Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.784

Phytochemical Analysis and Antibacterial Activity of *Stevia Rebaudiana Bertoni* Leaves Extract against *Streptococcus sanguis* (A Primary Inhabitant of Dental Plaque): In Vitro Study

Manar Ibrahim Ahmed[1], Safa Ali Hamad[2], Maha Abdulsalam[2]

[1] *Al-Bayan University College of Dentistry, Baghdad, Iraq*

[2] *Al-Hikma College University, Department of Dentistry, Baghdad, Iraq*

Abstract

Objective: Dental plaque is considered the primary causative agent in developing periodontal diseases. Early colonizers of dental plaque, such as Streptococcus sanguis (S.sanguis), are crucial in the succession steps of biofilm formation. As an alternative to the commonly used chlorohexidine (CHX), it is of interest to find naturally occurring antimicrobial substances from plants.

Materials and Methods: Volunteers were asked to provide plaque samples. Microscopic examination, gram stain, optochin test, catalase test and polymerase chain reaction were used to ensure the identification of S. sanguis. Stevia Rebaudiana Bertoni leaves extracted by 70% ethanol alcohol. Four experiments have been done in this study: the susceptibility of S. sanguis to stevia extract, the minimum inhibitory (MIC) and the minimum bactericidal (MBC) concentrations, and exploration of the extract effective constituents by using HPLC.

Results: Stevia extract had good antibacterial activity with varying inhibition zone diameters that were concentration dependent, but 0.2% CHX showed better activity with a statistically significant difference ($p < 0.05$). Both MIC and MBC were at 16 mg/ml. HPLC analysis confirmed the presence of antibacterial constituents: narigenin 25.76 ppm, catechin 30.25 ppm, coumarin 25.47 ppm, and kaempferol 4.59 ppm.

Conclusions: The antimicrobial activity of the alcoholic Stevia Rebaudiana Bertoni leaf extract was satisfactory. The study extract exhibited lower antibacterial activity at 512 mg/ml of stevia extract, while 0.2% CHX had superior activity overall. HPLC showed that the alcoholic leaves extract of Stevia Rebaudiana Bertoni contains several active antibacterial components: narigenin, catechin, coumarin and kaempferol.

Keywords: Streptococcus sanguis, Stevia Rebaudiana (Bertoni), Antibacterial Activity, High Performance Liquid Chromatography.

Citation: Ahmed MI, et al. (2025) Phytochemical Analysis and Antibacterial Activity of Stevia Rebauduana Bertoni Leaves Extract against Streptococcus sanguis (A Primary Inhabitant of Dental Place): In Vitro Study. Dentistry 3000. 2.a001 doi:10.5195/d3000.2025.784
Received: November 29, 2024
Accepted: December 18, 2024
Published: February 5. 2025
Copyright: ©2025 Ahmed MI, et al. This is an open access article licensed under a Creative Commons Attribution Work 4.0 United States License.
Email: Manar.i@albayan.edu.iq

Introduction

Many kinds of microbes call the mouth and gums their home [1]. The mouth supports distinct microbial communities, with each site containing an average of 50 species (a subset of 1,000 species capable of oral colonization) [2]. Oral surfaces are most often colonized by streptococci and actinomyces species, inter-bacterial streptococci binding has major implications for periodontal disease development in that several co-adhering organisms are periodontal pathogens. Decreased oxygen tensions favor population shifts, allowing strict anaerobes like Bacteroidaceae spp. and spirochaetes to flourish [3,4]. Biofilm in the form of supragingival and subgingival plaque causes periodontal disease [5]. Tamura et al. (2008) [6] postulated that some kinds of bacteria found above the gum line would entice free-living pathogens to the subgingival plaque, hence exacerbating periodontal disorders. Ximenez et al. (2000a,b) [7,8] discovered that

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.784

improving periodontal condition and reducing pathogenic bacterial species, including P. gingivalis, may be achieved by eliminating the supragingival plaque from periodontal patients. Biofilm bacteria produce a thin extracellular matrix that encloses and protects microbial populations from the environment, including chemotherapy attacks [9]. Mechanical methods are required to remove the dental biofilm on a regular basis, and antiseptics such as mouth rinse can aid in biofilm control.

Medicinal plants are becoming increasingly popular around the globe to treat a wide range of illnesses [10]. Stevia Rebaudiana (Bertoni) "Sweet Herb of Paraguay" is a zero-calorie sweetener native to South America, where it was initially ingested over 200 years ago by native people [11]. Stevia rebaudiana leaves contain over 100 phytochemicals, which are secondary metabolites that plants accumulate to defend themselves against microbial infections. They are active ingredients with therapeutic properties used to make medicines and drugs [12]. Stevia has anti-microbial, anti-hypertensive, anti-cariogenic, diuretic, anti-viral, anti-diarrheal, immunomodulatory, anti-mutagenesis, anti-teratogenesis and anti-allergic properties [13,14].

As far as we are aware, the potential antimicrobial properties of stevia extract against many microorganisms have been investigated, but no one proved the presence of an antibacterial effect against S. sanguis.

Material and Methods

The Research Ethics Review Board of the College of Dentistry Medical Ethical Committee at Baghdad University approved the protocol of this study. Stevia leaves were collected and extracted at University of Baghdad Research Unit of Aromatic and Medical Plants. By maceration according to Seidel, 2012 [15]; stevia leaves powder (100gm) was extracted with 1 liter of 70% ethanol alcohol. Using a laboratory spray dryer, the filtered ethanolic extract was spray dried to yield powder.

Prior to taking plaque samples, consents were obtained. Patients were required to refrain from using antibacterial mouthwash for a minimum of one month before the research. Brain Heart Infusion Broth, 1.5 mL, was added to sterile collection tubes as soon as samples were taken [16]. Samples were cultured on mitis salivarious blood agar aerobically for 24 hours at 37°C. Gram stain [17], hemolysis in blood agar [18], sensitivity to optochin [19], catalase reaction [20], and polymerase chain reaction [21] were all used to identify and characterize the isolated colonies.

The ABIOPureTM genomic DNA protocol was used to extract the DNA from bacterial growth. The concentration of DNA sample (20 ng/µl) was measured using a quantus fluorometer device. Macrogen Company provided the primers in lyophilized state: S. sanguis-F 5`-GGATAGTGGCTCAGGGCAGCCAGT T-3`, S. sanguis-R 5`-GAACAGTTGCTGGACTTGCTTGTC-3`, annealing temperature 70˚C and product size (Pb) 313 [21]. To obtain the stock solution, a concentration of 100 pmol/µl was achieved by dispersing lyophilized primers in 300µl of nuclease-free water. By mixing 10µl of stock primers with 90µl of nuclease-free water, a solution with a concentration of 10 pmol/µl was prepared. Following the manufacturer's instructions, a final solution of 20 µl was created by mixing 10 µl of master mix with 1 µl each of forward primer, reward primer, 6 µl of nuclease-free water, and 2 µl of the sample DNA.

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.784

The thermal cycling protocol was followed as directed by the manufacturer, with a reaction volume per run of 20 and a total of 30 PCR cycles. The PCR device was used to amplify the bacterial DNA samples with the following settings (Table 1).

Table 1. PCR program.

Stages	Temperature (°F)	Time (Min.)	Rounds
Preliminary denaturation	203	5	1
Denaturation	203	0.5	30
Annealing	158	0.5	
Elongation	161.6	1	
Definitive extension	161.6	7	1
Hold	50	10	

To verify PCR amplification, an agarose gel electrophoresis was performed. After sealing the edges of the gel tray with cellophane tapes, the agarose was poured into the tray and allowed to solidify at room temperature for 30 minutes. Then, the tray was filled with 1X TAE-electrophoresis buffer until the buffer reached a depth of 3-5 mm over the gel's surface. We loaded the PCR products immediately. Wells were immediately filled with 5µl of PCR product. After 60 minutes, the electricity was switched on at 100 volts per milliampere. The DNA molecule cycles between the cathode and anode ends. Gel imaging system was used to visualize the bands stained in ethidium bromide.

According to Cavalieri et al, (2005) [19], a suspension of S. oralis was prepared by using “direct colony suspension method”, confined colonies were picked from the agar media and grown in 1 ml mueller-hinton broth (MHB). The suspension was vortexed and adapted to a 0.600 absorbance at 600 nm, which corresponds to 0.5 McFarland using an absorbance plate reader [22].

Method for dispersing agar drops, as described by Cavalieri et al, 2005 [22] was used to test the activity of the study extract against S. sanguis. Using a Pasteur pipette under aseptic conditions, 6 mm wells of similar depth were created in each mueller hinton agar plate. 50 µl from each of stevia extract concentrations (512mg/ml, 256mg/ml, 128mg/ml, 64mg/ml, 32mg/ml and16mg/ml) were added to each well. The positive control was 0.2 % chlorhexidine gluconate (non-alcoholic), and the negative control was sterile distilled water. For 24 hours, the plates were maintained in an aerobic atmosphere at 37°C. Each well's inhibition zone was measured in (mm) using a ruler.

In order to determine the minimum inhibitory concentration (MIC), the experiment used two-fold serial broth micro-dilutions [19,22]. Between wells 1 and 9, 100 µl of brain-heart infusion broth (BHI) was added to two rows of a 96-well microplate. There was a two-fold serial dilution process starting from well 1 and continuing until well 9, after which 100 µl of the 512 mg/ml alcoholic stevia extract was added to each well in the appropriate row. Well 10 in the first row had 100 µl of broth and 100 µl of 0.2% CHX, whereas wells 11 and 12 had 100 µl of MHB alone; the whole first row was regarded as plain, devoid of bacteria. One hundred microliters of the test organism were added to each well in the second row. Well 10 in the second row served as the positive control, with 100 µl of 0.2% CHX in broth and 100 µl of bacterial suspension; in contrast, wells 11 and 12 in the negative control group contained 100 µl of MHB and 100 µl of bacterial suspension, respectively. Overnight, the microtiter plate was incubated at 37 ˚C in an aerobic environment. An "absorbance micro plate reader" calibrated to a wavelength of 600 nm was used to assess the turbidity of the wells after incubation.

Minimum bactericidal concentration (MBC) was established by placing 50 µl

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.784

portions from the minimum inhibitory concentration (MIC) well and the well immediately above it onto brain-heart agar plates and leaving them to incubate at 37 °C for 24 hours. The appearance of bacteria was then determined by looking at the agar plates [23].

An HPLC analysis was used to determine the concentrations of the active ingredients in an alcohol-based Stevia Rebaudiana leaf extract [24]. This test was carried out at the Ministry of Science and Technology/ Material's Research Department. To detect and quantify narigenin, catechin, coumarin and kaempferol, a stevia alcoholic extract was prepared for HPLC testing. Blend 1 gram of Stevia extract with 5 milliliters of HPLC-grade acetonitrile in a glass vial; cover with paraffin wax; vortex well; and subject to an ultrasonic bath set at 35 degrees Celsius for 15 minutes. The standards concentration was set to 5 part per million (ppm).

After filtering the solution with a 0.45 micro filter, 20 l of the solution was injected into an HPLC binary pump machine. To calculate the sample's concentration, the following formula was used:

"Conc. Of sample = (conc.of standard ×Area of sample)/(Area of standard) ×dilution factor"

The data were summarized by maximum, minimum, standard deviation (SD), standard error (SE), and mean values. Levene test, One Way Analysis of Variance (ANOVA), and Shapiro Wilk test were used. These tools were part of the Statistical Package for the Social Sciences (SPSS version -22, Chicago, Illinois, USA). P>0.05 was considered not significant, whereas P<0.05 was considered significant.

Results

Identification of S. sanguis: On MSA**,** S. sanguis colonies were small spherical colonies, blue in color with slightly raised surfaces and greatly adhere to the agar surface. On 40X magnification, cells of S. sanguis appeared as spherical purple cocci (gram- positive) and they were arranged in medium to long chains. Results from biochemical tests confirmed that the study organism is an alpha hemolytic, gram positive, catalase negative and optochin resistant streptococci. The PCR results validated the diagnosis of S. sanguis, the identification of S. sanguis was clarified by PCR findings (Figure1).

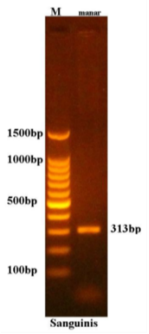

Figure 1. S. sanguis DNA amplification results. M stands for 100bp ladder marker, and Bp stands for base pair.

The zones of inhibition for S. Sanguis grew in diameter with increasing concentrations of the study extract; the greatest one was in chlorohexidine, and this difference was statistically significant ($p > 0.05$). Also, when comparing each group to each other and to chlorohexidine, the results demonstrated a significant difference using multiple comparisons among groups by Tukey's HSD (p more than 0.05). Tables 2, 3, and 4 show the results. According to an "absorbance microplate reader", MIC and MBC of the study extract against S. sanguis both were 16 mg/ml. Bacteriostatic activity has been observed with 0.2% CHX (0.1 percent in broth).

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.784

Table 2. Descriptive statistics for the diameter of the S. sanguis inhibition zone in different groups (Levene test= 0.829, p value= 0.558).

Groups/ (mg/ml)	Mean	(±SD)	(±SE)	Min.	Max.
16	12.4	0.418	0.187	12.000	13.000
32	15.1	0.418	0.187	14.500	15.500
64	17.3	0.447	0.200	17.000	18.000
128	19.8	0.570	0.255	19.000	20.500
256	22.6	0.548	0.245	22.000	23.000
512	26.3	0.274	0.122	26.000	26.500
CHX	28	0.500	0.224	27.500	28.500

Table 3. A one-way analysis of variance (ANOVA) was used to compare the groups' inhibitory zone diameters for S. sanguis. A p-value less than 0.05 is considered statistically significant.

ANOVA					
	Sum of squares	df	Mean square	F	P value
Between groups	996.143	6	166.024	774.778	0.000 Sig.
Within groups	6.000	28	0.214		
Total	1002.143	34			

Table 4. Tukey HSD analysis of multiple comparisons of S. sanguis inhibition zone diameter between groups.

Groups/ (mg\mL)		(Mean differenc e)	(P- value)	
16	32	-2.700	0.00000	Significant
	64	-4.900	0.00000	
	128	-7.400	0.00000	
	256	-10.200	0.00000	
	512	-13.900	0.00000	
	CHX	-15.600	0.00000	
32	64	-2.200	0.00000	
	128	-4.700	0.00000	
	256	-7.500	0.00000	
	512	-11.200	0.00000	
	CHX	-12.900	0.00000	
64	128	-2.500	0.00000	
	256	-5.300	0.00000	
	512	-9.000	0.00000	
	CHX	-10.700	0.00000	
128	256	-2.800	0.00000	
	512	-6.500	0.00000	
	CHX	-8.200	0.00000	
256	512	-3.700	0.00000	
	CHX	-5.400	0.00000	
512	CHX	-1.700	0.00006	

Using high-performance liquid chromatography, the presence of effective antibacterial phytochemical compounds was encountered at the following concentrations: narigenin (25.76 ppm), catechin (30.25 ppm), coumarin (25.47 ppm), and kaempferol (4.59 ppm) (Figure 2).

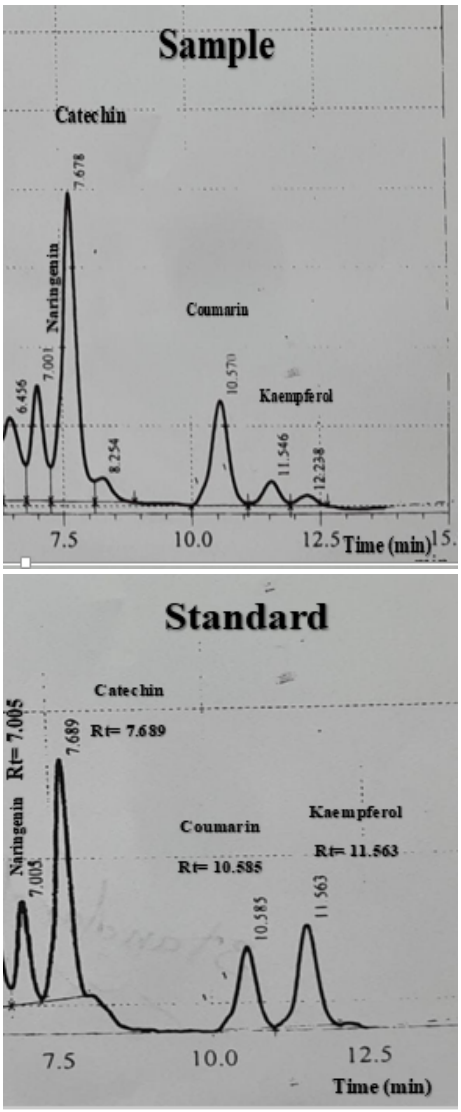

Figure 2. Narigenin, catechin, coumarin, and kaempferol were found in the study extract using HPLC. Rt: retention time.

Discussion

More and more studies are being conducted on plant extracts and the active ingredients in them from various parts of the world for their antibacterial activity [25]. Bacteria

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.784

cannot develop resistance to plant extracts because they are chemicaly complicated in structure, and any of its constituents may have an antimicrobial role [26].

According to studies, Stevia Rebaudiana (Bertoni) is a possible drug candidate with numerous nutritional and medicinal properties. It has been found to have anti-inflammatory, anti-gingivitis, anti-plaque, anti-cariogenic and health-promoting characteristics [27, 28]. The current study has been conducted and may be regarded the first report because of the previously stated characteristics and because stevia has no proven antibacterial activity on S. sanguis.

The antibacterial effect of the study extract against S. sanguis was found to be concentration dependent, as the concentration of the extract rose, the inhibition zone widened, suggesting that the quantity of soluble active chemicals in the extract was also rising. After comparing the advantages of stevia with the disadvantages of CHX, the research extract showed impressive results, with an average inhibitory zone width of 26.3 mm against S. sanguis at 512 mg/ml, which is comparable to the 28 mm seen at 0.2% CHX, we might come up with a satisfactory result. The extract's MIC and MBC were both found to be 16 mg/ml, showing that it exhibits bactericidal activity. Levison, (2004), as cited by Mogana et al., (2020) (29), said that if the mbc/mic ratio is less than 4, the agent is bactericidal. The similar outcome was reached by Parvekar et al., 2020 [30], who investigated the MIC / MBC for silver nanoparticles on Staph. aureus.

The exact mechanism of stevia's antibacterial action is unknown, although it could be linked to the existence of antimicrobial compounds such as: di-hydrodeoxy-streptomycin, diterpene glycosides, tannins, flavonoids, polyphenols and saponins [31-35]; and the extract's phytochemical components' synergism. Due to the interaction of multiple distinct chemicals in plant extracts, developing microbial resistance through genetic alterations driven by external stimuli is more challenging [36].

Many active compounds were found in alcoholic stevia extract by HPLC analysis, including narigenin, catechin, coumarin, and kaempferol. Narigenin is a bioactive flavonoid with good antibacterial properties, its concentration in the study extract was (25.76 ppm). Studies have reported its inhibitory action against: Escherichia coli, Staphylococcus aureus, Lactobacillus rhamnosus and Salmonella typhimurium [37, 38]. After being exposed to narigenin, by altering the ratio of certain fatty acids in their membranes, E. coli and Staphylococcus aureus cells demonstrated an increase in membrane fluidity. Narigenin also significantly down-regulated genes in bacteria involved in fatty acid production at higher doses [39].

Catechin is a polyphenol found in high concentration)30.25 ppm) in the study extract, it has antibacterial activity [40]. The mechanism of catechin action propose that the catechins influence the microbial membrane, altering its fluidity and lowering thiourea and cycloleucine flux [41]. The presence of the negatively charged lipopolysaccharide may explain why it is more effective against gram positive bacteria than gram negative bacteria, since it alters the structure of the membrane and damages the lipid bilayer [42,43].

Coumarin concentration in the study extract was (25.47ppm). Coumarin is a naturally occurring benzopyrone which is a potent inhibitor of DNA- gyrase, its derivatives exhibit antibacterial effect against both gram negative

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.784

and methicillin-resistant Staphylococcus aureus (MRSA), novobiocin, vancomycin, and teicoplanin-resistant Enterococci species are among the gram-positive bacteria that are most often seen [44,45].

The concentration of kaempferol in the study extract was (4.59 ppm). Kaempferol separated from Camellia oleifera demonstrated broad antibacterial activity against Staphylococcus aureus, E. coli, Salmonella enteriditis, Aspergillus niger, Bacillus thuringiensis and Rhizopus nigricans [46].

Conclusion

The results of this study demonstrate that the extract has an antibacterial effect and contains abundant antibacterial components; this might pave the way for further studies into its potential usage in oral hygiene products. Additional studies examining its toxicity and antibacterial effects on periodontal diseases are necessary to validate its use as a preventive intervention for oral health.

References

1- Abusleme, L., Dupuy, A. K., Dutzan, N., Silva, N., Burleson, J. A., Strausbaugh, L. D., Gamonal, J., & Diaz, P. I. (2013). The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. The ISME journal, 7(5), 1016–1025.

2- Griffen, A. L., Beall, C. J., Campbell, J. H., Firestone, N. D., Kumar, P. S., Yang, Z. K., Podar, M., & Leys, E. J. (2012). Distinct and complex bacterial profiles in human periodontitis and health revealed by 16S pyrosequencing. The ISME journal, 6(6), 1176–1185.

3- Aas, J. A., Paster, B. J., Stokes, L. N., Olsen, I., & Dewhirst, F. E. (2005). Defining the normal bacterial flora of the oral cavity. Journal of clinical microbiology, 43(11), 5721–5732.

4- Dewhirst, F. E., Chen, T., Izard, J., Paster, B. J., Tanner, A. C., Yu, W. H., Lakshmanan, A., & Wade, W. G. (2010). The human oral microbiome. Journal of bacteriology, 192(19), 5002–5017.

5- Haffajee, A. D., Dibart, S., Kent, R. L., Jr, & Socransky, S. S. (1995). Factors associated with different responses to periodontal therapy. Journal of clinical periodontology, 22(8), 628–636.

6- Tamura, A., Ara, T., Imamura, Y., Fujii, T. & Wang, P. (2008). The effects of antibiotics on in vitro biofilm model of periodontal disease. Eur J Med Res, 13, 439-445.

7- Ximénez-Fyvie, L. A., Haffajee, A. D. & Socransky, S. S. (2000a). Comparison of the microbiota of supra- and subgingival plaque in health and periodontitis. J Clin Periodontol, 27, 648-57.

8- Ximénez-Fyvie, L. A., Haffajee, A. D., Som, S., Thompson, M., Torresyap, G. & Socransky, S. S. (2000b). The effect of repeated professional supragingival plaque removal on the composition of the supra- and subgingival microbiota. J Clin Periodontol, 27, 637-47.

9- Saini, R., Saini, S. & Sharma, S. (2011). Biofilm: A dental microbial infection. Journal of natural science, biology, and medicine, 2, 71-75.

10- Rafiq, K., Sherajee, S. J., Sufiun, M., Mostofa, M., Alam, A. & Barman, B. (2011). Comparative efficacy of stevia leaf (stevia rebaudiana bertoni), methi seeds (trigonella foenum-graecum) and glimepiride in streptozotocin induced rats. International Journal, 2229, 7472.

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.784

11- Ashwell, M.)2015(. Stevia, nature's zero-calorie sustainable sweetener: A new player in the fight against obesity. Nutrition today, 50, 129.

12- Shakya, A. K. (2016). Medicinal plants: Future source of new drugs. International Journal of Herbal Medicine, 4, 59-64.

13- Abou-Arab, A. E., Abou-Arab, A. A. & Abu-Salem, M. F. (2010). Physico-chemical assessment of natural sweeteners steviosides produced from Stevia rebaudiana Bertoni plant. African Journal of Food Science, 4, 269-281.

14- Yildiz-Ozturk, E., Nalbantsoy, A., Tag, O. & Yesil-Celiktas, O. (2015). A comparative study on extraction processes of Stevia rebaudiana leaves with emphasis on antioxidant, cytotoxic and nitric oxide inhibition activities. Industrial Crops and Products, 77, 961-971.

15- Seidel, V. (2012). Initial and bulk extraction of natural products isolation. Natural products isolation, 27-41.

16- Abd Noor, H.J, 2020. Potential Effect of Gold Nanoparticles against Streptococcus mitis and Streptococcus oralis (Primary Periodontal Colonizeres) (Doctoral dissertation, University of Baghdad).

17- Koneman E, Schreckenberge P, Allens S, et al. Diagnostic microbiology. 4th Edn. J.B. Lippincott Co.USA. 1992.

18- Buxton R. Blood agar plates and hemolysis protocols. ASM Micro Library 2013.

19- Cavalieri, S., Harbeck, R., Mccarter, Y., Ortez, J., Rankin, I., Sautter, R., Sharp, S. & Spiegel, C. (2005). Manual of antimicrobial susceptibility testing. American Society for Microbiology. Pan American Health Organization: Washington, DC, USA.

20- Reiner K. Catalases test protocol. ASM Micro Library 2013.

21- Hoshino, T., Kawaguchi, M., Shimizu, N., Hoshino, N., Ooshima, T. & Fujiwara, T. (2004). PCR detection and identification of oral streptococci in saliva samples using gtf genes. Diagn Microbiol Infect Dis, 48, 195-9.

22- Abdulbaqia, H. R., Himratul-Aznita, W. H. & Baharuddin, N. A. (2016). Anti-plaque effect of a synergistic combination of green tea and Salvadora persica L. against primary colonizers of dental plaque. Archives of oral biology, 70, 117-124.

23- Parvekar, P., Palaskar, J., Metgud, S., Maria, R. & Dutta, S. 2020. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against Staphylococcus aureus. Biomaterial investigations in dentistry, 7, 105-109.

24- Gini, T. G. & Jeya Jothi, G. (2018). Column chromatography and HPLC analysis of phenolic compounds in the fractions of Salvinia molesta Mitchell. Egyptian Journal of Basic and Applied Sciences, 5, 197-203.

25- Nayan, R.B. and Shukla, V.J. 2011. Antibacterial and antifungal activities from leaf extracts of Cassia fistula: An ethnomedicinal plant. J Adv Pharm Technol Res.2(2): 104–109

26- Tariq, M., Gore, M. & Aruna, K. (2014). Antibacterial and synergistic activity of

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.784

ethanolic Ajwain (Trachyspermum ammi) extract on ESBL and MBL producing uropathogens. Int J Pharm Pharm Sci, 6, 278-84.

27- De Slavutzky, S. M. B. (2010). Stevia and sucrose effect on plaque formation. Journal für Verbraucherschutz und Lebensmittelsicherheit, 5, 213-216.

28- Contreras, M. 2013. Anticariogenic properties and effects on periodontal structures of Stevia rebaudiana Bertoni. Narrative review. Journal of Oral Research, 2, 158-166.

29- Mogana, R., Adhikari, A., Tzar, M. N., Ramliza, R. & Wiart, C. 2020. Antibacterial activities of the extracts, fractions and isolated compounds from Canarium patentinervium Miq. against bacterial clinical isolates. BMC Complementary Medicine and Therapies, 20, 55.

30- Parvekar, P., Palaskar, J., Metgud, S., Maria, R. & Dutta, S. 2020. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against Staphylococcus aureus. Biomaterial investigations in dentistry, 7, 105-109.

31- Brambilla, E., Cagetti, M. G., Ionescu, A., Campus, G. & Lingström, P. (2014). An in vitro and in vivo comparison of the effect of Stevia rebaudiana extracts on different caries-related variables: a randomized controlled trial pilot study. Caries research, 48, 19-23.

32- Fasiha A, Shahid B, Faiz-Ul-Hassan S. Nutritional and Medicinal Properties of Stevia Rebaudiana. Curre Res Diabetes & Obes J (2020); 13(4): 555867.

33- Shakya, A. K. (2016). Medicinal plants: Future source of new drugs. International Journal of Herbal Medicine, 4, 59-64.

34- Raut, D. & Aruna, K. (2017). Antimicrobial activity of Stevia rebaudiana against antibiotic resistant ESBL producing uropathogens and evaluation of its antioxidant activity. Int. J. Adv. Res. Biol. Sci, 4, 110-118.

35- Lemus-Mondaca, R., Vega-Gálvez, A., Rojas, P., Stucken, K., Delporte, C., Valenzuela-Barra, G., Jagus, R. J., Agüero, M. V. & Pasten, A. (2018). Antioxidant, antimicrobial and anti-inflammatory potential of Stevia rebaudiana leaves: effect of different drying methods. Journal of Applied Research on Medicinal and Aromatic Plants, 11, 37-46.

36- Moussaoui, F. & Alaoui, T. (2016). Evaluation of antibacterial activity and synergistic effect between antibiotic and the essential oils of some medicinal plants. Asian Pacific Journal of Tropical Biomedicine, 6, 32-37.

37- Parkar, S. G., Stevenson, D. E., & Skinner, M. A. (2008). The potential influence of fruit polyphenols on colonic microflora and human gut health. International journal of food microbiology, 124(3), 295–298.

38- Zhang, Y., Wang, J. F., Dong, J., Wei, J. Y., Wang, Y. N., Dai, X. H., & Deng, X. M. (2013). Inhibition of α-toxin production by subinhibitory concentrations of naringenin controls Staphylococcus aureus pneumonia. Fitoterapia, 86, 92-99.

39- Wang, L. H., Zeng, X. A., Wang, M. S., Brennan, C. S., & Gong, D. (2018). Modification of membrane properties and fatty acids biosynthesis-related genes in Escherichia coli and Staphylococcus aureus:

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.784

Implications for the antibacterial mechanism of naringenin. Biochimica et biophysica acta. Biomembranes, 1860(2), 481–490.

40- Toda, M., Okubo, S., Ikigai, H., Suzuki, T., Suzuki, Y., Hara, Y. and Shimamura, T. (1992) Microbiol. Immunol. 36, 999-1001.

41- Ring, K., Ehle, H., Foit, B. and Schwarz, M. (1977) Flavonoids and Bioflavonoids, Proc. 5th Symp. (Farkas, L., Abor, H. and Kally, F., eds.), pp. 427-437. 18

42- Perrissoud, D., Maignan, M.F. and Anderset, G. (1981) Int. Cong. Symp. Ser.-R. Soc. Med. 47, 21-25.

43- Hajime Ikigai, Taiji Nakae, Yukihiko Hara, Tadakatsu Shimamura, (1993). Bactericidal catechins damage the lipid bilayer, Biochimica et Biophysica Acta (BBA) - Biomembranes, 1147, 132-136.

44- Thati, B., Noble, A., Rowan, R., Creaven, B. S., Walsh, M., Mccan, M., Egan, D. & Kavanagh, K. 2007. Mechanism of action of coumarin and silver (I)–coumarin complexes against the pathogenic yeast Candida albicans. Toxicology in vitro, 21, 801-808.

45- Qin, H.-L., Zhang, Z.-W., Ravindar, L. & Rakesh, K. 2020. Antibacterial activities with the structure-activity relationship of coumarin derivatives. European journal of medicinal chemistry, 112832.

46- Qiu, Y., HE, D., Yang, J., MA, L., Zhu, K. & Cao, Y. 2020. Kaempferol separated from Camellia oleifera meal by high-speed countercurrent chromatography for antibacterial application. European Food Research and Technology, 246, 2383-2397.