protein pair, the best binding pose was selected based on binding affinity (kcal/mol), where more negative values indicate stronger predicted interactions (Trott & Olson, 2010; Alhossary et al., 2015). Protein–ligand interactions, including hydrogen bonds and hydrophobic contacts, were analysed using Biovia Discovery Studio visualization tools. In addition, pharmacokinetic and toxicity properties were predicted using ADMETLab 2.0. Key descriptors such as molecular weight, lipophilicity (LogP), solubility (LogS), topological polar surface area (TPSA), drug-likeness (QED), and Lipinski and Veber rules were evaluated alongside toxicity alerts including CYP inhibition, P-glycoprotein interaction, and carcinogenicity risks (Lipinski et al., 2001; Veber et al., 2002; Brenk et al., 2008; Baell & Holloway, 2010; Bickerton et al., 2012; Xiong et al., 2021).

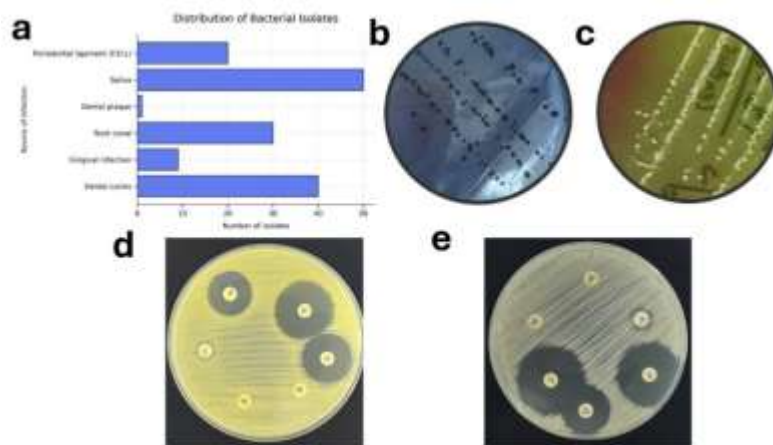
Result

Clinical Isolates and Source Distribution

Initially, there were 150 oral clinical samples obtained from various patients suffering from oral infections. After carrying out primary cultures and biochemistry, 30 potential pathogens were selected. Finally, 10 bacteria with high probability of identification (probability >90%) using the VITEK 2 automated identification system were chosen to proceed with the antibiotic sensitivity test.

Figure 3: Isolation and antibacterial activity of *Agave americana* leaf extract against oral bacterial isolates. (a) Distribution of bacterial isolates according to the source of infection among clinical dental samples. (b) Growth of *Streptococcus* spp. on Mitis Salivarius Agar showing characteristic blue colonies. (c) Growth of *Staphylococcus aureus* on Mannitol Salt Agar (MSA), exhibiting yellow colonies and yellow discoloration of the medium due to mannitol fermentation. (d–e) Antibiotic susceptibility testing of selected oral bacterial isolates using the Kirby–

Bauer disk diffusion method, demonstrating variable zones of inhibition and indicating differences in antimicrobial susceptibility and resistance patterns among the isolates.



In figure (3a, b,c) The distribution of clinical isolates according to the source of infection shows that a total of 150 oral samples were initially collected from different clinical sites. The highest proportion of samples was obtained from saliva ($n = 50$), followed by dental caries ($n = 40$) and root canal infections ($n = 30$), indicating that these sites represent the main reservoirs of oral bacterial pathogens. Moderate numbers of isolates were obtained from periodontal infections ($n = 20$) and gingival samples ($n = 9$), while the lowest number of samples was recorded from dental plaque ($n = 1$).

The distribution of clinical samples in the present study showed that saliva, dental caries, and root canal infections were the main sources of recovered oral bacterial isolates. The lower number of isolates from gingival infections and periodontal ligament samples may be attributed to the localized nature of these infections and the lower bacterial shedding compared with saliva. Collectively, these findings reinforce the importance of saliva and dental caries as major ecological reservoirs for oral pathogens and highlight their significance in the diagnosis and management of oral infections.

Selection and Identification of Clinical Isolates

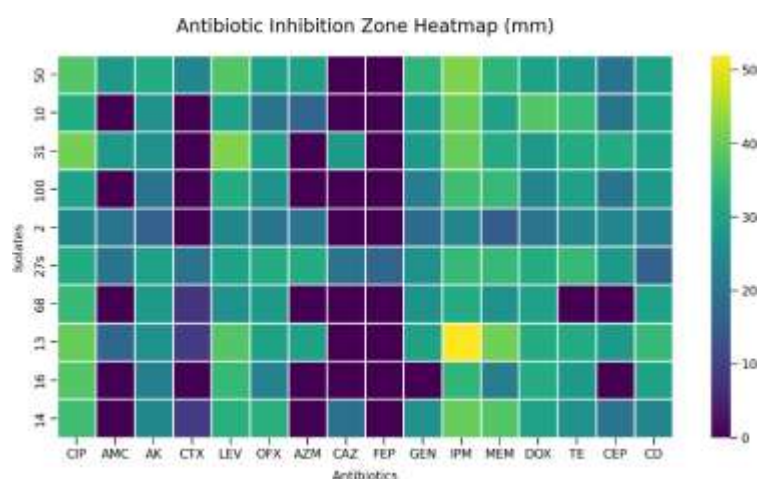
In total, 150 samples from clinical cases of the mouth were collected and isolated for bacteriological tests. From these samples, 30 samples that showed signs of pathogenicity were selected using cultural and biochemical methods of isolation. After further identification using the VITEK 2 system, 10 samples whose identification probability was greater than 90% were selected. This involved the use of samples from *Staphylococcus* and *Streptococcus* genera.

Antibiotic Susceptibility Testing

Figure 3d,e showed the antibiotic susceptibility profiles of ten selected oral bacterial isolates against seventeen antimicrobial agents. Considerable variation in inhibition zone diameters was observed, indicating substantial differences in susceptibility among the tested isolates. Overall, imipenem (IPM) and meropenem (MEM) exhibited the greatest antibacterial activity, producing the largest inhibition zones across most isolates, with values reaching up to 52 mm, thereby demonstrating their high efficacy against the tested pathogens. Ciprofloxacin (CIP) and levofloxacin (LEV) also showed good inhibitory activity against several isolates.

In contrast, reduced susceptibility and resistance were frequently observed for several cephalosporins, including cefotaxime (CTX), ceftazidime (CAZ), and cefepime (FEP), as well as azithromycin (AZM), with some isolates exhibiting no detectable inhibition. Notably, *Staphylococcus lentus*, *Staphylococcus aureus*, and *Staphylococcus hominis* displayed multidrug-resistant phenotypes, characterized by resistance or reduced susceptibility to multiple antibiotic classes. Conversely, *Staphylococcus xylosus*, *Staphylococcus epidermidis*, and *Staphylococcus simulans* were generally more susceptible, exhibiting comparatively larger inhibition zones against several antibiotics.

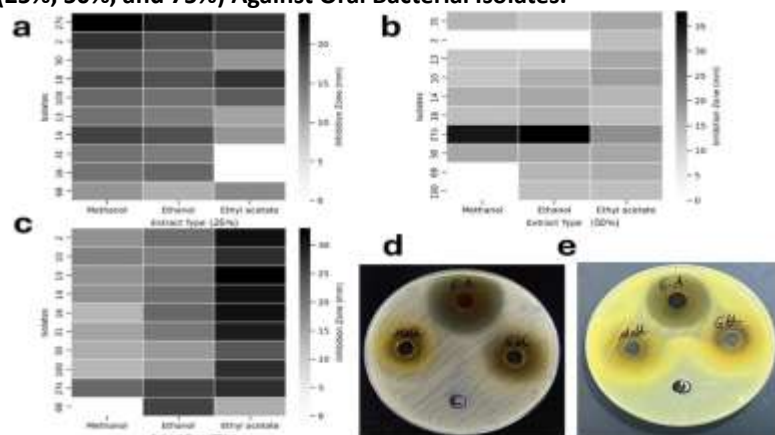
Collectively, these findings reveal marked heterogeneity in the antibiotic susceptibility patterns of oral bacterial isolates, with carbapenems remaining the most effective antimicrobial agents, whereas resistance to several commonly used antibiotics was widespread among the tested strains.



Concentration-Dependent Antibacterial Activity of *Agave americana* extract Against Oral Bacterial Isolates

The results demonstrated that *Agave americana* extract exhibited variable antibacterial activity depending on both the extraction solvent and the bacterial isolate, as shown in (Figure, 5a). At 25% concentration, the extracts showed moderate inhibitory effects, with ethanol and methanol extracts generally producing consistent activity across several isolates. However, some isolates, such as *Staphylococcus lentus*, *Streptococcus pseudoporcinus*, and *Staphylococcus aureus*, exhibited weak or no inhibition with these solvents, while limited activity was still observed with ethyl acetate extract. At 50% concentration, an overall increase in antibacterial activity was evident. Ethanol and methanol extracts showed improved inhibition against most isolates, such as *Streptococcus pseudoporcinus* demonstrating the highest sensitivity across all solvents, reaching inhibition zones of approximately 35–38 mm. Despite this improvement, certain strains maintained partial resistance, particularly toward methanol and ethanol extracts, further emphasizing variability in bacterial susceptibility as shown in (Figure, 5b). The highest antibacterial activity was observed at 75% concentration, confirming a clear concentration-dependent effect. At this level, ethyl acetate extract exhibited the strongest inhibitory activity, reaching a maximum inhibition zone of approximately 33 mm. Overall, the results indicate that increasing extract concentration enhances antimicrobial efficacy, with ethyl acetate showing superior performance at higher concentrations as shown in (Figure, 5c). Inhibition zones was recorded using different solvent as shown in (Figure, 5c, 5d)

Figure 4: Comparative Heatmap Analysis of Antibacterial Activity of Plant Extracts at Different Concentrations (25%, 50%, and 75%) Against Oral Bacterial Isolates.



It presents three heatmaps illustrating the antibacterial activity of plant extracts (methanol, ethanol, and ethyl acetate) against oral bacterial isolates at (a) 25%, (b) 50%, and (c) 75% concentrations. The heatmaps display inhibition zone diameters (mm), represented using a grayscale gradient ranging from light (low or no inhibition) to dark/black (high antibacterial activity). Each row corresponds to a bacterial isolate, while each column represents a type of solvent extract. Comparative visualization highlights the influence of extract type and concentration on antimicrobial efficacy across isolates, (d) shows the inhibition zone of each extract against *Streptococcus* spp., (e) shows the inhibition zone of each extract against *Staphylococcus* spp.

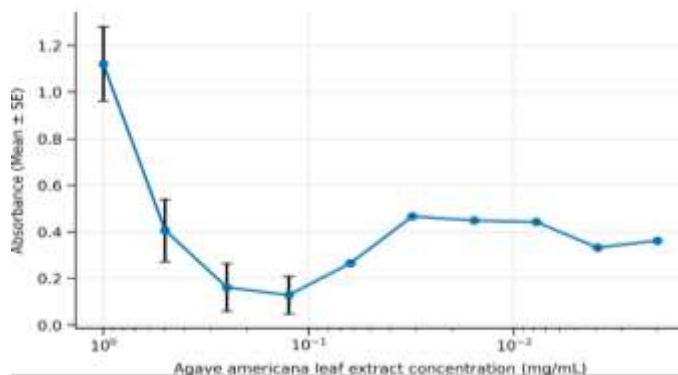
Determination of Minimum Inhibitory Concentration (MIC) of *Agave americana* extract

The antibacterial activity of *Agave americana* leaf extract was evaluated using a broth microdilution assay across concentrations ranging from 0.002 to 1 mg/mL Figure (6). A concentration-dependent reduction in bacterial growth was observed, as indicated by changes in absorbance values.

The highest absorbance value was recorded at 1.0 mg/mL (1.121 ± 0.275), whereas a marked decrease in absorbance was observed with subsequent dilutions. The lowest absorbance value was obtained at 0.125 mg/mL (0.129 ± 0.139), indicating the greatest inhibition of bacterial growth at this concentration. At concentrations below 0.125 mg/mL, absorbance values increased again, ranging from 0.266 at 0.0625 mg/mL to 0.467 at 0.0312 mg/mL, suggesting reduced antibacterial efficacy at lower extract concentrations.

The dose-response profile demonstrated that bacterial growth inhibition was most pronounced at 0.125 mg/mL, which was therefore identified as the minimum inhibitory concentration (MIC) of the *Agave americana* leaf extract under the tested conditions. The observed increase in absorbance at concentrations lower than the MIC further supports the inability of these concentrations to effectively suppress bacterial growth.

Figure 5: Effect of *Agave americana* leaf extract concentration on bacterial growth determined by broth microdilution assay.



Data are presented as mean absorbance $\pm$ standard error (SE, $n = 3$). The absorbance decreased with increasing extract concentration, reaching the lowest value at 0.125 mg/mL, indicating the strongest inhibitory effect and suggesting this concentration as the putative minimum inhibitory concentration (MIC) under the tested conditions.

Synergistic Activity of *Agave americana* extract with Antibiotics

The synergistic antibacterial activity of 75% *Agave americana* leaf extract in combination with commonly used antibiotics was evaluated against the selected clinical oral bacterial isolates Figure (7). The results demonstrated considerable variability in synergistic responses among both bacterial isolates and antibiotic classes.

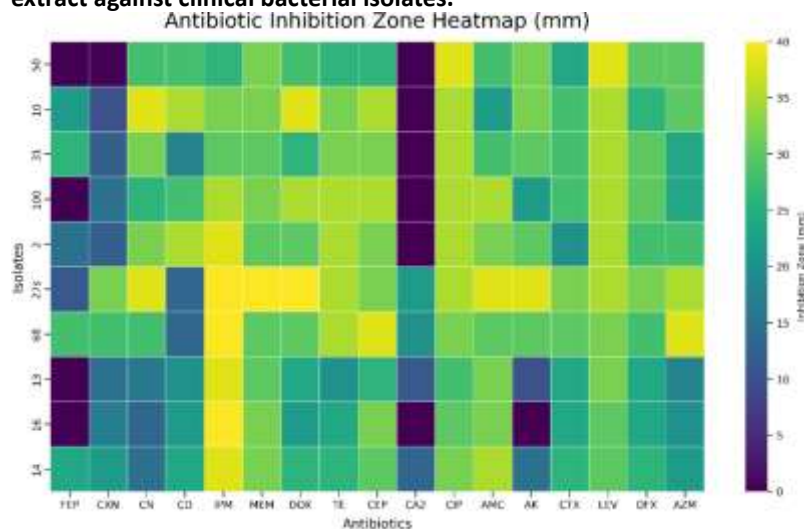
The strongest synergistic effects were observed when the extract was combined with the carbapenem antibiotics imipenem (IPM) and meropenem (MEM). These combinations produced the largest inhibition zones, reaching up to 40 mm in several isolates, including *Streptococcus pseudoporcinus*, *Streptococcus sobrinus*, and *Staphylococcus hominis*. Similarly, doxycycline (DOX) exhibited substantial enhancement in antibacterial activity in the presence of the extract, with inhibition zones comparable to those observed for the carbapenems.

Moderate synergistic effects were detected for gentamicin (CN) and tetracycline (TE), indicating that the extract enhanced the efficacy of these antibiotics, although to a lesser extent than that observed for carbapenems and

doxycycline. In contrast, combinations involving cefepime (FEP) and ceftazidime (CA2) showed minimal or no enhancement against most tested isolates, as reflected by small or absent inhibition zones.

Overall, the synergistic assay demonstrated that *A. americana* extract can substantially enhance the antibacterial activity of selected antibiotics, particularly carbapenems and doxycycline. The observed enhancement may be attributed to the phytochemical constituents of the extract, including phenolic compounds and other secondary metabolites, which have been reported to increase bacterial membrane permeability, inhibit efflux pumps, disrupt biofilm formation, and thereby improve antibiotic efficacy (Bouarab-Chibane et al., 2019; Langeveld et al., 2014). These findings suggest that *A. americana* extract may serve as a promising adjuvant to conventional antibiotic therapy for the treatment of resistant oral bacterial infections.

Figure 6: Heatmap of the synergistic antibacterial activity of antibiotics combined with 75% *Agave americana* extract against clinical bacterial isolates.



Heatmap illustrating the inhibition zone diameters (mm) produced by the combination of 75% *Agave americana* extract and different antibiotics against ten clinical bacterial isolates. Rows represent bacterial isolates and columns represent the tested antibiotics.

Color intensity corresponds to the size of the inhibition zone, with darker colors indicating lower antibacterial activity and brighter colors indicating stronger synergistic activity. Inhibition zones ranged from 0 to 40 mm. Abbreviations: FEP, cefepime; CXN, cefalexin/cephalosporin; CN, gentamicin; CD, clindamycin; IPM, imipenem; MEM, meropenem; DOX, doxycycline; TE, tetracycline; CEP, cephalothin; CA2, ceftazidime; CIP, ciprofloxacin; AMC, amoxicillin-clavulanic acid; AK, amikacin; CTX, cefotaxime; LEV, levofloxacin; OFX, ofloxacin; AZM, azithromycin.

Antioxidant activity, hemolytic activity, and cytotoxicity assessment of *Agave americana* extract

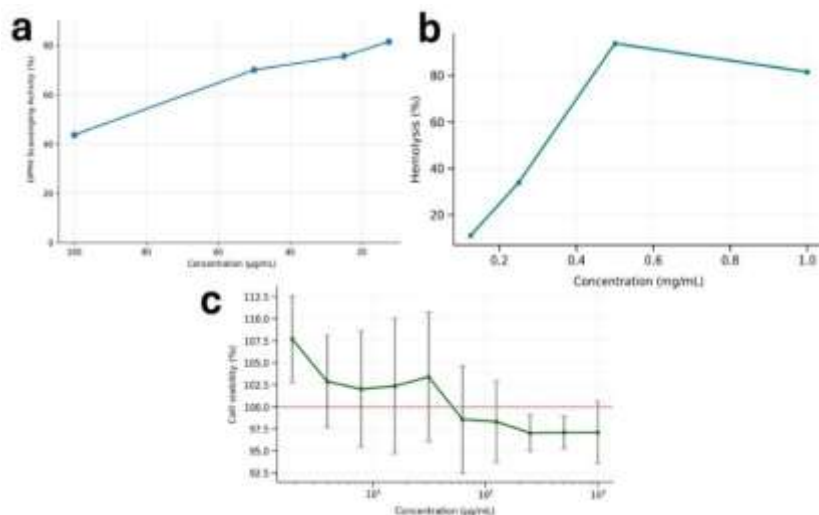
The biological activities and safety profile of *Agave americana* leaf extract are presented in Figure 8. The antioxidant activity assay demonstrated a concentration-dependent increase in DPPH radical scavenging activity (Figure 8a). The extract exhibited scavenging activities of approximately 40%, 70%, 76%, and 81% at concentrations of 100, 50, 25, and 12.5 µg/mL, respectively, indicating strong antioxidant potential.

The hemolytic activity assay revealed a concentration-dependent increase in erythrocyte lysis up to 0.5 mg/mL (Figure 8b). Hemolysis increased from 11.16% at 0.125 mg/mL to 33.97% at 0.25 mg/mL and reached a maximum value of 93.87% at 0.5 mg/mL. A slight decrease in hemolytic activity was observed at 1.0 mg/mL, where hemolysis was 81.61%. These findings indicate that the extract possesses membrane-disruptive activity at elevated concentrations.

The cytotoxicity assessment against HEK-293 cells demonstrated that *A. americana* extract exhibited minimal toxicity throughout the tested concentration range (Figure 8c). Cell viability remained above 97% at all concentrations tested (1.9–1000 µg/mL). Notably, viability values exceeded 100% at lower concentrations (1.9–31.25 µg/mL), reaching a maximum of 107.71% at 1.9 µg/mL, suggesting a possible stimulatory effect on cellular metabolic activity. At higher

concentrations, cell viability remained stable between 97.03% and 98.58%, confirming the biocompatibility of the extract toward normal human cells. Collectively, these results demonstrate that *A. americana* possesses considerable antioxidant activity while exhibiting low cytotoxicity toward HEK-293 cells, supporting its potential as a safe natural bioactive agent.

Figure 7: Antioxidant, Hemolytic and cytotoxic Activities of *Agave americana* Leaf Extract at Different Concentrations.



(a) DPPH scavenging activity of *Agave americana* extract showing increased antioxidant activity at lower concentrations.

(b) Hemolytic activity demonstrates a concentration-dependent increase with a peak at 0.50 mg/mL followed by a slight decline.

(c) Cytotoxicity profile of the extract against HEK-293 cells determined by the MTT assay. Cell viability values are presented as mean $\pm$ SD. The dashed red line indicates 100% cell viability. The extract-maintained cell viability above 97% across the tested concentration range (1.9–1000 μ g/mL), indicating low cytotoxicity toward normal human kidney cells.

FTIR Analysis of *Agave americana* extract

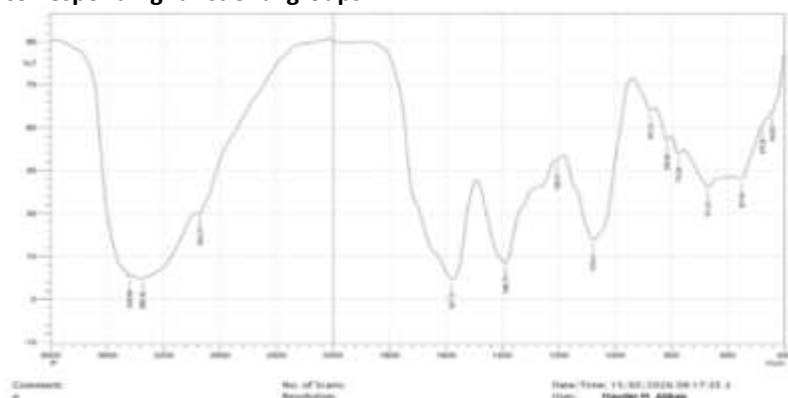
The FTIR spectrum of *Agave americana* leaf extract revealed the presence of several characteristic absorption bands corresponding to different functional groups. The broad and intense absorption band observed at 3412.07–3308.86 cm^{-1} was assigned to O–H stretching vibrations, indicating the presence of hydroxyl-containing compounds such as phenolics, flavonoids, and alcohols.

A prominent absorption band at 2933.37 cm^{-1} was attributed to aliphatic C–H stretching vibrations, suggesting the occurrence of long-chain hydrocarbons, lipids, and other aliphatic constituents. Additionally, the band detected at 1597.77 cm^{-1} corresponded to C=C stretching vibrations of aromatic rings and/or amide functional groups, indicating the presence of aromatic compounds and nitrogen-containing metabolites.

The absorption peak at 1037.7 cm^{-1} was assigned to C–O stretching vibrations, which are commonly associated with alcohols, esters, carbohydrates, and glycosidic compounds. Furthermore, several bands were observed within the fingerprint region (873.74–446.66 cm^{-1}), reflecting the complex chemical composition of the extract and supporting the presence of diverse secondary metabolites.

Overall, the FTIR profile confirmed the occurrence of multiple bioactive functional groups in *A. americana* leaf extract, including hydroxyl, aliphatic, aromatic, and oxygenated functionalities, as shown in figure (9). These findings suggest the presence of various phytochemical classes, such as phenolic compounds, flavonoids, alcohols, esters, carbohydrates, and other secondary metabolites, which may contribute to the biological activities observed in the present study.

Figure 8: FTIR spectrum of *Agave americana* leaf extract showing the major absorption bands and their corresponding functional groups.



The broad peak at 3412.07–3308.86 cm^{-1} corresponds to O–H stretching vibrations, while the band at 2933.37 cm^{-1} is attributed to aliphatic C–H stretching. The absorption band at 1597.77 cm^{-1} indicates aromatic C=C and/or amide group vibrations, whereas the band at 1037.7 cm^{-1} corresponds to C–O stretching. Peaks within the fingerprint region (873.74–446.66 cm^{-1}) further indicate the presence of diverse phytochemical constituents in the extract.

GC–MS Analysis of *Agave americana* extract

GC–MS analysis of *Agave americana* leaf extract identified ten phytochemical constituents with varying relative abundances (Table 1). The detected compounds comprised heterocyclic compounds, fatty acid derivatives, aromatic compounds, and other secondary metabolites, indicating a chemically diverse phytochemical profile.

Among the identified compounds, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- was the most abundant constituent, accounting for 40.30% of the total chromatographic area and exhibiting a very high identification quality. The second major component was 2H-Pyran-2-one, representing 32.26% of the extract composition. Together, these two compounds constituted approximately 72.56% of the total detected constituents, highlighting the predominance of pyran-based derivatives in the extract.

Other compounds were present in moderate amounts, including Bis(2-ethylhexyl) phthalate (8.18%) and Hexanoic acid, 3-hydroxy-, methyl ester (6.41%). Several minor constituents were also detected, namely Hydroxytoluic acid (3.03%), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (2.43%), 1-Pyrrolidinecarbonitrile (2.37%), 1-Butanol/Cycetacide (1.95%), Benzofuran, 2,3-dihydro- (1.68%), and Propane (1.39%). These compounds individually contributed less than 5% of the total chromatographic area but may collectively influence the biological properties of the extract.

Retention times ranged from 3.263 to 28.764 min, with the later-eluting compound, Bis(2-ethylhexyl) phthalate, exhibiting the highest retention time, suggesting a comparatively less volatile nature than the remaining detected compounds. The predominance of pyran derivatives, together with the presence of fatty acid esters and aromatic compounds, demonstrates the richness of the extract in potentially bioactive phytochemicals.

Overall, GC–MS profiling revealed that *A. americana* leaf extract is dominated by pyran-related compounds, with additional contributions from fatty acid derivatives and aromatic constituents. These findings provide a comprehensive chemical characterization of the extract and establish a phytochemical basis for further investigation of its biological activities.

Table 1. Major phytochemical compounds identified by GC–MS analysis of *Agave americana* extract.

Peak No.	Retention Time (RT)	Area %	Identified Compound	Quality
1	3.263	2.37	1-Pyrrolidinecarbonitrile	Low
2	3.524	1.95	1-Butanol / Cycetacide	Moderate

3	3.824	32.26	2H-Pyran-2-one	High
4	4.333	2.43	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	Moderate
5	7.098	40.30	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Very High
6	8.233	1.68	Benzofuran, 2,3-dihydro-	Moderate
7	8.787	6.41	Hexanoic acid, 3-hydroxy-, methyl ester	Moderate
8	15.003	3.03	Hydroxytoluic acid	Low
9	16.920	1.39	Propane	Low
10	28.764	8.18	Bis(2-ethylhexyl) phthalate	Moderate

Selection of Receptor Proteins for Molecular Docking

To investigate the potential molecular interactions underlying the biological activity of *Agave americana* leaf extract, five selected phytochemicals identified by GC–MS analysis were subjected to molecular docking against three *Staphylococcus aureus* target proteins. The receptor proteins included Sortase A (srtA), DNA gyrase subunit A (gyrA), and DNA gyrase subunit B (gyrB), which were retrieved from the UniProt database and employed as receptor models in the docking workflow (Table 2).

The selected targets represent essential bacterial proteins involved in virulence and cellular survival. Sortase A is responsible for anchoring surface proteins to the bacterial cell wall, whereas DNA gyrase subunits A and B play critical roles in DNA topology regulation and replication. Consequently, these proteins are regarded as promising targets for the development of antibacterial agents.

Protein identities and accession numbers were standardized according to the UniProt Knowledgebase. The binding-site labels used in the docking experiments correspond to the centre coordinates of the selected amino acid residues within each receptor structure. The docking protocol focused on the predefined binding regions and did not specifically target the catalytic sites of the respective proteins.

Table 2. Protein targets, UniProt identifiers, and receptor models used in the docking workflow.

Target	Protein/receptor description	UniProt ID	Binding-site label
srtA	Sortase A-labeled receptor model; cell-wall anchoring/virulence-associated target	Q2FV99	184
gyrA	DNA gyrase subunit A-labeled receptor model; DNA topology-control target	P20831	123
gyrB	DNA gyrase subunit B-labeled receptor model; ATPase/topoisomerase-associated target	P66937	508

Molecular Docking Interaction Analysis

Representative docking poses were analyzed to characterize the binding interactions of the major phytochemicals from *Agave americana* leaf extract with three bacterial targets, namely Sortase A (srtA), DNA gyrase subunit A (gyrA), and DNA gyrase subunit B (gyrB) Figure (10).

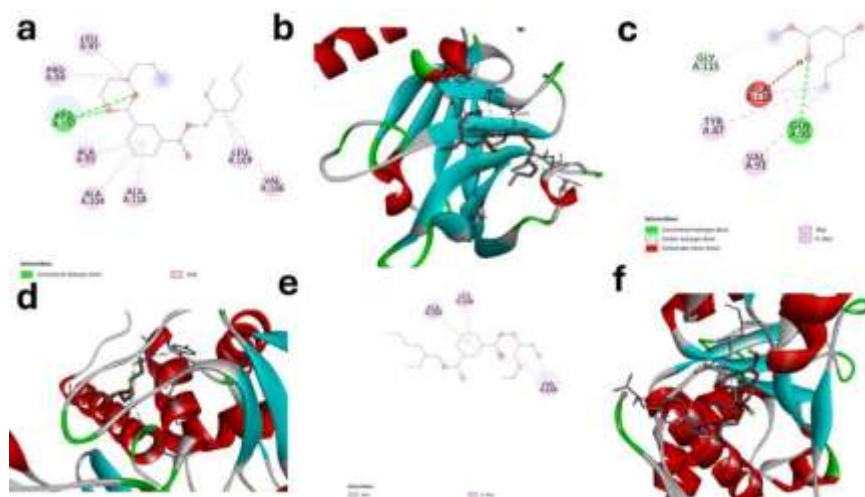
The interaction profile of 1,3-benzenedicarboxylic acid bis(2-ethylhexyl) ester with srtA demonstrated a mixed polar–hydrophobic binding pattern (Figure 10 a, b). The ligand formed a conventional hydrogen bond with ARG197, which served as the principal anchoring residue within the binding pocket. Additional stabilization was provided by multiple hydrophobic alkyl interactions involving ALA92, PRO94, LEU97, ALA104, ALA118, VAL166, and LEU169. The three-

dimensional binding pose confirmed that the ester oxygen group was oriented toward ARG197, while the hydrophobic side chains extended into nonpolar regions of the binding cavity, supporting strong ligand accommodation within the receptor.

For *gyrA*, hexanoic acid, 3-hydroxy-, methyl ester displayed a combination of polar and hydrophobic interactions (Figure 10 c, d). The ligand formed a conventional hydrogen bond with GLN95 and a carbon–hydrogen bond with GLY115, alongside hydrophobic contacts with TYR87 and VAL91. An unfavourable donor–donor interaction involving ALA118 was also observed; however, the overall interaction network remained favourable due to the presence of multiple stabilizing contacts. The three-dimensional docking pose showed the ligand positioned between loop and helical regions, allowing simultaneous engagement of polar and nonpolar residues within the binding site.

In contrast, the interaction of 1,3-benzenedicarboxylic acid bis(2-ethylhexyl) ester with *gyrB* was primarily driven by hydrophobic interactions (Figure 10 e, f). The ligand established alkyl and π -alkyl interactions with ALA509, LEU544, and PHE644, while no conventional hydrogen bonds were detected. The corresponding three-dimensional structure revealed that the ligand occupied a predominantly hydrophobic pocket, where extensive van der Waals and hydrophobic interactions likely contributed to complex stabilization. This binding mode suggests that favourable receptor–ligand fitting rather than directional hydrogen bonding was the major contributor to binding within *gyrB*. Overall, the docking analysis demonstrated distinct interaction patterns among the three receptor proteins. Binding to *srtA* and *gyrA* involved both hydrogen-bonding and hydrophobic interactions, whereas *gyrB* binding was predominantly mediated by hydrophobic contacts. These findings support the potential of *A. americana* phytochemicals to interact with multiple antibacterial targets and provide a molecular basis for the antimicrobial activity observed experimentally.

Figure 9: Molecular docking interactions of selected phytochemicals with bacterial target proteins.



(a) Two-dimensional interaction map of 1,3-benzenedicarboxylic acid bis(2-ethylhexyl) ester docked with Sortase A (*srtA*), showing hydrogen-bonding with ARG197 and hydrophobic interactions with surrounding residues.

(b) Three-dimensional binding pose of the *srtA*–ligand complex.

(c) Two-dimensional interaction map of hexanoic acid, 3-hydroxy-, methyl ester docked with DNA gyrase subunit A (*gyrA*), showing interactions with GLN95, GLY115, TYR87, VAL91, and ALA118.

(d) Three-dimensional binding pose of the *gyrA*–ligand complex.

(e) Two-dimensional interaction map of 1,3-benzenedicarboxylic acid bis(2-ethylhexyl) ester docked with DNA gyrase subunit B (*gyrB*), highlighting hydrophobic interactions with ALA509, LEU544, and PHE644.

(f) Three-dimensional binding pose of the *gyrB*–ligand complex showing ligand accommodation within the hydrophobic binding pocket. Green dashed lines indicate hydrogen bonds, light-green dashed lines represent carbon–hydrogen bonds, red dashed lines indicate unfavorable donor–donor interactions, and purple dashed lines denote alkyl or π -alkyl interactions.

Discussion

The present study investigated the antibacterial potential of *Agave americana* leaf extract against oral bacterial isolates and explored its phytochemical composition, antioxidant activity, cytotoxicity, and molecular interactions with selected bacterial targets. The findings collectively demonstrate that *A. americana* possesses promising bioactive properties that may support its future application as a natural antimicrobial agent in oral healthcare.

A total of 150 clinical oral samples was collected and subjected to bacteriological examination. Initial isolation and screening using conventional cultural and biochemical methods resulted in the selection of 30 isolates exhibiting characteristics associated with pathogenic bacteria. The identified isolates belonged predominantly to the genera *Staphylococcus* and *Streptococcus*, both of which are frequently associated with oral infections and opportunistic pathogenicity. The predominance of these bacterial genera reflects the ecological complexity of the oral cavity, where saliva, teeth, gingival tissues, and root canal systems provide diverse microbial niches that facilitate bacterial colonization and persistence (Rajasekaran et al., 2024). The recovery of a substantial proportion of isolates from dental caries is in agreement with previous studies demonstrating that caries-associated biofilms are characterized by microbial dysbiosis, enhanced acid production, and progressive demineralization of dental hard tissues, thereby creating favourable conditions for the proliferation of pathogenic microorganisms (Spatafora et al., 2024). In addition, saliva serves as an important reservoir and transmission medium for oral bacteria because of its continuous contact with oral surfaces and its role in regulating the oral microbial community (Li et al., 2022).

The isolation of *Staphylococcus* and *Streptococcus* species in the present study is consistent with their recognized involvement in oral and dental diseases. Members of the genus *Streptococcus*, particularly viridans streptococci, are among the primary colonizers of the oral cavity and play a significant role in dental plaque formation and the development of dental caries. Likewise, *Staphylococcus* species have been increasingly reported in oral infections and are capable of contributing to biofilm formation and persistent infections within the oral environment. These findings highlight the importance of monitoring oral bacterial populations and exploring alternative antimicrobial agents for the management of oral pathogens.

The bacterial isolates included members of the genera *Staphylococcus* and *Streptococcus*, which are frequently implicated in oral infections and biofilm-associated diseases. Antimicrobial susceptibility testing revealed considerable variability in resistance patterns among the isolates. Carbapenems, particularly imipenem (IPM) and meropenem (MEM), exhibited the strongest antibacterial activity, whereas resistance was common against several cephalosporins and azithromycin. These findings are consistent with the growing concern regarding antimicrobial resistance among oral pathogens and emphasize the need for alternative therapeutic approaches.

In this context, *A. americana* extract demonstrated notable antibacterial activity, producing inhibition zones ranging from 14 to 33 mm against all tested isolates. Importantly, no complete resistance to the extract was observed, suggesting that the phytochemical constituents may exert their effects through mechanisms distinct from those of conventional antibiotics. Similar antibacterial activity of *A. americana* has been reported previously by Jayalakshmi et al. (2024) and Pandey et al. (2019), although the present study specifically targeted clinically relevant oral bacterial isolates. The broad-spectrum inhibition observed here supports the potential of *A. americana* as a source of natural antibacterial compounds.

The extract also enhanced the antibacterial efficacy of several antibiotics when used in combination. The strongest synergistic effects were observed with the carbapenems imipenem and meropenem, while doxycycline also exhibited marked enhancement. In contrast, cefepime and ceftazidime showed little or no synergistic improvement. These findings suggest that the interaction between *A. americana* phytochemicals and antibiotics is both antibiotic- and isolate-dependent. Similar synergistic effects of plant-derived phytochemicals have been reported in previous studies and are often attributed to increased membrane permeability, inhibition of bacterial defense mechanisms, or enhanced intracellular antibiotic uptake (Jayalakshmi et al., 2024; Pandey et al., 2019).

The MIC analyses further confirmed the antimicrobial efficacy of the extract. A concentration-dependent inhibitory effect was observed, with bacterial growth decreasing as extract concentration increased. The lowest absorbance value, representing maximal growth inhibition, was recorded at the MIC concentration, whereas bacterial regrowth was evident at lower concentrations. Complete bactericidal activity was achieved only at the highest concentration tested (1.0 mg/mL), indicating that higher concentrations are required for bacterial killing than for growth inhibition. These findings agree with those reported by Hammuel (2011), who demonstrated concentration-dependent antimicrobial effects of cactus-derived extracts using similar MIC and MBC methodologies.

Consistent with the phytochemical findings, the extract exhibited strong antioxidant activity in the DPPH assay. Interestingly, radical scavenging activity increased as concentration decreased, reaching a maximum of 81.5%. The ability of the extract to neutralize free radicals is likely related to the abundance of phenolic and pyran-containing compounds identified by FTIR and GC–MS analyses. Similar antioxidant activity has been reported for *A. americana* by Pandey et al. (2019), supporting the role of this species as a natural source of antioxidant molecules.

Evaluation of hemolytic activity revealed a concentration-dependent cytotoxic response toward human erythrocytes. Minimal hemolysis was observed at the lowest concentration tested (11.16%), whereas hemolytic activity increased markedly at higher concentrations, reaching 93.87% at 0.5 mg/mL. Although these findings indicate that the extract possesses membrane-disrupting properties at elevated concentrations, lower concentrations appeared relatively safe. Similar concentration-dependent hemolytic effects have been reported for aqueous plant extracts by Shegute and Wasihun (2020). Therefore, while the extract exhibits considerable biological activity, careful dose optimization remains essential for potential therapeutic applications.

Interestingly, assessment of cytotoxicity using HEK293 cells demonstrated an excellent biosafety profile. Cell viability remained above the 70% threshold defined by ISO 10993-5, reaching 97.11% even at the highest concentration tested. Moreover, low concentrations promoted cell proliferation, with viability increasing to 107.71%. These findings contrast with reports by Abdulla et al. (2022), who observed concentration-dependent cytotoxicity in other *A. americana*-derived preparations, suggesting that extraction method, solvent system, and phytochemical composition strongly influence biological safety. The present extract therefore appears considerably safer than some previously reported formulations while retaining potent biological activity.

The pronounced antibacterial activity can be partially explained by the phytochemical composition of the extract. FTIR analysis revealed the presence of hydroxyl, aliphatic, aromatic, and oxygenated functional groups associated with phenolics, flavonoids, alcohols, esters, carbohydrates, and other secondary metabolites. Such compounds have been widely associated with antimicrobial properties through mechanisms including membrane disruption, enzyme inhibition, and interference with bacterial metabolism. The diversity of functional groups observed suggests that multiple phytochemicals may act synergistically to produce the antibacterial effects recorded in this study.

GC–MS analysis provided further chemical characterization and identified ten compounds within the extract. The most abundant constituents were 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (40.30%) and 2H-pyran-2-one (32.26%), together accounting for more than 70% of the detected compounds. Additional constituents included bis(2-ethylhexyl) phthalate and hexanoic acid, 3-hydroxy-, methyl ester. Pyran derivatives have previously been associated with antimicrobial and antioxidant activities, while fatty acid esters may contribute to membrane-targeting effects. These findings are in agreement with Ahumada-Santos et al. (2013), who reported that *Agave* species contain biologically active phytochemicals capable of exerting antioxidant and antimicrobial effects. The phytochemical profile identified in the present study therefore provides a chemical basis for the biological activities observed.

To further explore the molecular basis of antibacterial activity, molecular docking was performed against three *Staphylococcus aureus* targets: Sortase A (srtA), DNA gyrase subunit A (gyrA), and DNA gyrase subunit B (gyrB). Although the docking scores ranged from –5.4 to –3.4 kcal/mol, indicating moderate rather than high-affinity binding, several biologically relevant interactions were identified. The strongest interaction was observed between 1,3-benzenedicarboxylic acid bis(2-ethylhexyl) ester and srtA (–5.4 kcal/mol), where ARG197 formed hydrogen bonds while surrounding hydrophobic residues contributed additional stabilization. A similar ligand exhibited favorable binding to gyrB through predominantly hydrophobic interactions involving ALA509, LEU544, and PHE644. In contrast, the best gyrA ligand was hexanoic acid, 3-hydroxy-, methyl ester, which formed hydrogen-bonding interactions with GLN95 and GLY115 together with hydrophobic contacts involving TYR87 and VAL91. Given the established roles of Sortase A and DNA gyrase in bacterial virulence and replication (Mazmanian et al., 2001; Marraffini et al., 2006; Collin et al., 2011; Schneewind and Missiakas, 2019), these interactions provide plausible molecular support for the antibacterial effects observed experimentally.

Overall, the findings indicate that *Agave americana* leaf extract possesses significant antibacterial and antioxidant activities supported by a chemically diverse phytochemical profile. The combination of experimental antimicrobial efficacy, favorable biosafety toward HEK293 cells, and biologically relevant docking interactions suggests that *A. americana* represents a promising source of natural bioactive compounds for future antimicrobial applications. However, further studies involving compound isolation, molecular dynamics simulations, and in vivo validation are required to confirm the mechanisms underlying its biological activities.

Conclusion

Agave americana leaf extract exhibited significant antibacterial activity against oral bacterial isolates, with additional synergistic effects when combined with selected antibiotics. The extract demonstrated concentration-dependent inhibitory and bactericidal activities, supported by strong antioxidant potential. FTIR and GC–MS analyses revealed a diverse phytochemical profile dominated by pyran derivatives and other bioactive compounds that may contribute to the observed biological effects. Although hemolytic activity increased at higher concentrations, the extract showed excellent biocompatibility toward HEK293 cells, indicating a favorable safety profile within the tested range. Molecular docking further supported the antibacterial findings by demonstrating favorable interactions between major phytochemicals and key bacterial targets, including Sortase A and DNA gyrase enzymes. Collectively, these findings highlight *A. americana* as a promising source of natural antimicrobial and antioxidant agents, warranting further studies to isolate active compounds and validate their therapeutic potential in vivo.

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