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Antibacterial and Antibiofilm Properties of Jicama (*Pachyrhizus erosus*) Seed Extract against *Streptococcus mutans*: Identification of Key Bioactive Compounds Using Bioautography and LC-MS/MS Analysis

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ABSTRACT

Streptococcus mutans (*S. mutans*) is the primary bacterium responsible for dental caries, forming biofilms that enhance resistance to antimicrobial treatments. This study aimed to evaluate the antibacterial and antibiofilm activities of jicama seed extract (*Pachyrhizus erosus*) against *S. mutans*, as well as to identify the bioactive compounds responsible for these effects. Jicama seeds were extracted using a 70% ethanol solution, and antibacterial activity was assessed using the disc diffusion method, while antibiofilm properties were analyzed via microplate assays. Thin-layer chromatography and bioautography were conducted to identify active compounds further characterized by LC-MS/MS analysis. The results indicated that a 70% concentration of jicama extract inhibited bacterial growth, with inhibition zones increasing proportionally with extract concentrations. Bioautographic and LC-MS/MS analysis confirmed the presence of antibacterial compounds such as alkaloids and flavonoids, including hypaphorine, trigonelline, glabridin, and gancaonin. This compound significantly inhibited *S. mutans* biofilm formation. The LC-MS/MS results also revealed various bioactive compounds with potential antibacterial properties. These findings suggest that jicama seed extract could serve as a natural antibacterial agent, with potential applications in dental care. Further studies should explore optimal extraction methods to enhance its effectiveness and safety in clinical use.

Keywords: Antibiofilm, Jicama seed, *Streptococcus mutans*, flavonoid, Trigonelline

Introduction

Streptococcus mutans (*S. mutans*) is a primary microorganism responsible for dental caries. This Gram-positive bacterium, commonly found in the oral cavity, synthesizes adhesive glucans from sucrose via the action of glucosyltransferases (GTFs). These glucans facilitate firm bacterial adhesion to tooth surfaces, while the additional binding is supported by glucan-binding proteins (Gbp proteins) produced by *S. mutans*.[1] Utilizing carbohydrates, *S. mutans* produces acidic metabolites that lead to enamel demineralization and destruction, ultimately causing dental caries.[2] Dental plaque, a soft, sticky biofilm comprising proteins and bacteria, is present in approximately 80% of individuals with plaque-related dental conditions and is associated with persistent clinical infections.[3,4]

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Recent studies have indicated that biofilms protect *S. mutans* from host immune responses, antimicrobial drugs, nutrient scarcity, and pH variations, which suggests that biofilm formation is a significant factor contributing to bacterial drug resistance.[5] Therefore, controlling *S. mutans* growth and inhibiting biofilm formation are major targets in treating bacterial infections.

Jicama (*Pachyrhizus erosus*), a member of the Fabaceae family, contains seeds rich in various secondary metabolites, including flavonoids, tannins, quinones, saponins, alkaloids, and triterpenoids. These metabolites demonstrate potential antibacterial properties by inhibiting bacterial growth through mechanisms such as DNA or RNA synthesis disruption, interference with cell membrane permeability, and damage to bacterial cell walls.[6] Several studies have highlighted the antibacterial potential of jicama seed extracts. For instance, a 70% ethanol extract of jicama seeds has demonstrated bactericidal activity against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*.[7] Currently, no studies have specifically examined the antibacterial potential of jicama seed 70% ethanol extract against *S. mutans* and its biofilm, according to a thorough review of the existing literature.

This study aims to evaluate the antibacterial activity and antibiofilm properties of 70% ethanol extract from jicama seeds against *S. mutans*. Additionally, we seek to identify the classes of bioactive compounds present in the jicama seed extract that exhibit antibacterial properties through bioautographic analysis. Finally, LC-MS/MS analysis will be conducted to identify specific chemical compounds in the extract that contribute to its antibacterial potential against *S. mutans*. The bioautographic technique is particularly relevant as it

allows for the identification of bioactive compounds responsible for inhibiting bacterial growth, by visually detecting zones of inhibition on the chromatographic plate. Additionally, by identifying different classes of bioactive compounds, we can gain insights into the potential mechanisms of action of the jicama seed extract. Furthermore, LC-MS/MS analysis is critical as it provides detailed chemical identification, allowing for a precise determination of which compounds contribute to the antibacterial activity against *S. mutans*. LC-MS/MS will thus complement the bioautographic analysis by offering structural information on the bioactive metabolites and their potential role in modulating bacterial growth and biofilm formation. Together, these methods provide a comprehensive approach to understanding the antibacterial potential of jicama seed extract.
The discovery of a compound responsible for the antibiofilm and antibacterial effects against *S. mutans* represents the key novelty of this research.

Materials and Methods

Sample Preparation and Extraction
Jicama seeds (*Pachyrhizus erosus*) were sourced from plantations in Rancabungur District, Bogor Regency (-6.537206, 106.737063) with authenticity confirmed by the National Innovation Research Agency (registration number B-3049/II.62/DI.05.07/9/2022). A total of 2 kg of healthy seeds were cleaned, dried at 40°C for three days, ground, and passed through a 40-mesh sieve.[8] For extraction, 500 g of the resulting powder was macerated in 2000 mL of 70% ethanol (Merck, German) for 24 hours. The solution was filtered, and the residue was re-macerated twice with fresh ethanol. The combined filtrates were concentrated with a vacuum rotary evaporator (Rotavapor, R-300, Büchi, Switzerland) at 45°C and further thickened in a water bath (Memmert Water Bath WTB, Memmert, Germany) at 50°C to yield a concentrated extract. Determination of moisture and ash content was carried out following the Association of Official Analytical Collaboration (AOAC) protocol.[9]

Antibacterial Activity Analysis
To assess antibacterial activity, *S. mutans* suspensions (1.5×10^8 CFU/mL) were prepared, and 2 mL of this suspension was added to tubes containing 8 mL of jicama seed extract at concentrations ranging from 30% to 100%.[10] After thorough mixing, the tubes were incubated at 37°C for 24 hours. Samples were then plated on nutrient agar (Merck, USA) and incubated to determine the Minimum Bactericidal Concentration (MBC) based on the absence of visible bacterial growth. Additionally, the Kirby-Bauer disc diffusion method was used to assess the inhibitory zone. Discs impregnated with the extract and a 100 ppm Amoxicillin disc (positive control) were placed on inoculated agar plates and incubated at 37°C for 24 hours. Inhibition zones were measured to determine the antibacterial efficacy.[11]

TLC-Bioautographic Analysis
Thin Layer Chromatography (TLC) was performed on silica plates (1 cm x 10 cm) (TLC Silica gel 60 F254, Canada), which were preheated at 105°C for 10 minutes to remove moisture.[12,13] A 70% solution of jicama seed extract was applied, and the plates were developed in a mobile phase of n-hexane (Merck, Germany) and ethyl acetate (Merck, Germany) (7:3).[14] Post-development, the plates were treated with various reagents: Dragendorff's (Merck, Germany) for alkaloids, $FeCl_3$ (Merck, Germany) for tannins, $AlCl_3$ (Merck, Germany) for flavonoids, and Liebermann-Burchard (Merck, Germany) reagent for terpenoids and saponins. All reagents and solutions used in the bioautography assay were of analytical grade with purity of >99%. Spots were visualized under UV light at 254 nm and 366 nm using a UV-Vis lamp (Camag UV Cabinet Lamp 4 Dual Wave, 254/356 nm, 2x8 W, Camag, Switzerland). Bioautography was conducted by placing the chromatogram on nutrient agar inoculated with *S. mutans* and incubating at 37°C for 24 hours. Clear zones around spots indicated antibacterial activity, allowing the identification of active compounds.[15,16]

Antibiofilm Activity Analysis
The antibiofilm properties of the jicama seed extract were evaluated using a microplate assay. Nutrient Broth (NB) (Himedia, USA) medium (50 µL) was added to each well, followed by 100 µL of *S. mutans* suspension and extract concentrations ranging from 6.25% to 100% in sterile distilled water. The plate was incubated at 37°C for 24 hours, after which the wells were rinsed, dried, and stained with 1% crystal violet. Following an additional wash, 200 µL of 96% ethanol was added to dissolve the stain, and absorbance was measured at 595 nm using a BioTek Epoch 2 Microplate Spectrophotometer (Agilent Technologies, USA) to determine the level of biofilm inhibition.[17]

LC-MS/MS Analysis
The chemical composition of the jicama seed extract was analysed using Liquid Chromatography-Mass Spectrometry (LC-MS) (UHPLC Vanquish Tandem Q Exactive plus Orbitrap HRMS ThermoScientific, Germany). The Accucore C18 column (100 x 2.1 mm, 1.5 µm) (ThermoScientific, Germany) was used with a mobile phase consisting of H_2O + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B). A gradient elution program was applied, with a flow rate of 0.2 mL/min and a column temperature set at 30°C. The mass range analyzed was 100-1500 m/z, in positive ionization mode, to identify bioactive compounds responsible for the antibacterial effects.[18] All solvents used in the LC-MS/MS analysis were of LC-MS grade.

Data Analysis
Data analysis was performed to determine the concentrations of jicama seed extract exhibiting antibacterial activity against *S. mutans*. A Completely Randomized Design (CRD) with Analysis of Variance (ANOVA), followed by a post hoc Duncan test, was performed using Prism 10 for macOS (GraphPad Software, 2024) and IBM SPSS Statistics version 26 (2019).

Results and Discussions

Extraction yield of jicama seed
The extraction process of jicama seeds yielded 39.0% for the powder and 22.03% for the viscous extract, as shown in Table 1. The moisture content of the powder was 4.79 ± 0.36%, about twice that of the extract (2.39 ± 0.26%), while the ash content was also higher in the powder (3.05 ± 0.98%) compared to the extract (2.18 ± 0.21%). Interestingly, the yield of the extract obtained from the maceration process, 22.3%, was higher than the 19.1% reported by a previous study,[7] likely due to differences in growth location and extraction methods. Factors such as the soil composition and climate in Rancabungur District, Bogor Regency, where the seeds were sourced, may have contributed to this increase, along with using 70% ethanol as a solvent and the re-maceration process, which enhanced the extraction efficiency. The differences in moisture and ash content can be attributed to the drying process used for the powder, which likely retained higher levels of these components, while the extraction and evaporation steps for the viscous extract resulted in a more concentrated form of bioactive compounds (Table 1).

Antibacterial activity of jicama seed extract
The antibacterial efficacy of jicama seed extract against *S. mutans* was demonstrated using the disc diffusion method. A minimum bactericidal concentration of 70% was sufficient to initiate inhibitory effects, resulting in a zone of inhibition measuring 4.97 mm. Higher concentrations showed a positive correlation with larger inhibition zones, with the maximum inhibition at 100% concentration reaching 12.56 mm. In comparison, the positive control (amoxicillin, 100 ppm) exhibited a significantly larger inhibition zone of 22.54 mm ($P < 0.05$), highlighting its superior antibacterial effect. In contrast, the negative control showed no inhibition. Figure 1B visualizes the inhibition zones at varying extract concentrations, and Figure 1C shows the minimum bactericidal concentration (MBC) range from 30% to 100%. These findings are consistent with previous studies,[19] which demonstrated significant inhibition of *S. mutans* using a 90% ethanol-based jicama extract. Nevertheless, the potency of jicama extract was lower

compared to propolis, with MIC and MBC values of 293 µg/mL and 1172 µg/mL, respectively,[20] and green tea extract, with a lower MIC of 150 mg/mL,[21] *Caesalpinia sappanl* with MIC 17%,[22] as well as *Rhizophora mucronata Leaves Extract with MIC 0.78%.*[23]

Table 1: Yield, moisture content, and ash content of jicama seed powder and extract

No.	Parameter	Jicama Powder	Jicama Extract
1.	Yield (%)	39.00	22.03
2.	Moisture Content (%)	4.79 ± 0.36	2.39 ± 0.26
3.	Ash Content (%)	3.05 ± 0.98	2.18 ± 0.21

Thin-layer chromatography (TLC) and bioautography analysis
Figure 2 illustrates the phytochemical testing using thin-layer chromatography (TLC) analysis, showing that secondary metabolites such as flavonoids, alkaloids, quinones, and saponins contribute to inhibiting *S. mutans*. TLC analysis revealed distinct Rf values and color changes for each compound: flavonoids (Rf 0.71–0.72, greenish with Aluminum Chloride), alkaloids (Rf 0.62–0.65, reddish with Dragendorff reagent), quinones (Rf 0.40–0.42, black with Ferric Chloride), and saponins (Rf 0.33–0.35, brownish with Lieberman-Burchard reagent). Four active compounds were identified through contact TLC-bioautography, while terpenoids and tannins were not detected, requiring further investigation.

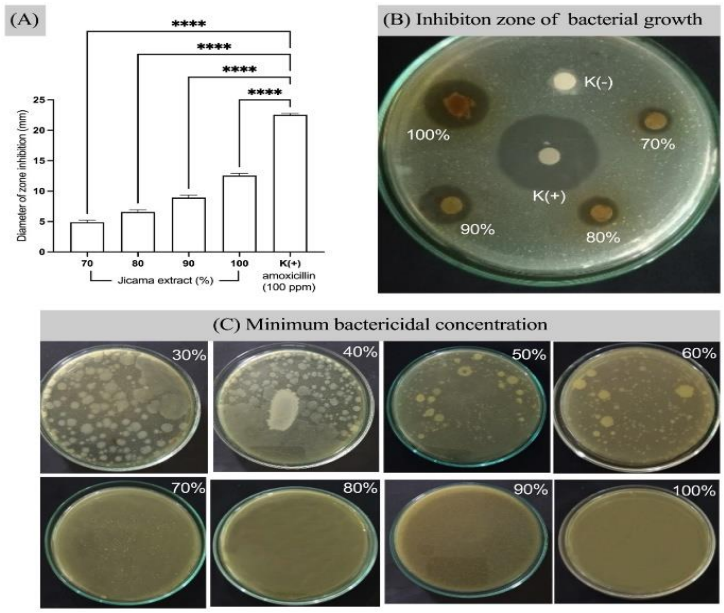

Figure 1: Antibacterial activity of jicama seed extract against *S. mutans*. (A) Diameter of inhibition zones for *S. mutans* treated with various concentrations of jicama seed extract and amoxicillin (100 ppm), showing significant differences (****P < 0.0001) as determined by Bartlett's test. Significant differences among groups (P < 0.05) were further analyzed using ANOVA followed by Duncan's post hoc test, indicated by different letters (a, b, c, d). (B) Inhibition zones of *S. mutans* growth at different jicama extract concentrations (70%, 80%, 90%, 100%), with K (+) as the positive control (amoxicillin) and K(-) as the negative control. (C) Minimum bactericidal concentration of jicama extract against *S. mutans* at concentrations ranging from 30% to 100%

The TLC-bioautography results presented in Figure 3 indicate the presence of antibacterial compounds in jicama seed extract with varying inhibitory zones against *S. mutans*. In both replicate 1 (r1) and replicate 2 (r2), the Rf values (retention factors) for the four observed zones (a, b, c, and d) consistently displayed the inhibitory zone. The Rf of the inhibitory zone was in line with the TLC Rf spot which exhibited the existence of flavonoids, alkaloids quinone, and saponin compounds (Figure 3).

Antibiofilm analysis
The results presented in Figure 4 demonstrate the differential effects of chlorhexidine and jicama seed extract on biofilm formation by *S. mutans*. Chlorhexidine (Figure 4-a) effectively inhibits biofilm formation at low concentrations, achieving near-complete inhibition (0.0125% to 0.05%). The inhibitory effect remains robust, at higher concentrations, peaking around 45% inhibition at 0.2%, suggesting a consistent antibacterial activity across concentrations. In contrast, jicama seed extract (Figure 4-b) exhibits a significantly weaker antibiofilm effect (P < 0.05) than chlorhexidine. Interestingly, the inhibitory effect of jicama seed extract increases with concentration, from approximately 20% inhibition at 6.25% to 80% inhibition at 100%, indicating a dose-dependent antibiofilm effect. These findings align with the half-maximal inhibitory Concentration (IC50) of jicama seed extract, which inhibits biofilm formation of *S. mutans* at 23.82 ± 8.19%. This is notably less potent compared to colchicine, which has an IC50 of 0.34 ± 0.16%, and both alcoholic and aqueous green tea extracts, which exhibit minimum biofilm inhibitory concentrations (MBICs) ranging from 3.1–12.5 mg/mL and 6.5–50 mg/mL, respectively.[2]

The higher concentrations required for jicama seed extract to achieve antibiofilm effects compared to green tea and propolis may be attributed to its higher protein content of 28.27 g/100 g, contrasting with the lower protein content in green tea (1.05 ± 0.00%).[24] This suggests that the high protein content in jicama seeds could contribute to the reduced efficacy observed in biofilm inhibition.

Phytochemical analysis
The LC-MS/MS analysis presented in Figure 5 and summarized in Table 2 identified various bioactive compounds in jicama seed extract that demonstrate significant antibacterial properties. The chromatogram revealed peaks corresponding to different classes of compounds, including alkaloids, flavonoids, and phenolics. Notably, the alkaloids identified included hypaphorine, trigonelline, and γ-mangostin, along with isoquinoline alkaloids such as 1,2,3,9,10-pentamethoxy-7H-

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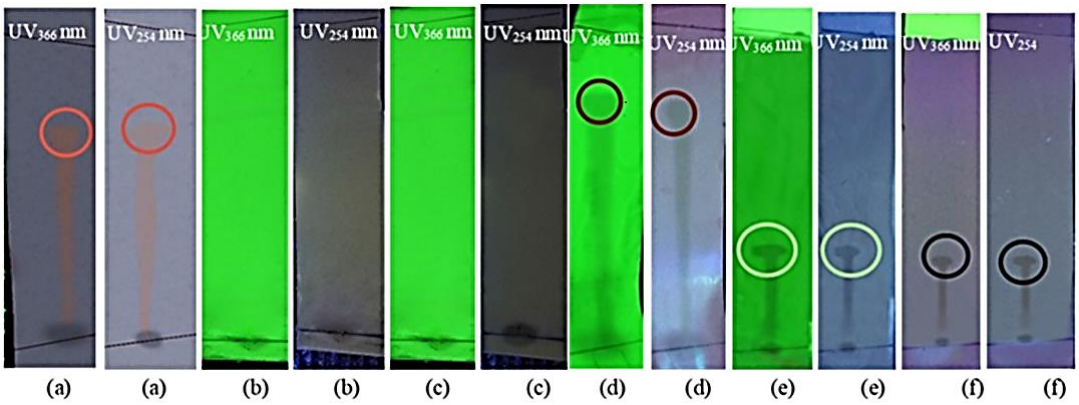

Figure 2: Phytochemical testing results of alkaloid, terpenoid, tannin, flavonoid, quinone, and saponin compounds on thin-layer chromatography (TLC) plates visualized under UV light (366 nm and 254 nm). Spot (a) represents alkaloid compounds, spot (b) indicates terpenoid compounds, spot (c) shows tannin compounds, spot (d) identifies flavonoid compounds, spot (e) highlights quinone compounds and spot (g) reveals saponin compounds

dibenzo[de,g]quinolin-7-one (oxopurpureine). These alkaloids inhibit bacterial growth by disrupting nucleic acid synthesis, altering membrane permeability, inhibiting metabolism, and blocking efflux pumps.[25] Hypaphorine exhibited antibacterial activity against Gram-positive bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, and *Mycobacterium smegmatis*. Trigonelline, which is also present in roasted coffee, exhibits activity against *S. mutans* and demonstrates antibiofilm effects by inhibiting quorum sensing. Quorum sensing is a cell-density-dependent communication mechanism that regulates biofilm formation and virulence characteristics.[30–32]

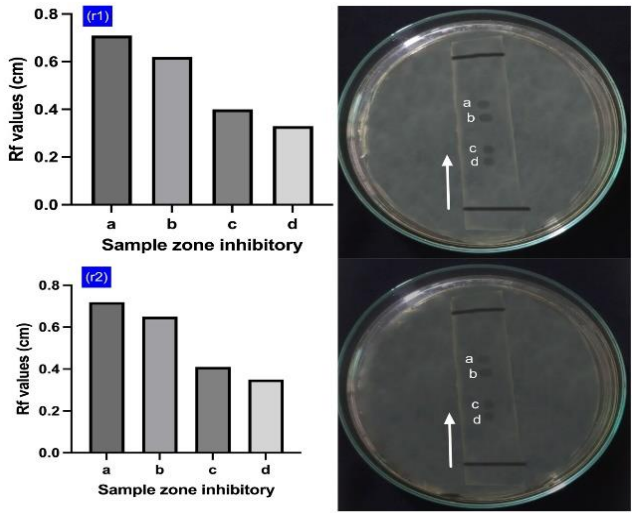

Figure 3: TLC-bioautography of antibacterial compounds against *S. mutans* from jicama seed extract, with (r1) and (r2) representing replicate 1 and replicate 2, respectively.

Bioautographic analysis also indicated the presence of flavonoids in the jicama seed extract. These compounds exert antibacterial effects through mechanisms such as inhibiting nucleic acid synthesis, disrupting membrane functions, and inhibiting biofilm formation. The identified flavonoids include flavonols such as dihydromyricetin (DHM) and retusin, isoflavonoids such as glabridin and gancaonin, and prenylated xanthones like γ-mangostin. Glabridin exhibited both antibacterial and antibiofilm effects against *S. mutans*, with a minimum inhibitory concentration (MIC) ranging from 6.25 to 12.5 µg/mL, and its mechanism of action involves increasing membrane permeability.[33,34] Gancaonin, a 6-prenylated isoflavone, was also effective against *S. mutans* at a low MIC of 1 to 2 µg/mL, as well as against other Gram-positive bacteria, including vancomycin-resistant *Enterococcus faecium*.[35,36] Although gancaonin's impact on *S. mutans* biofilm formation remains underexplored, its low MIC suggests its potential as an antibacterial agent.

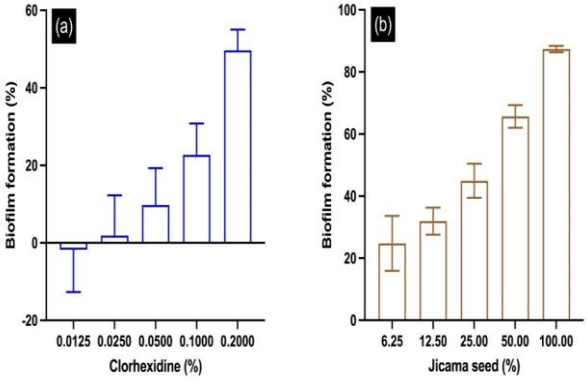

Figure 4: Comparative effects of chlorhexidine (a) and jicama seed extract (b) on biofilm formation of *S. mutans* at varying concentrations

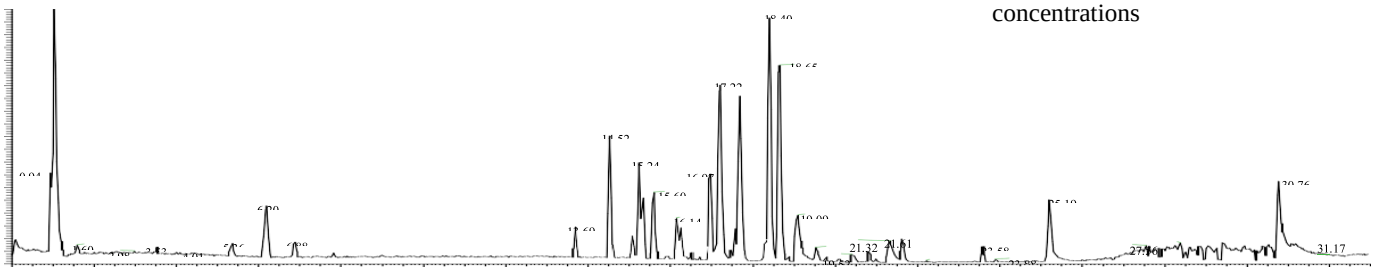

Figure 5: LC-MS/MS base peak chromatogram of jicama seed extract analyzed in positive ESI mode

Table 2: Bioactive compounds identified in jicama seed extract via LC-MS/MS analysis and their reported antibacterial activities

No	Compound Name	Formula	RT [min]	Molecular Weight (MW)	Class	Antibacterial Activity	References
1	1-*O*-Phosphonopentitol	$C_5H_{13}O_8P$	1.1	232.03	Sugar alcohol	Not specified	
2	Methyl alpha-aspartylphenylalaninate	$C_{14}H_{18}N_2O_5$	6.191	294.12	Dipeptide, sweetening agent	Not specified	
3	L-Canavanine	$C_5H_{12}N_4O_3$	1.008	176.09	Non-proteinogenic amino acid	Insecticidal properties	
4	L-Histidine	$C_6H_9N_3O_2$	1.01	155.07	Amino acid	Not specified	
5	L-(+)-Arginine	$C_6H_{14}N_4O_2$	1.015	174.11	Amino acid	Not specified	
6	L-Glutamic acid	$C_5H_9NO_4$	1.071	147.05	Amino acid	Not specified	
7	L-Tyrosine	$C_9H_{11}NO_3$	1.599	181.07	Amino acid	Not specified	
8	Dihydromyricetin (DHM)	$C_{15}H_{12}O_8$	6.872	320.05	Flavonol	Antibacterial and antibiofilm against *S. aureus*, *E. coli*, *P. aeruginosa* [46]	[46]
9	Retusin	$C_{19}H_{18}O_7$	16.568	358.10	Flavonol	Antibacterial against *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*[47]	[47]
10	Hypaphorine	$C_{14}H_{18}N_2O_2$	6.182	246.14	Indole alkaloid	Antibacterial against Gram-positive bacteria including *B. cereus*, *S. aureus*, *M. smegmatis*[26]	[26]
11	Trigonelline	$C_7H_7NO_2$	1.086	137.05	Alkaloid	Antibacterial and antibiofilm against *S. mutans* [28] and Pseudomonas[29]	[28,29]
12	γ-Mangostin	$C_{23}H_{24}O_6$	17.01	396.16	Prenylated xanthone	Antibacterial against Gram-positive bacteria including *MRSA*[42]	[42]
13	Combretastatin A-4	$C_{18}H_{20}O_5$	16.369	316.13	Stilbenoid phenol	Effective against *S. aureus*[48]	[48]
14	Glabridin	$C_{20}H_{20}O_4$	18.225	324.13	Isoflavone	Reduced biofilm viability of *S. mutans*, MIC range 6.25 to 12.5 µg/mL[34]	[34]
15	Myricetin 3-O-beta-D-galactopyranoside	$C_{21}H_{20}O_{13}$	8.969	480.09	Flavonoid-3-*O*-glycoside	Inhibited biofilm formation of *S. aureus*[49,50]	[49,50]

Other phenolic compounds identified include γ-mangostin, DL-Cerulenin, and alterperylenol, which exhibited notable antibacterial activity, particularly against *S. aureu.s.* [37–42] However, their specific antibacterial activity against *S. mutans* has not yet been thoroughly studied. Unlike flavonoids, phenolic compounds typically do not directly inhibit glycolytic activities or glucosyltransferases (GTFs) in *S. mutans* but rather exert antibacterial effects by broadly interfering with bacterial metabolic processes or cellular integrity.

The antibacterial effects of jicama seed extract arise from interactions among its constituent bioactive compounds. The high protein content of jicama seeds, measured at 28.27 g/100 g, may interfere with the effectiveness of compounds like trigonelline, glabridin, and gancaonin, necessitating higher concentrations of the extract to achieve effective minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC). Furthermore, the presence of rotenone, an insecticidal compound, poses a challenge to applying jicama seed extract due to its potential toxicity. Rotenone has been associated with oxidative damage at the cellular level and neuronal loss, contributing to Parkinson's disease.[44] The World Health Organization classifies rotenone as moderately hazardous, with an oral LD50 (lethal dose for 50% of the population) ranging from 132 to 1500 mg/kg in rats.[43] However, processing methods such as drying, roasting, and maceration have been shown to reduce rotenone content by up to 80%.[44] Moreover, improvements in protein content and bioavailability through boiling, fermenting, and malting processes may help minimize the negative interactions between protein content and bioactive compounds.[45]

Conclusion

This study confirmed the antibacterial and antibiofilm potential of jicama seed extract against *S. mutans*. At a minimum bactericidal concentration of 70%, the extract effectively inhibited bacterial growth, with higher concentrations yielding larger inhibition zones. Bioautography and LC-MS/MS analyses revealed key bioactive compounds, including alkaloids, flavonoids, and phenolics, with notable contributions from hypaphorine, trigonelline, glabridin, and gancaonin. The findings highlight jicama seeds as a promising natural source of antibacterial agents against *S. mutans*, suggesting potential applications in dental care. Further studies should refine extraction methods and evaluate clinical safety and efficacy.

Conflict of Interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors declare that the work presented in this article is original, and that any liability for claims related to the content of this article will be borne by them.

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