ANTIMICROBIAL ACTIVITY OF EXTRACT OF NATURAL SWEETNER SYNSEPALUM DULCIFICUM AGAINST CARIES CAUSING BACTERIA STREPTOCOCCUS MUTANS – AN IN-VITRO STUDY

Dr Vijai S[1], Dr Anusree Sadanandan[2*], Dr Rahmath A[3], Dr Amrutha V R[4], Dr Kavya Krishnan[5]

Abstract:
This in-vitro study aims to evaluate the antimicrobial activity of extract of natural sweetner *Synsepalum dulcificum* with that of xylitol against caries producing pathogen Streptococcus mutans. *Synsepalum dulcificum*, commonly known as miracle plant, miracle berry plant or red berry is an evergreen shrub belonging to the Sapotaceae family. It has a unique taste modifying feature that results in an increase in sweetness and decrease in sourness. This taste modifying function is due to its content, Miraculin. The taste-modifying protein, miraculin is extracted with 0.5 M NaCl solution. The various concentrations of extracts of miraculin are tested on Streptococcus mutans using agar well diffusion method. The length of inhibition zone was measured in millimeters (mm) from the edge of the well to the edge of the inhibition zone produced against the bacterial culture. The extracts were assessed in an effort to validate potential activity of the miracle fruit against the pathogen. This study reveals that the miraculin content having superior antimicrobial property at the concentration of 60 µL when compared to xylitol. This article provides an insight into the non-cariogenic property of natural sweetner miraculin. Since dental caries is a matter of concern worldwide, so sugar substitutes having non-cariogenic property can play an important role in maintaining dental health.

Keywords: Miracle fruit (*Synsepalum dulcificum)*, Miraculin, Zone of inhibition, Sugar substitute, Xylitol, Non-Cariogenic sweetner

[1] MDS, Professor and Head of the Department, Department of Pediatric and Preventive Dentistry, Malabar Dental College and Research Centre, Manoor, Edappal, Malappuram, Kerala.
[2*] MDS, Senior Lecturer, Department of Pediatric and Preventive Dentistry, Malabar Dental College and Research Centre, Manoor, Edappal, Malappuram, Kerala.
[3] Professor and Head of the Department, Department of Physiology, Malabar Dental College and Research Centre, Manoor, Edappal, Malappuram, Kerala.
[4,5] CRRI, Malabar Dental College and Research Centre, Manoor, Edappal, Malappuram, Kerala.

***Corresponding Author:-** Dr Anusree Sadanandan
*MDS, Senior Lecturer Malabar Dental College and Research Cdentre Manoor, Edappal, Malappuram district Kerala, PIN : 679582

DOI: - 10.48047/ecb/2023.12.Si6.739

INTRODUCTION:

Dental caries is the most common type of oral health problem globally caused by the interaction of bacteria, mainly *Streptococcus mutans*, and sugary foods on tooth enamel. Despite credible scientific advances and the fact that caries is preventable, the disease continues to be a major public health problem. In developing countries, changing lifestyles and dietary patterns are markedly increasing the incidence of caries. The World Health Organization (WHO) in 2018 reports that 60–90% of children are affected by dental caries. Its consequences can affect the immediate and long-term quality of life of the child's family and can have significant social and economic consequences beyond the immediate family as well. Even-though the etiology of caries is multifactorial and has been well established, dental caries is frequently associated with a poor diet [1] and bad oral health habits.

The dentist often has the opportunity to provide advice regarding the importance of diet and the role of sugar in caries formation. Reducing the amount of sugar in the diet of humans, especially children, is an important consideration in preventing caries. The use of sucrose substitutes/sugar substitutes have contributed a major part in the prevention of dental caries. Sugar substitutes are tooth-friendly as they are not fermented by the microflora of the dental plaque. Xylitol being a sucrose substitute gained a lot of attention in dentistry with its immense contribution to improved oral health.[2] Xylitol is a five-carbon sugar alcohol first discovered in 1891 by Emil Fischer, a German chemist.[3] Long-term xylitol consumption leads to the emergence of xylitol-resistant (X-R) mutans streptococci.[4] Thus newer researches in the field of sugar substitutes becomes necessary to find new leads for better non-cariogenic sugar substitutes other than xylitol.

Miracle fruit (*Synsepalum dulcificum*) or magic fruit, is an evergreen shrub belonging to the Sapotaceae family. The miracle plant was first discovered in West and Central Africa, specifically in countries like Congo, Ghana and Nigeria.[5] It has a unique taste modifying feature that results in an increase in sweetness and decrease in sourness. This taste modifying function is due to its content, miraculin[6] and it is 4 lakh times sweeter than sucrose. Miraculin is a single polypeptide glycoprotein consisting of two sugars linked to two amino acid residues and with molecular weight ranging from 24,000 to 45,000 Da.[7] The exact mechanism of miraculin action is not entirely clear. However, based on the known information a theoretical model is proposed to explain the power of miraculin to modify taste perception from sour to sweet. This model suggests that miraculin binds directly to the sweet taste receptor T1R2-T1R3 extracellularly within the taste buds of the tongue epithelium's plasma membrane.[8] While it can bind to the receptors under neutral pH conditions, miraculin only activates the receptor in the presence of an acid, thus rendering sour foods to be perceived as sweet. The receptor undergoes a structural change in the presence of protons (H^+), causing the carbohydrate portion of the miraculin molecule to bind to the sweet receptor site, leading to a pH-dependent (between pH 4.8 and 6.5) activation of human sweet taste receptor cells.[8] The most interesting aspect of this mechanism is that unlike sugary substances, Miracle Fruit alone does not evoke a sweet taste, but alters the perception of sweet in the presence of H^+ ions. Apart from miraculin other pharmacological active compounds have been indentified in the plant including alkaloids (dihydro-feruloyl-5-methoxytyramine, N-cis-caffeoyltyramine, N- cis-feruloyl- tyramine), lignins (+-syringaresinol, + -episiringaresinon), phytosterols, triterpenoids, phenolic acids, flavanoids, and aminoacids.[9] A number of pharmacological activities of *S. dulcificum* have been reported in literature, which justifies some of its ethno-medicinal uses that includes anti-diabetic, anti-cancer, anti-hyper urecemia, anti-convulsant, antioxidant capacity, lowering blood cholesterol and cosmetic functions.[10]

The impetus of the study was paucity of literature about the relevance and use of *Synsepalum dulcificum* against caries-causing micro-organisms like Streptococcus mutans, in addition of possessing known medicinal properties. Therefore, in the present research, we aimed to evaluate the antimicrobial activity of extract of natural sweetner *Synsepalum dulcificum* with that of xylitol against caries producing pathogen Streptococcus mutans by agar well diffusion method.

Figure 1: Miracle Fruit

MATERIALS AND METHODS:

This study was an experimental in-vitro, and its target population was *Streptococcus mutans*.

Collection and Processing of Miracle fruit:

For the study, fresh fruits were cultivated and collected from Kerala agricultural University, Mannuthi, Thrissur, Kerala. The pulps of the fruit free from skin and seed were smashed and lyophilized. The lyophilized pulps were kept at -20°C before use. For extracting the desired protein, miraculin, 10g of the lyophilized pulps were suspended in 100ml of water and homogenized for 2 minutes. The homogenates were centrifuged at 13000 rpm for 30 minutes. The pink color supernatant which had no sweet inducing activity was discarded. The sediment was thoroughly washed with water and then extracted three times with 60 ml of 0.5 M NaCl. Each extraction was followed by centrifugation at 13000 rpm for 30 minutes. The supernatant produced and pooled after this procedure was colorless with pH of 4 showing high sweet inducing activity. Miraculin was purified from the pooled supernatant by ammonium sulfate fractionation and this purified miraculin is used for the anti-microbial study.[11 12]

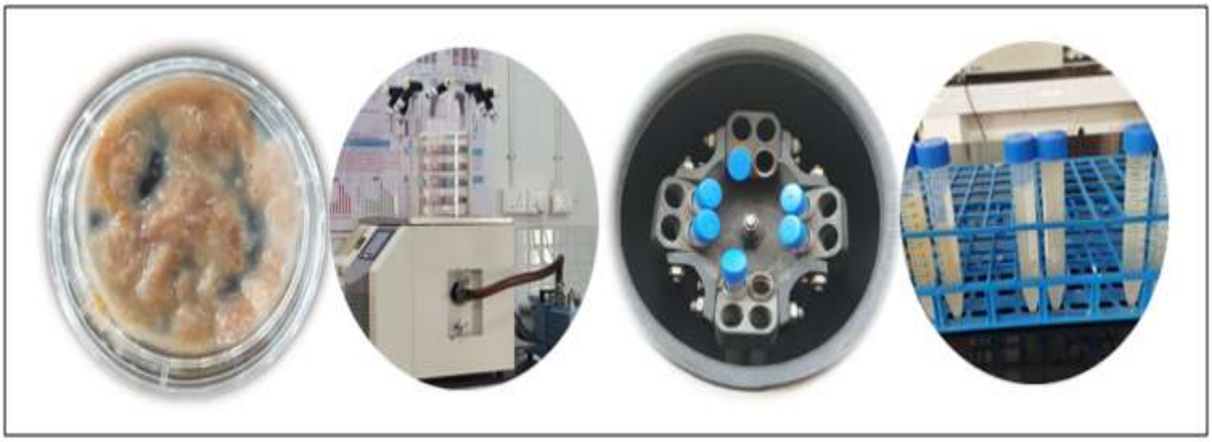


Figure 2: Extraction of Miraculin

Microbiological procedures:
Pure strains of S. mutans (ATCC 25175) is used in the present study. The microorganisms were maintained in Rogosa agar medium (HiMedia Laboratories, Mumbai, India). The antimicrobial property of different concentration of miraculin extract against the selected microorganism was assessed in terms of zone of inhibition with xylitol as control.

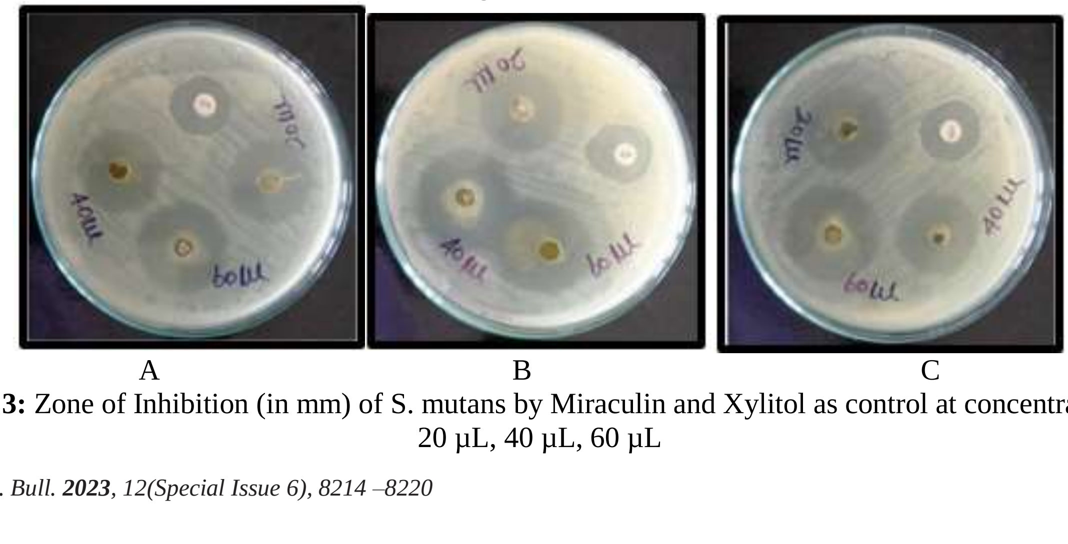
Zone of inhibition
For conducting zone of inhibition test, agar well diffusion method was adopted. For this we prepared Muellar Hinton agar (MHA). The bacterial strains of caries causing bacteria S.mutans were swaped on the agar media, and this procedure was done in triplicates.

After drying the media, wells were made in the agar plates with well cutter. 20 µL, 40 µL, 60 µL of the miraculin extract and Xylitol as control were added to each wells followed by overnight incubation at 37 ^{o}C. The antibacterial activity was interpreted from the diameter of inhibition zone which was observed as a clear zone surrounding each well on the agar plate. The zone of inhibition was measured in millimeters using vernier caliper. No statistical tests were employed as they were not required and the data obtained were appraised observationally.

RESULTS

Zone of inhibition
The miraculin extract showed the anti-microbial property by inhibiting Streptococcus mutans when assessed using all three concentrations of 20 µL, 40 µL, 60 µL (Table 1). At concentration of 40 µL, miraculin showed anti-microbial activity which is at par with the control group xylitol in all three agar plates with zone of inhibition of 8 mm respectively.

In all the 3 plates, xylitol had the same level of antimicrobial activity with zone of inhibition of 8 mm. This study reveals that the miraculin content has superior antimicrobial property at the concentration of 60 µL when compared to xylitol by forming the zone of inhibition of 11mm, 12mm, 12mm respectively.



A B C

Figure 3: Zone of Inhibition (in mm) of S. mutans by Miraculin and Xylitol as control at concentrations of 20 µL, 40 µL, 60 µL

BACTERIA	MEDIA	CONC 20 µL	CONC 40 µL	CONC 60 µL	CONTROL XYLITOL
STREPTOCOCCUS MUTANS (ATCC 25175)	MUELLER HINTON AGAR	06	08	11	08
		06	08	12	08
		07	08	12	08

Table 1: Zone of Inhibition (in mm) of S. mutans by Miraculin

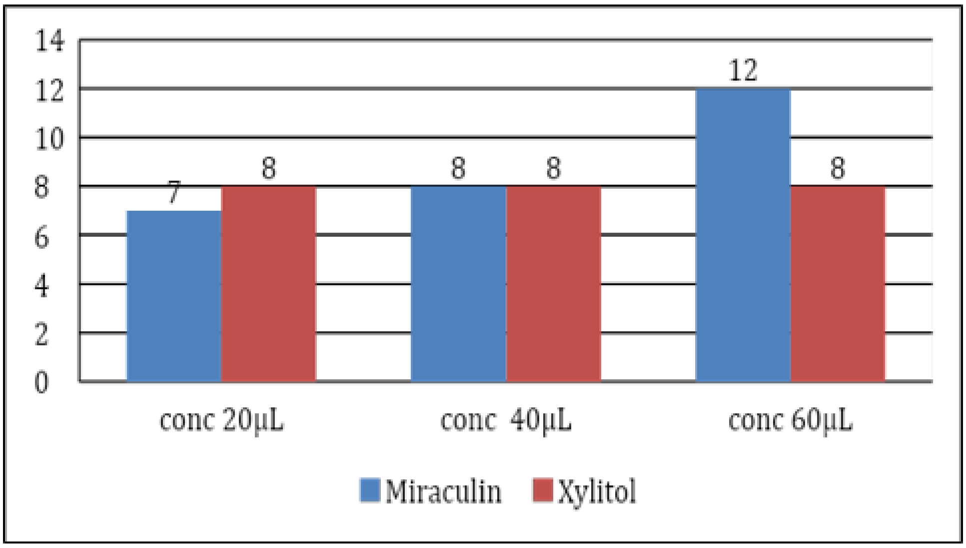


Figure 4: Graphical representation of the result showing the superior antimicrobial property of Miraculin at 60 µL

DISCUSSION

Dental caries is a chronic disease which can affect us at any age. The term "caries" denotes both the disease process and its consequences, that is, the damage caused by the disease process. Dental caries has a multifactorial etiology in which there is interplay of three principal factors: the host (saliva and teeth), the microflora (plaque), and the substrate (diet), and a fourth factor: time. The role of sugar (and other fermentable carbohydrates such as highly refined flour) as a risk factor in the initiation and progression of dental caries is overwhelming. Whether this initial demineralization proceeds to clinically detectable caries or whether the lesion is remineralized by plaque minerals depends on a number of factors, of which the amount and frequency of further sugar consumption are of utmost importance.

Technically the term "sugar" applies to two classifications of carbohydrates: free-form monosaccharides (simple sugar) which include the more common glucose, fructose, and galactose and disaccharides (two simple sugar molecules linked together) which include the most common sucrose, lactose, and maltose. Naturally occurring sugars are available in fruits, vegetables, grains, and dairy foods. Sweeteners are added sugars that are used as ingredients to both satisfy our taste and in some cases provide added energy. Grouping sweeteners as "nutritive" or "nonnutritive" acknowledges a difference in the amount of energy provided by the sweetener. Nutritive sweeteners may be referred to as caloric and include sugars and sugar alcohols. Nonnutritive sweeteners offer no energy and can sweeten with little volume. Both sugar alcohols and nonnutritive sweeteners can replace the sugars and are sometimes referred to as sugar substitutes, sugar replacers, or alternative sweeteners.

Currently, the risk of dental caries is related both to the amount and the frequency of intake of sugar.[12] Technically the term "sugars" applies to two classifications of carbohydrates: free-form mono-saccharides also known as simple sugars which include the more common glucose, fructose, and galactose and the second group disaccharides which include the most common sucrose, lactose, and maltose. Naturally occurring sugars are available in fruits, vegetables, grains, and dairy foods. Sweeteners are added sugars that are used as ingredients to both satisfy our taste and in some cases provide added energy. Sugar substitutes are food additives that are used to mimic the sweet taste of sugar. Sugar substitutes can either be intense or bulk sweeteners. Sugar substitutes can be categorised into two major groups: intense sweeteners (noncaloric) like aspartame, saccharin, sulfame, glycyrrhizin, and so forth and bulk sweeteners (caloric) like sorbitol, xylitol, mannitol, and so forth. Intense sweeteners are sugar substitutes that are usually many times sweeter than sucrose. Their sweetness may overcome sweetness of glucose up to 8000 times thus a much less amount of sweetener is used and only a minute fraction of the caloric content of sucrose is consumed. Sugar substitutes are mainly categorized into natural and synthetic sugar substitutes; the latter are generally referred to as artificial sweeteners. Natural sugar substitutes are those that are found naturally in certain fruits and vegetable, yet they may not be found in adequate amounts suitable for major extraction and consumption.[14]

Grouping sweeteners as “nutritive” or “nonnutritive” acknowledges a difference in the amount of energy provided by the sweetener. Nutritive sweeteners may be referred to as caloric and include sugars and sugar alcohols. Non-nutritive sweeteners offer no energy and can sweeten with little volume. Both sugar alcohols and nonnutritive sweeteners can replace the sugars and are sometimes referred to as sugar substitutes, sugar replacers, or alternative sweeteners.[15]

It is imperative to remember that the usefulness of a sugar substitute has to be looked upon not only from a cariological but also from a nutritional, toxicological, economic, and technical point of view. When evaluating a non-sugar sweetener in relation to dental caries, it is important to consider the potential for metabolism by oral microorganisms and dental plaque, the influence of consumption on cariogenic microorganisms, and the risk of microbial adaptation to the sweetener. Many alternative sweeteners have been extensively researched and their consumption gained much popularity among which xylitol was the most studied candidate. AAPD, 2020 recognizes that the large dose and at high frequency of xylitol used in clinical trials may be unrealistic in clinical practice. The usefulness of a sugar substitute must be looked upon not just from cariological but additionally from nutritional, toxiological, economical and technical point of view. [16]

A substitute for sugar must have few properties such as [17]

- Sufficient sweetening power with no unpleasant aftertaste.
- Be non carcinogenic and non-mutagenic.
- Reasonably inexpensive as well as easily available
- Thermo stable with little or no calories.

The natural sweet taste receptor modulation may establish a safer sugar substitutes than artificially low calorie sweeteners currently available in market. [18] The key candidate of our study, miraculin, a natural sweetner was first seperated and purified in 1968[19] shows taste modifying characteristics in acidic pH and last around 30 min [20]. The sweetening effect takes place when miraculin binds to taste cell membranes near the sweet receptor site. Consequently, a conformational change occurs in the receptor membrane allowing the carbohydrate portion of miraculin to bind to the sweet receptor site and producing sensation of sweetness. Presence of protons (H^+) is required during the conformational change[21].

The present study revealed that the miraculin content having superior anti-microbial property at the concentration of 60 µL when compared to conventional xylitol. This was at par with the study conducted by Ebrahim et al., 2020, water extract of miracle fruit leaves had antibacterial activity against S. mutans and S. sobrinus. However, no inhibitory activity of water extract of miracle fruit leaves against L. salivarius[22] is reported. Miracle fruit is a good source, not only for flavor and color, but also antioxidant activity for functional food applications [23.] Miracle fruit has several health benefits, and the commonly explored use of miracle fruit is to adopt it as a sugar substitutes for diabetics and those suffering from obesity[17]. Miracle fruit produces a comparable perceptual if at between sugar and artificial low calorie sweeteners when consumed with acidic foods [24]. Study conducted by Soares et al.,2010 indicate that miracle fruit can be used for the treatment of dysgeusia, which might ultimately leads to better eating [25]. According to Qutieshat et al.,2018 miraculin induced sweetness has evoked a significant increase in parotid salivary flow [26].

The use of non-cariogenic sweets can be recommended by professionals in the clinical settings as an important adjunct to reducing dental caries risk in individuals especially in children. Trusted information about different sugar substitutes should be collected from authorized sites and press releases by government organizations. This can avoid misconception about the various food additives such as sugar substitutes.

CONCLUSION:

This Research has focused solely on the effects of various concentrations of Miraculin extract on caries producing organism Streptococcus mutans. Here we were able to find out that the Miraculin extract has significant antimicrobial activity against Streptococcus mutans.

FINANCIAL SUPPORT AND SPONSORSHIP
Nil

CONFLICT OF INTEREST STATEMENT
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCE:

1. Davies GN. Early childhood caries--a synopsis. *Community Dent Oral Epidemiol*. 1998;26:106– 16.
2. Guptha M . Sugar Substitutes: Mechanism, Availability, Current Use and Safety Concerns – An Update. Open Access Maced J Med Sci. 2018 Oct 25; 6(10): 1888- 1894.
3. Zacharis C (2012) Xylitol. In: O'Donnell K, Kearsley MW (eds) Sweeteners and sugaralternatives in food technology, 2nd edn. Wiley & Sons, UK
4. Trahan L, Söderling E, Dre´an MF, Chevrier MC, Isokangas P. Effect of xylitol consumption on the plaque-saliva distribution of mutans streptococci and the occurrence and long-term survival of xylitol-resistant strains. J Dent Res 1992: 71: 1785–1791.
5. He Z, Tan JS, Lai OM, Ariff AB. Optimization of conditions for the single step IMAC purification of miraculin from Synsepalum dulcificum. Food chemistry. 2015 Aug 15;181:19-24.Sulaini and Sabran, 2018
6. Doddawad VG,SS,CSV,BM, Gurupadayya BM . Insight on Synsepalum dulcificum: A Bioactive Compound on Taste Modification and Its Biological properties.Int J Nutr Pharmacol Neurol Dis 2022;12:93-8
7. Lim, T.K., 2012. Edible Medicinal and Non-medicinal Plants, 1. Springer.
8. Misaka T. Molecular mechanisms of the action of miraculin, a taste-modifying protein. InSeminars in cell & developmental biology 2013 Mar 1 (Vol. 24, No. 3, pp. 222-225). Academic Press.
9. Chen, C., Wang, Y., Wang, H., 2010b. Chemical constituents from the roots of Synsepalum dulcificum. Chem. Nat. Compd. 46 (3), 448–449.
10. Akinmoladun AC, Adetuyi AR, Komolafe K, Oguntibeju OO. Nutritional benefits, phytochemical constituents, ethnomedicinal uses and biological properties of Miracle fruit plant (*Synsepalum dulcificum* Shumach. & Thonn. Daniell). Heliyon. 2020 Dec 29;6(12):e05837.
11. Theerasilp S, Hitotsuya H, Nakajo S, Nakaya K, Nakamura Y, Kurihara Y. Complete amino acid sequence and structure characterization of the taste-modifying protein, miraculin. J Biol Chem.1989;264:6655-6659.
12. Theerasilp S, Kurihara Y. Complete purification and characterization of the taste-modifying protein, miraculin. J Biol Chem.1988;263:11536-11539.
13. Newbrun E. Cariology 3th ed (1989) ,Quentessence Publishing Co.
14. Shafer. Textbook of Oral pathology, 5 Edition, Elsevier; 2006:567-658.
15. V. B. Duffy and G. H. Anderson, "Position of the American Dietetic Association: use of nutritive and nonnutritive sweeteners," Journal of the American Dietetic Association, vol. 98, no.5, pp. 580–587, 1998
16. T. Ikeda, "Sugar substitutes: reasons and indications for their use," International Dental Journal,vol.32,no.1,pp.33–43,198
17. Roberts MW and Wright JT.Food sugar substitutes:a brief review for dental clinicialns.J clin Pediatr Dent.2002;27(1):1-55.
18. Kant R. Sweet proteins- potential replacement for artificial low calorie sweeteners. Nutr J 2005;4:5.
19. Kurihara K, Beidler LM. Taste Modifying protein from miracle fruit. Science. 1968;161:1241- 1243.
20. Rodrigues JF, da Silva Andrade R, Bastos SC, Coelho SB, Pinheiro AC. Miracle fruit: An alternative sugar substitute in sour beverages. Appetite. 2016 Dec 1;107:645-53.
21. Kurihara K, Beidler LM. Taste Modifying protein from miracle fruit. Science. 1969;222:1176- 1179.
22. Ibrahim HA, Kassim NK, Che Soh NAA, Othman Z, Tuan Ismail TNN (2020). Antibacterial property of Synsepalum dulciffcum leaves aqueous extract against oral pathogens and its chemical compounds. Arch Orofac Sci, 15(1): 55–64.
23. He Z, Tan JS, Abbasiliasi S, Lai OM, Tam YJ, Ariff AB. Phytochemicals, nutritionals and antioxidant properties of miracle fruit Synsepalum dulcificum. Industrial Crops and Products. 2016 Aug 1;86:87-94.
24. Yamamoto C, Nagai H, Takahashi K, Nakagawa S, Yamaguchi M, Tonoike M, Yamoto T. Cortical representation of taste-

modifying action of miracle fruit in humans. Neuroimage 2006;33:1145-1151.

25. Soares H, Cusnir M, Schwartz M, Pizzolato J, Lutzky J, Campbell R, Lilenbaum R. Treatment of taste alterations in chemotherapy patients using the " miracle fruit": preliminary analysis of a pilot study. Paper presented at the ASCO Annual Meeting Proceedings .2010.
26. Qutieshat AS, Aouididi RM. The effect of miraculin on the low rate of parotid salivary secretion. J Dent Res Rev 2018;5:105-10
27. Ibrahim HA, Kassim NK, Che Soh NAA, Othman Z, Tuan Ismail TNN (2020).
28. Antibacterial property of Synsepalum dulci□cum leaves aqueous extract against oral pathogens and its chemical compounds. Arch Orofac Sci, 15(1): 55–64. https://doi.org/10.21315/aos2020.15.1.