Original Article

The Effect of Nonnutritive Sweeteners on the Antimicrobial Activity of Eucalyptus Extracts against Salivary Mutans Streptococci (*in-vitro* Study)

AL-Qaralusi, D. M[1 *], Al-Mizraqchi, A. S[2]

1. Department of Basic sciences, College of Dentistry, University of Baghdad, Baghdad, Iraq
2. Medical Microbiology, Department of Basic Sciences, College of Dentistry, University of Baghdad, Baghdad, Iraq

How to cite this article: **AL-Qaralusi, DM, Al-Mizraqchi, AS. The Effect of Nonnutritive Sweeteners on the Antimicrobial Activity of Eucalyptus Extracts against Salivary Mutans Streptococci (*in-vitro* Study). Archives of Razi Institute. 2023;78(4):1203-11.**

DOI: 10.32592/ARI.2023.78.4.1203

Article Info:
Received: 3 September 2022
Accepted: 10 December 2022
Published: 31 August 2023

Corresponding Author's E-Mail:
dalyamustafa5559555@gmail.com

ABSTRACT

The eucalyptus tree is an excellent source of antimicrobial agents; it is used in many oral cure products. The bitter taste of these agents could compromise their usage. Therefore, fortifying the extracts with non-nutritive sweeteners could be a promising procedure for masking their unpleasant taste. This study was an *in vitro* evaluation of the antimicrobial activity of eucalyptus (alcoholic and aqueous) extracts against salivary *Streptococci mutans*. It aimed to investigate the effect of non-nutritive sweeteners on the antimicrobial activity of these extracts against salivary *S. mutans*. The test microbes were sensitive to different concentrations of eucalyptus alcoholic and aqueous extract, and the inhibition zone increased as the concentration of the extracts increased. All the Mutans isolates were killed at a concentration of 75 mg/ml for the alcoholic extract and 175 mg/ml for the aqueous extracts. In this experiment, the concentration of up to 15% stevia and up to 5% sucralose did not affect the antimicrobial activity of eucalyptus alcoholic extract. While the concentration of up to 1% of stevia and sucralose did not interfere with the antimicrobial activity of aqueous eucalyptus extract against salivary *S. mutans*. An increase in the concentration of non-nutritive sweeteners in this experiment appeared to interfere with the antimicrobial activity of eucalyptus extract against salivary *S. mutans.*

Keywords: Eucalyptus, Mutans streptococci, Nonnutritive sweeteners, Stevia, Sucralose

1. Introduction

Many people have been afflicted by dental caries worldwide, which is a long-term condition of the teeth. It is a disease with many interacting factors, including food, saliva, and microbes as well as the surface of the tooth that is prone to decay. Dysbiosis of the indigenous commensal oral microbiota on tooth surfaces is the etiology of this endogenous infection. As they break down carbohydrates, acids created by plaque microbes are responsible for the demineralization of enamel and dentine, respectively, in carious lesions. Acid production is influenced by many factors, such as the microbiota composition, type and frequency of sugar intake, saliva flow rate, salivary buffering ability, and pH parameters, which play a crucial role in the caries process (1).

Since *S. mutans* meet all the criteria for being a caries-causing bacterium, it was previously assumed that those with a high level of *S. mutans* colonization had an increased risk of developing tooth decay (2). Several cross-sectional studies have examined the role of *S. mutans* in the development of dental caries (3). According to previous research, people with active caries had a more significant concentration of *S. mutans* in their saliva and plaque, compared to people without active caries. In addition, long-term research has shown that the number of people affected by dental caries increases with time (4).

Around 700 species of flowering trees, shrubs, and mallees belong to the eucalyptus genus, which is part of the Myrtaceae family. The "Eu", which means "true " and "calyptus" which means "to cover" are the origins of the name Eucalyptus, which refers to the lower bud made up of the calyx and corolla sections that seal the flower until it blossoms (5). Eucalyptus leaves contain a wide range of bioactive compounds, including 1,8-cineole, macrocarpals (phloroglucinol-sesquiterpenes), monoterpenes (limonin, à-pinene, ß-pinene, p-cymene), alkaloids, eucalyptin, phenols, flavonoids, oleanolic acid, tannins, and terpenes (6).

Eucalyptol, a naturally occurring cyclic ether and monoterpenoid, is one of the most important bioactive ingredients discovered in the eucalyptus plant. It has a camphor-like odor and spicy cooling taste (7). The actions of 1,8-cineole on airways are particularly interesting due to their well-known anti-inflammatory and pectolytic effects (6). They have long been utilized to treat respiratory ailments. Many previous studies have shown the antimicrobial activity of ethanol extract from eucalyptus leaves against oral microorganisms due to the specific activity of 1,8-cineole against periodontopathic and cariogenic bacteria, such as *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *S. mutans, and S. sobrinus* (8, 9).

Non-nutritive sweeteners are food additives with a sweet taste similar to sugar but contain far less food energy than sugar-based sweeteners (10). Chemical synthesis or the production of plant extracts are two methods of obtaining them. They are used as an additive in baked goods, confections, dairy products, sweets, preserves, soft drinks, and tabletop sweeteners (11). They are also used as carbohydrate replacements for people with diabetes mellitus and in chewing gum and candies to minimize the risk of dental caries (12).

Stevia (*Stevia rebaudiana Bertoni*), a perennial shrub from the Asteraceae family, is native to South America; however, the plant is grown all over the world (13). Its sweetening power is 200-300 times greater than sucrose due to the numerous Steviol glycosides of the leaf, making it an excellent sugar substitute in the food and pharmaceutical industries (14). After being presented with sufficient data confirming the safety of using stevia as a manufactured sweetener, FDA issued a "no objection" designation as generally recognized as safe (GRAS) in December 2008 (13, 14).

Sucralose is the most regularly used artificial sweetener worldwide. Due to its lack of calories, sucralose is permitted in a wide variety of food and beverage products all over the world. It was discovered in 1976 and awarded FDA approval in 1998 for use as a sugar substitute in 15 food and beverage categories, and marketed under the brand name Splenda (15). Based on a weight-for-weight comparison, sucralose is predicted to have a sweetness potency of 600 times

more than sugar. Sucralose (1/600th) is a sweetener that can be used to replace a small amount of sugar while maintaining the same sweetness (16). The body does not break down the majority of ingested sucralose; hence, it is non-caloric (17).

Eucalyptus leaves have been used in dental products, such as mouth washing agents, toothpaste, and chowing gums (18). The problem with using eucalyptus extracts is the bitterness and intense taste; therefore, it needs an additive to be more acceptable to the users. Hence, this study aimed to assess the antimicrobial activity of eucalyptus (alcoholic and aqueous) extracts against salivary *S. mutans* through *in vitro* evaluation. Moreover, it aimed to investigate the effect of non-nutritive sweeteners on the antimicrobial activity of these extracts against salivary *S. mutans*.

2. Materials and Methods

2.1. Study Design Sampling and Methods

The study samples were collected from students at the College of Dentistry, University of Baghdad. First, stimulated saliva samples were collected under standard conditions according to Thylstrup and Fejerskov (19).

In order to obtain 25 microbial samples, dental students with no medical history aged 18-22 years old were selected to participate in this study. Each individual was instructed to chew a piece of Arabic chewing gum (0.5 g) for 5 min to stimulate salivary flow, and then saliva was collected in sterilized screw-capped bottles. Finally, the collected saliva was homogenized by a vortex mixer for 2 min. It should be mentioned that 10-fold serial dilutions were prepared using phosphate buffer saline (17).

For each isolate, 0.1 ml was taken from dilutions 10^{-3} and 10^{-5} and spread in duplicate on Mitis-salivarius-bacitracin (MSB) agar plates, which were incubated anaerobically for 48 h at 37 °C, and then aerobically for a further 24 h. As part of the identification process, a colony was isolated from MSB agar plates and tested for Gram stain, motility, and catalase activity under sterilized conditions. Capacity of *S. mutans* to ferment mannitol was tested using a Cystine Trypticase-mannitol medium. Fresh green eucalyptus leaves were collected from a local tree in Baghdad city and identified by a botanist. Afterward, the leaves were thoroughly cleansed and rinsed.

Furthermore, the extract was prepared according to the instructions provided by Richardson (20). Accordingly, 500 ml of distilled water was added to 100 g of the leaves, then cut by a blender at very low speed for 20 s and left for 24 h. Subsequently, the macerate was first filtered by a clean towel and then by filter paper (Wattman No. 1), after which the solution was poured into glass Petri dishes and left to dry at room temperature. Afterward, the resultant powder of aqueous extract was collected in a tightly closed container and preserved in the refrigerator until being used to prepare a different concentration.

Moreover, another 100 g of cleaned eucalyptus leaves were added to 500 ml of absolute (100%) ethanol alcohol to prepare the alcoholic extract. A stock solution of 300 mg/ml was prepared by mixing the extract powder with distilled water for the aqueous extract and dimethyl sulfoxide (DMSO) for the alcoholic extracts. Subsequently, it was homogenized with a vortex mixer and filtered by a Millipore filter of 0.45 µm and then 0.22 µm. Final concentrations of eucalyptus alcoholic and aqueous extract of 50, 100, 150, 200, and 250 mg/ml were prepared from the stock material by dissolving extract powder in distilled water for the aqueous and in DMSO for the alcoholic extract.

Two types of non-nutritive sweeteners were bought from a local market: stevia in the form of powder and sucralose in the form of an aqueous compound of 12.5% concentration. The final concentrations of stevia were prepared in 1%, 5%, and 10% by dissolving the powder in distilled water. Moreover, the final concentrations of sucralose that were prepared from the stock compound after dilution in distilled water were 1%, 2%, and 3%. All the non-nutritive sweeteners were sterilized by a Millipore filter of 0.22 µm. Agar well

diffusion technique was applied to study the antibacterial effects of both eucalyptus extracts, stevia and sucralose, against the isolates that spread on Brain Heart Infusion Agar (BHI-A).

In total, 10 isolates of *S. mutans* were used for this purpose. The density of activated microbial inoculum was adjusted to that of the McFarland standard turbidity (0.5 for bacterial isolates) to approximate microbial cell density (1.5 $x10^8$ CFU/ml) by adding more bacterial inoculum or more sterile saline. Wells of equal sizes and depths were prepared using a Kork porer 6 mm in the agar. These wells were filled with 100 µl of a different concentration of the extracts separately. It should be noted that distilled water and DMSO were controlled for the aqueous and alcoholic extracts. Plates were incubated aerobically for 24 h at 37 °C and the inhibition zone diameters were measured using a scientific ruler. The minimum bactericidal concentration (MBC) was determined for the eucalyptus alcoholic and aqueous extracts, which was conducted using the agar streaking method.

According to Wiegand, Hilpert (21), different eucalyptus extracts were prepared by tube dilution in BHI-B, inoculation with 0.1 ml of fresh microbial inoculum, and then aerobic incubation for 24 h. A sterile microbiological lobe was dipped in different extract concentrations and then stroke on BHI-A. All these Petri dishes were incubated for 24 h at 37 °C, including the control plates (negative control, which contained BHI-A with microbial inoculums without the addition of the extract, and positive control plates, which contained BHI-A and different concentrations of eucalyptus aqueous and alcoholic extract without microbial inoculums). The MBC was determined as the lowest concentration of the extracts that killed the microorganisms. Each petri dish was checked and examined for microbial growth. Moreover, after determining the MBC value for the extracts against the test microbes, the previously mentioned concentrations of stevia and sucralose were added to the MBC of the extracts according to Wiegand, Hilpert (21) to investigate the effect of non-nutritive sweeteners on the antimicrobial activity of eucalyptus alcoholic and aqueous extracts against salivary *S. mutans*.

2.2. Statistical analysis

The recorded data were examined by using the General Linear Model (univariate factorial analysis of variance). It is a statistical test used to find the effect of two or more categorical variables (independent) on the quantitative dependent variable and measure the main interaction effect of those independent variables. Moreover, Tukey's honestly significant difference post-hoc test was used as well and the data were represented as minimum, maximum, mean, and standard deviation values. A *P* value of less than 0.05 was considered statistically significant.

3. Results

The results showed that *S. mutans* isolates were sensitive to the eucalyptus alcoholic and aqueous extracts, and the diameter of the inhibition zone was found to increase as the concentrations of the extracts increased against the test microbes (Figure 1). There was a significant difference between the different concentrations of each eucalyptus extract in terms of their antimicrobial activity ($P<0.05$), as summarized in tables 1-3. In this experiment, the MBC of eucalyptus alcoholic extract against *S. mutans* was 75 mg/ml, while that of the aqueous extract was 175 mg/ml (Figure 2). The results showed that the *S. mutans* isolates were more sensitive to the alcoholic extract, compared to the aqueous extract (Figures 1 and 2). Addition of 1% of stevia or sucralose to the MBC of the experimental extracts did not affect the antimicrobial activity of these extracts. Furthermore, an increase in the concentration of stevia up to 15% did not affect the antimicrobial activity of the alcoholic extract against *S. mutans*. Moreover, an increase in the concentration of sucralose up to 5% did not affect the antimicrobial activity of the alcoholic extract. However, the addition of concentration beyond 1% of stevia or sucralose to the MBC of aqueous eucalyptus extract interfered with the antimicrobial activity of the aqueous extract.

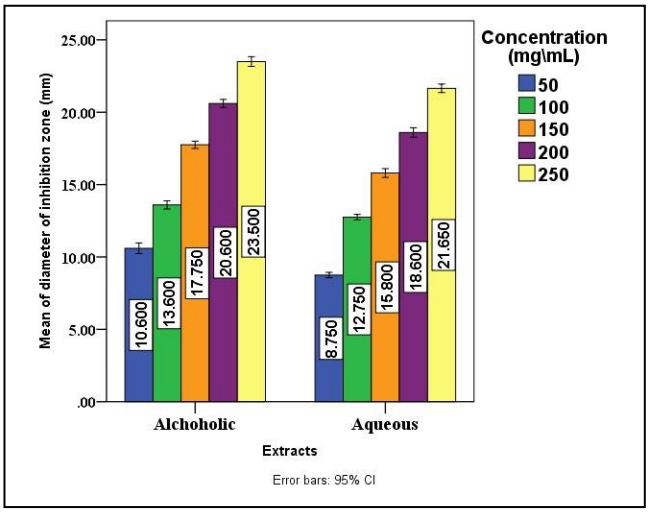

Figure 1. Mean diameter of inhibition zone of different concentrations of eucalyptus alcoholic and aqueous extracts against MS

Table 1. Descriptive and statistical test of the diameter of inhibition zone of Mutans streptococci (10 isolates) between concentrations of both eucalyptus extracts

Extract	Concentration (mg/ml)	Minimum	Maximum	Mean	±SD	F	*P*-value*
Eucalyptus alcoholic	50	10.000	11.500	10.600	0.516	1665.493	0.000
	100	13.000	14.000	13.600	0.394		
	150	17.000	18.000	17.750	0.354		
	200	20.000	21.000	20.600	0.394		
	250	23.000	24.000	23.500	0.471		
Eucalyptus aqueous	50	8.500	9.000	8.750	0.264	1551.067	0.000
	100	12.500	13.000	12.750	0.264		
	150	15.000	16.500	15.800	0.422		
	200	18.000	19.000	18.600	0.459		
	250	21.000	22.000	21.650	0.412		

*=significant at $P<0.05$

Table 2. Multiple Comparisons between concentrations of Eucalyptus extracts against MS (10 isolates) by Tukey Honestly Significant Difference (Tukey HSD)

Concentrations (mg\ml)		Eucalyptus alcoholic		Eucalyptus aqueous	
		MD	*P*-value	MD	*P*-value*
50	100	-3.000	0.00000	-4.000	0.00000
	150	-7.150	0.00000	-7.050	0.00000
	200	-10.000	0.00000	-9.850	0.00000
	250	-12.900	0.00000	-12.900	0.00000
100	150	-4.150	0.00000	-3.050	0.00000
	200	-7.000	0.00000	-5.850	0.00000
	250	-9.900	0.00000	-8.900	0.00000
150	200	-2.850	0.00000	-2.800	0.00000
	250	-5.750	0.00000	-5.850	0.00000
200	250	-2.900	0.00000	-3.050	0.00000

* = significant at $P<0.05$

Table 3. Descriptive and statistical test of the diameter of inhibition zone (mm) against MS (10 isolates) between eucalyptus extract concentrations

Concentration (mg/ml)	Extract	Minimum	Maximum	Mean	±SD	F	*P*-value*
50	Eucalyptus alcoholic	10.000	11.500	10.600	0.516	105.488	0.00000
	Eucalyptus aqueous	8.500	9.000	8.750	0.264		
100	Eucalyptus alcoholic	13.000	14.000	13.600	0.394	22.269	0.00001
	Eucalyptus aqueous	12.500	13.000	12.750	0.264		
150	Eucalyptus alcoholic	17.000	18.000	17.750	0.354	117.200	0.00000
	Eucalyptus aqueous	15.000	16.500	15.800	0.422		
200	Eucalyptus alcoholic	20.000	21.000	20.600	0.394	123.288	0.00000
	Eucalyptus aqueous	18.000	19.000	18.600	0.459		
250	Eucalyptus alcoholic	23.000	24.000	23.500	0.471	105.488	0.00000
	Eucalyptus aqueous	21.000	22.000	21.650	0.412		

* = significant at $P< 0.05$

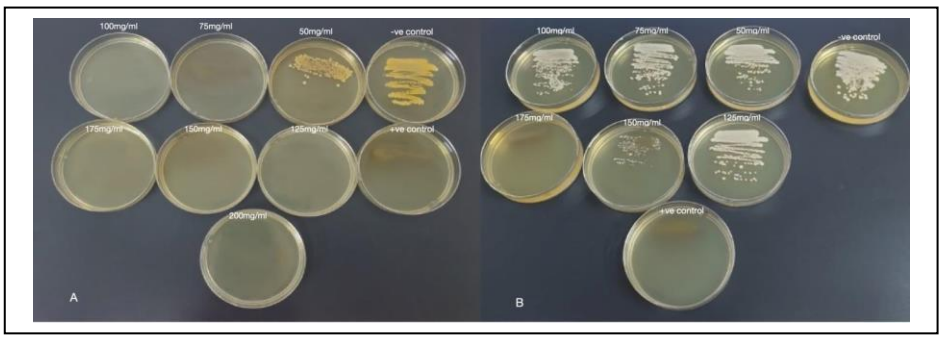

Figure 2. Minimal bactericidal concentration against Mutans streptococci of (A) Eucalyptus alcoholic extract. (B) Eucalyptus aqueous extract

4. Discussion

Oral diseases are among the most frequent diseases in the world, bringing considerable health and economic concerns to individuals who suffer from them and resulting in a considerably lower quality of life for them (22). The most globally widespread and consequential oral disorders are dental caries and periodontal disease (22), and oral hygiene routines continue to be the primary prevention measures against them. Dentists have long used various equipment and chemicals to keep the oral health of their patients in prime condition (23). Scientists have been obliged to hunt for novel antibacterial compounds from other sources, such as medicinal plants (24).

Streptococci mutans collectively are a prokaryotic group of microorganisms that inhabit the oral cavity and are responsible for the beginning and propagation of dental caries, relying on various virulence factors (1). Results of the current study showed that the most significant inhibition zone belonged to eucalyptus alcoholic extract against both of the experimental microbes, compared to aqueous eucalyptus extracts. This result is in line with those of another research (25), in which the antimicrobial activity of aqueous and alcoholic crude extracts of *Eucalyptus camaldulensis* leaves was studied. In the aforementioned study, the water extracts were less effective than ethanolic extracts in terms of their antimicrobial activity against some of the pathogenic bacteria.

A significant difference between the alcoholic and aqueous extracts may be due to the presence of oxygenated monoterpenes, like 1,8-cineole, and the

monoterpenes hydrocarbons, like α-Pinene, β-Pinene, and limonin (26). These compounds were insoluble in water and miscible with an organic solvent, like ethanol (27). Therefore, the experimental microbes showed a higher level of sensitivity against alcoholic extract, compared to the aqueous one. At the same time, the aqueous extract was rich in water-soluble compounds, like flavonoids, tannins, and phenols, which have antimicrobial activity against various microbes (28).

In this study, the addition of stevia and sucralose in concentrations up to 1% had no effect on the antimicrobial activity of all experimental extracts against *S. mutans* at which all these microbial isolates were killed at the same MBC of the extracts prior to the addition of non-nutritive sweeteners (NNS). Maybe there were no significant interactions between the NNS at a concentration of 1% and less for both sweeteners with the extracts, which could affect the major antimicrobial molecules found in these extracts, such as total polyphenols, catechins, tannins, flavonoids, and the other substances. This finding was consistent with those of another study (29) performed on the effect of the addition of NNS on the total phenolic compounds in tea which revealed that the addition of stevia at a concentration of 0.1 g to 100 ml of green and black tea aqueous extract separately had no significant effect on it.

Furthermore, the results were in line with those of another research (30), which discussed a suggested mechanism for the effect of sweeteners on the radical scavenging activity of phenolic compounds in black and green. Findings of the above-mentioned study revealed that no significant interactions occurred between aspartame glycosides and phenolic compounds in tea samples. Moreover, the antimicrobial activity of eucalyptus alcoholic extract against *S. mutans* appeared to be unaffected by the addition of 5%, 10%, and even 15% of stevia or 2%, 3%, 4%, and even 5% of sucralose. Maybe it was due to the presence of the potent compound in the alcoholic extract, such as limonene, a monoterpene unsaturated hydrocarbon with low polarity and weak hydrogen bond basicity. That is used as an alternative to many solvents in the extraction processes or cleaning applications and is also used in organic transformations. Besides, it might have been due to the presence of 1,8-cineole, a saturated oxygenated terpene, which is used as a green solvent because of its safety and pharmacological profiles (27). These compounds were insoluble in water but miscible with ether, ethanol, and chloroform, making the eucalyptus alcoholic extract rich in these compounds. They may also dissolve other substances that mix with them, including high concentrations of stevia and sucralose.

Authors' Contribution

Study concept and design: D. M. A. and A. S. A.
Acquisition of data: D. M. A.
Analysis and interpretation of data: D. M. A.
Drafting of the manuscript: D. M. A. and A. S. A.
Critical revision of the manuscript for important intellectual content: D. M. A. and A. S. A.
Statistical analysis: A. S. A.
Administrative, technical, and material support: A. S. A.

Ethics

The scientific committee approved the protocol of this study at the Basic Science Department, College of the Dentistry, University of Baghdad, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Strużycka I. The oral microbiome in dental caries. Pol J Microbiol. 2014;63(2):127.
2. Ryan M. Advancing oral health through science and technology. SAGE Publications Sage CA: Los Angeles, CA; 2018. p. 857-8.
3. Sounah SA, Madfa AA. Correlation between dental caries experience and the level of Streptococcus mutans

and lactobacilli in saliva and carious teeth in a Yemeni adult population. BMC Res Notes. 2020;13(1):1-6.

4. Kassebaum NJ, Smith AG, Bernabé E, Fleming TD, Reynolds AE, Vos T, et al. Global, regional, and national prevalence, incidence, and disability-adjusted life years for oral conditions for 195 countries, 1990–2015: a systematic analysis for the global burden of diseases, injuries, and risk factors. J Dent Res. 2017;96(4):380-7.
5. Chandorkar N, Tambe S, Amin P, Madankar C. A systematic and comprehensive review on current understanding of the pharmacological actions, molecular mechanisms, and clinical implications of the genus Eucalyptus. Phytomedicine Plus. 2021;1(4):100089.
6. Daus M, Wunnoo S, Voravuthikunchai SP, Saithong S, Poldorn P, Jungsuttiwong S, et al. Phloroglucinol–meroterpenoids from the leaves of Eucalyptus camaldulensis Dehnh. Phytochemistry. 2022;200:113179.
7. Galan DM, Ezeudu NE, Garcia J, Geronimo CA, Berry NM, Malcolm BJ. Eucalyptol (1, 8-cineole): an underutilized ally in respiratory disorders? J Essent Oil Res. 2020;32(2):103-10.
8. Hans VM, Grover HS, Deswal H, Agarwal P. Antimicrobial efficacy of various essential oils at varying concentrations against periopathogen Porphyromonas gingivalis. J Clin Diagn Res. 2016;10(9):16.
9. Müller-Heupt LK, Vierengel N, Groß J, Opatz T, Deschner J, von Loewenich FD. Antimicrobial activity of Eucalyptus globulus, Azadirachta indica, Glycyrrhiza glabra, Rheum palmatum extracts and rhein against Porphyromonas gingivalis. Antibiotics. 2022;11(2):186.
10. Yudha MT, Misnaniarti M. Effectiveness of chewing marshmallow containing betel chew on salivary streptococcus mutans in 12 years old children of smpn 29 palembang J Kesehat Masy. 2021;5(2):1068-72.
11. Farhat G, Dewison F, Stevenson L. Knowledge and perceptions of non-nutritive sweeteners within the UK adult population. Nutrients. 2021;13(2):444.
12. Purohit V, Mishra S. The truth about artificial sweeteners–are they good for diabetics? Elsevier; 2018. p. 197-9.
13. Ahmad J, Khan I, Blundell R, Azzopardi J, Mahomoodally MF. Stevia rebaudiana Bertoni.: An updated review of its health benefits, industrial applications and safety. Trends Food Sci Technol. 2020;100:177-89.
14. Ghaheri M, Adibrad E, Safavi SM, Kahrizi D, Soroush A, Muhammadi S, et al. Effects of life cycle and leaves location on gene expression and glycoside biosynthesis pathway in Stevia rebaudiana Bertoni. Cell Mol Biol. 2018;64(2):17-22.
15. Martyn D, Darch M, Roberts A, Lee HY, Yaqiong Tian T, Kaburagi N, et al. Low-/no-calorie sweeteners: a review of global intakes. Nutrients. 2018;10(3):357.
16. Tennant DR, Vlachou A. Potential consumer exposures to low/no calorie sweeteners: a refined assessment based upon market intelligence on use frequency, and consideration of niche applications. Food Addit Contam Part A. 2019;36(8):1173-83.
17. Green LH, Goldman E. Practical Handbook of Microbiology Fourth Edition. 2021.
18. Barad MK, Ishnava KB, Chauhan JB. Anticariogenic activity and phytochemical studies of crude extract from some Indian plant leaves. J Intercult Ethnopharmacol. 2014;3(2):85.
19. Thylstrup A, Fejerskov O. Textbook of clinical cariology: Munksgaard; 1996.
20. Richardson PM. Phytochemical methods. By JB Harborne. Brittonia. 1985;3(37):309-.
21. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc. 2008;3(2):163-75.
22. Peres MA, Daly B, Guarnizo-Herreño CC, Benzian H, Watt RG. Oral diseases: a global public health challenge–authors' reply. Lancet. 2020;395(10219):186-7.
23. Naseri-Salahshour V, Abredari H, Sajadi M, Sabzaligol M, Karimy M. The effect of oral health promotion program on early dental decay in students: A cluster randomized controlled trial. J Caring Sci. 2019;8(2):105.
24. Janakiram C, Venkitachalam R, Fontelo P, Iafolla TJ, Dye BA. Effectiveness of herbal oral care products in reducing dental plaque & gingivitis–a systematic review and meta-analysis. BMC Complement Med Ther. 2020;20(1):1-12.
25. Abubakar E-MM. Antibacterial potential of crude leaf extracts of Eucalyptus camaldulensis against some pathogenic bacteria. Afr J Plant Sci. 2010;4(6):202-9.
26. Ali LF, Hussein NSM. The Biological Activity of Eucalyptus rostrata Leaves Extraction against E. coli and Staphylococcus aureus isolated from Iraqi Patients. Iraqi J Sci. 2018:1806-10.
27. Campos JF, Scherrmann M-C, Berteina-Raboin S. Eucalyptol: a new solvent for the synthesis of heterocycles containing oxygen, sulfur and nitrogen. Green Chem. 2019;21(6):1531-9.

28. Syukri DM, Nwabor O, Ergantara RI. Preliminary phytochemical, antioxidants, and antibacterial properties of eucalyptus aqueous leaf extract against Streptococcus pyogenes. IOP Conference Series: Earth and Environmental Science; 2019.Sci. 305 012067.
29. Korir M, Wachira F, Wanyoko J, Ngure R, Khalid R. The fortification of tea with sweeteners and milk and its effect on in vitro antioxidant potential of tea product and glutathione levels in an animal model. Food Chem. 2014;145:145-53.
30. Shalaby EA, Mahmoud GI, Shanab SM. Suggested mechanism for the effect of sweeteners on radical scavenging activity of phenolic compounds in black and green tea. Front Life Sci. 2016;9(4):241-51.