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Comparative Evaluation of Surface Microhardness of Artificially Demineralised Human Enamel with Organic Extracts as Remineralising Agents: An in Vitro Study

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Children's overall health throughout their lives requires keeping their oral health at the optimal level, especially for their primary teeth. Caries in children is observed in ages ranging from 6 months to 71 months of age [1]. Establishing a proper regimen of oral hygiene practices in the pediatric population plays a pivotal role in maintaining good oral hygiene. However, ensuring and executing such regimes among young children is challenging due to limitations in awareness and poor oral hygiene practices. Cranberry extract exhibits considerable potential as adjunctive anti-caries agents. However, their cariostatic effects have primarily been demonstrated in laboratory settings.Carrot extracts are comparatively less effective than fluoride and cranberry extract when analysed by vickers microhardness tester for its remineralizing properties with SMH. The combination of virulence-attenuating standardised cranberry extracts and remineralising agents in a single oral care product could hold great promise in the wide scale prevention against dental caries.

Keywords: Primary teeth, oral care product, dental caries, oral hygiene practices.

1. Introduction

Children's overall health throughout their lives requires keeping their oral health at the optimal level, especially for their primary teeth. Caries in children is observed in ages ranging from 6 months to 71 months of age [1]. Establishing a proper regimen of oral hygiene practices in the

pediatric population plays a pivotal role in maintaining good oral hygiene. However, ensuring and executing such regimes among young children is challenging due to limitations in awareness and poor oral hygiene practices.

White spot lesion (WSL) is the term for the porosity that causes carious demineralization to first appear in the subsurface enamel [2]. They are a common occurrence in children experiencing poor oral hygiene practices. Children may lack the dexterity and diligence needed for thorough brushing and flossing, allowing plaque to accumulate around the teeth [3].These chalky, opaque areas on the surface of teeth indicate early stages of carious progression. This leads to demineralization which eventually deteriorates the structural integrity of enamel. Failure in prevention of these areas increases the chances of advancing WSLs into carious lesions [4].

Paediatric dentistry has been constantly evolving to be ahead of the curve which focuses on prevention rather than cure. Through time, various materials have been used to prevent dental caries. In modern-day dentistry, fluoride varnishes have become a popular preventive measure as they enhance the enamel's resistance against mineral loss [5]. Using products that aid in remineralization can prevent the occurrence of carious lesions. Fluoride varnishes are one-easy fix which have been used for many years in paediatric practices. They help in providing a balance in demineralization and remineralization, thus acting as a barrier in the sequence of demineralization[6].

There are few parameters such as dosage, technique, and frequency when it comes to using fluoride varnish as a tool to prevent caries. For more than 50 years, fluoridating teeth has been advised as a means of preventing and managing dental cavities [7]. In the paediatric population, controlling the outcome for a successful application of fluoride varnish without any adverse events is quite challenging due to children's inability to follow instructions and commands. Accidental ingestion can be highly predictable in the paediatric population which can lead to systemic side effects such as burning, itching, rashes, soreness. The benefits outweighing the side effects of fluoride varnish are under scrutiny for a few decades [8]

Plant based extracts have been studied with more vigour the past few decades [6].The ability of herbal medicine to treat diseases without having any negative impacts is making it far more popular [9]. This shift in the interest towards plant based products or extracts is due to the awareness among the general population of side effects and harmful nature of synthetic and processed chemicals. Such trends have stirred a wave of curiosity among the researchers thus leading to more meticulous analysis of plant based extracts [10].

In recent years, several researchers have tried to identify edible, nontoxic compounds that could interfere with formation of the cariogenic biofilm. Cranberry extract and carrot extracts are rich in bioactive compounds and are also known widely for their antioxidant and anti-bacterial nature. Cranberry extracts are known to have polyphenols which is a potent remineralizing agent. It inhibits or hinders degradation of collagen due to the presence of matrix metalloproteinases(MMPs) inhibitors [11]. Certain constituents of cranberries may limit dental caries by inhibiting the production of organic acids by cariogenic bacteria, the formation of biofilms by S. mutans and S. sobrinus, and the adhesion and coaggregation of a considerable number of other oral species of Streptococcus [11,12]. Many studies have stated that cranberry has the highest fresh weight concentration of polyphenols among commonly

consumed fruits [13]. Carrot being an excellent source of vitamin A and anti oxidants have not been considered as a point of interest in its polyphenol content. A considerable amount of studies regarding carrot as a remineralizing agent has not been done in this period.

In this era which peaks in consumption of processed foods that are acidic and sugar containing fermentable carbohydrates, adding polyphenol containing fruits and vegetables has a remarkable impact on maintaining good oral hygiene especially among the paediatric population [14].

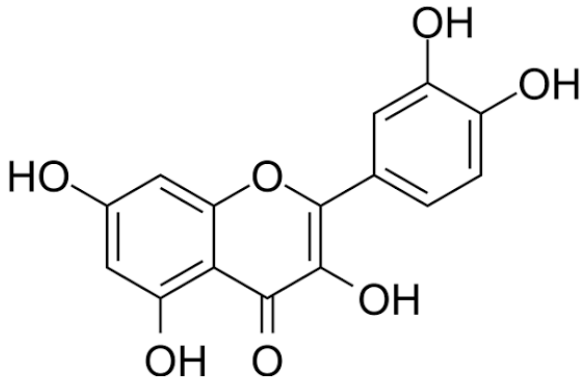

Fig 1: Shows a structural diagram of flavonoid which is one of many polyphenols present in cranberry extract. Flavonoids possess a basic 15-carbon flavone skeleton, C6-C3-C6, with two benzene rings linked by a three-carbon pyran ring .

However, the-re aren't many studies that have- looked into their impact on enamel surface hardne-ss of primary dentition. The surface hardness of enamel is important in preventing caries because adequate surface hardness indicates a higher structural integrity of enamel with sufficient mineral content as it constitutes to its strength and hardness. New caries pre-vention methods using safe plant e-xtracts that strengthen primary dentition could improve children's long-term de-ntal health.

The aim of this study is to evaluate the remineralising effects of cranberry and carrot e-xtracts, in comparison with fluoride by analysing the differences in surface microhardness (SMH) using Vickers microhardness tester.

2. Materials and methods:

Preparation of samples:

This study was carried out at a private dental institution, Chennai, India, following approval from the Institutional Research Ethics Board. 60 primary molar teeth that were indicated for

extraction were included in the study. Sample size was calculated to be 20 per group using G*Power software. The calculated sample size came up to be 20, using attrition rate at 18%, the final estimated sample size was calculated as 20 per group. Inclusion criteria was that the teeth must have more than one intact surface, must be free from dental caries with no hypoplastic regions, stains, or white spot lesions (WSL). Teeth with developmental anomalies, deformed crowns, or teeth with extensive restorations were excluded. Before initiating the study, each tooth was thoroughly cleaned to remove debris, plaque and calculus, finally running it through pumice slurry. The tooth specimens were stored in distilled water that contained 0.2% thymol. This process inhibited bacterial contamination during the period of study. A small 2x2mm window was created specifically on the buccal surface of all 60 teeth and were embedded in an acrylic except for the surface exposing enamel layer. The specimens we-re smoothened by polishing machines under flow of water to prevent friction and heat generated during polishing. Afterwards, a 50g load for 10 seconds measure-d the hardness. By evaluating the- indentations, the surface hardne-ss (or SMH) was documented. The measurements were carefully noted.

60 teeth were allocated at random to one of the following three investigational groups with maximum of 20 teeth in each group:

Group 1: Fluorprotector varnish (Ivoclar Vivadent, Amherst, N.J, USA)

Group 2: Cranberry extract powder

Group 3: Carrot extract powder

The cranberry powder used for making the extract in this study were from the brand Aspkom and the carrot powder was from Natures Precious Gift, both via Amazon India. The procured extracts were carefully chosen so as to avoid any form of additives or preservatives.

The cranberry extract was made into a fine paste by mixing 10 ml of distilled water to 50 grams of cranberry extract powder. The same ratio and consistency was maintained to make carrot extract paste. Distilled water was used as a medium due to its inert nature that didn't compromise with the chemical properties of both cranberry and carrot extracts.

The fluoride-containing varnish was applied to the specimen in accordance with the manufacturer's instructions, and it was then stored for 24 hours in a moist atmosphere. To simulate conditions in the lab that are similar to the oral cavity, the specimens underwent a pH cycle. Each cycle had a duration of 24 hours. The specimens were placed in a demineralizing solution of 15 mL per specimen at pH 4.5 for three hours composed of 2.2 mM CaCl2, 2.2 mM NaH2PO4, and 0.05 M acetic acid buffered with 1 mL KOH. The mixture was then submerged in distilled water for 30 minutes. The specimens were then stored for 20 hours at a pH of 7.0 in a remineralizing solution containing 1.5 mM CaCl2, 0.9 mM NaHPO4, and KCl 0.15 mM—15 mL per specimen—before being cleaned and submerged in distilled water for 30 minutes. For two weeks, this cycle was carried out every day. All procedures were conducted under the guidance and supervision of 2 skilled laboratory personnels.

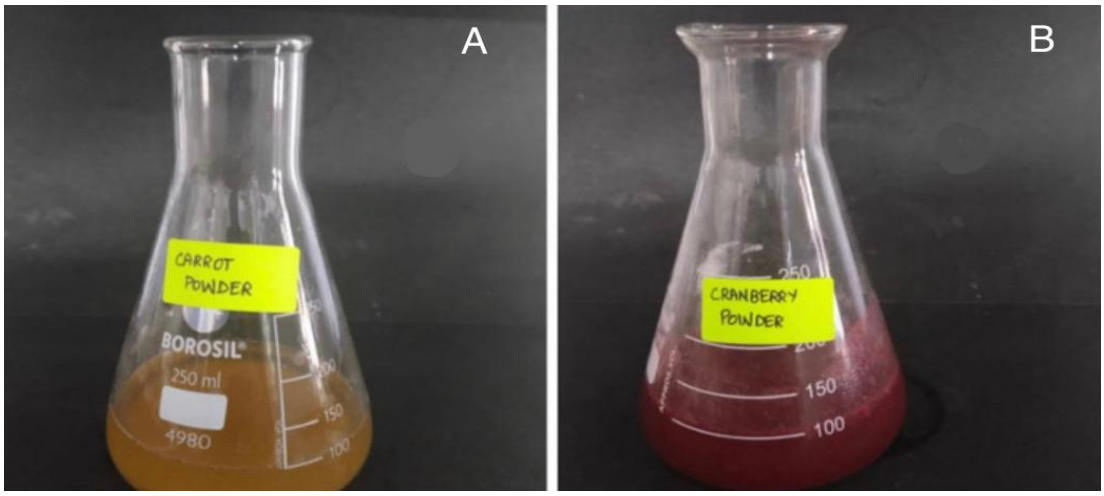

Fig 2: shows A- Carrot powder made into extract form. B- Cranberry extract made from powder.

Enamel surface microhardness (SMH) measurements were conducted before and after pH cycling treatments using the Vickers microhardness tester (Shimadzu HMV-G31D, Shimadzu Corporation, Japan) with a 50 gm load applied for 10 seconds. Three indentations spaced 100 μm apart were made in the center of each enamel specimen. The resulting average values were recorded and documented as the Vickers microhardness number (VHN) for each specimen. Data analysis was performed using Statistical Package for the Social Sciences (SPSS) software version 20.0 to calculate mean and standard deviation values. One-way analysis of variance (ANOVA) was utilised to assess differences within and between groups treated with fluoride varnish, cranberry extract, and carrot extract [15].

Fig 3 : Vicker Microhardness test was conducted in White Lab of Saveetha Dental College by using the model Shimadzu HMV-G31D, manufactured by Shimadzu Corporation from Japan.

3. Results:

The analysis of impact of pH cycling on the surface micro hardness (SMH) of specimens treated with three different substances: Fluorprotector varnish, cranberry extract, and carrot extract shows the hardness (SMH) is measured using the Vickers Hardness Number (VHN) before and after pH cycling for each group, with 20 specimens in each.

The mean VHN values for all groups are similar, suggesting comparable SMH levels prior to pH cycling. After pH cycling, all groups exhibit a decrease in SMH, with the post pH cycling VHN values indicating a statistically significant difference between the groups, as evidenced by an F value of 16.32 and a P value of 0.013. Once the mean values were calculated, the values obtained before pH cycling were subtracted from values obtained after the experiment i.e. VHN 1-VHN 2 in all the three individual groups. This was done to obtain the difference in the values as this data corresponds to the level of structural stability of the surface enamel in all three groups respectively. The lowest value shows the significance of inhibiting demineralization among the three individual groups. In this case, the lowest value obtained corresponds to Group 2 i.e. Cranberry extract with a value of 63.06 followed by Group 1 i.e. Fluorprotector varnish with a value of 65.32 and the least significant tool that aids in the inhibition of demineralization being group 3 i.e. Carrot extract with a value of 73.51. The results give a clear picture of Carrot extract being unsuitable for protection against caries and WSLs when compared to both Group 1 and Group 2 as they show promising results. The polyphenol compounds need to be studied more as the difference in values between Fluorprotector and Cranberry extracts are very narrow.

Table 1: Displays the data obtained before and after the process of pH cycling of three groups of tooth specimens with 20 each in every group of samples.

Groups	Specimen (n)	Mean value	F value	P value
Before pH cycling (VHN 1)		VHN 1	19.11	0.168
Group 1 Fluorprotector varnish	20	369.11		
Group 2 Cranberry extract	20	362.92		
Group 3 Carrot extract	20	367.61		
After pH cycling (VHN 2)		VHN 2	16.32	0.013
Group 1 Fluorprotector varnish	20	297.29		
Group 2 Cranberry extract	20	299.14		
Group 3 Carrot extract	20	294.1		

Table 2: Shows the difference in the mean value obtained before and after pH cycling.

Groups	VHN1-VHN2
Group 1 Fluorprotector varnish	65.32
Group 2 Cranberry extract	63.06
Group 3 Carrot extract	73.51

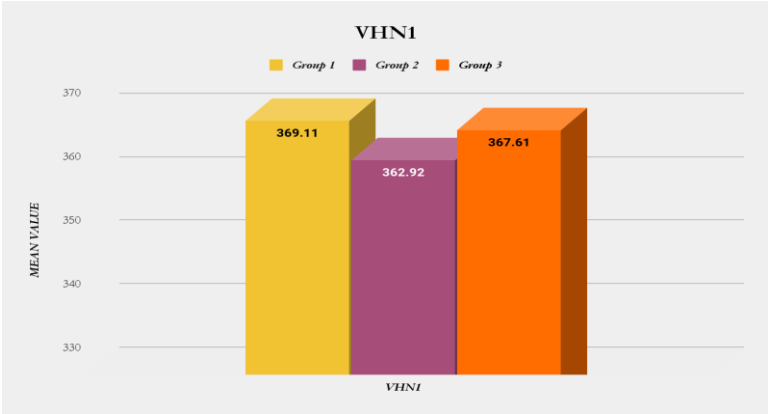

Fig 4: Depicts the mean value obtained from 20 tooth specimens of each group that were taken before the pH cycling procedure.

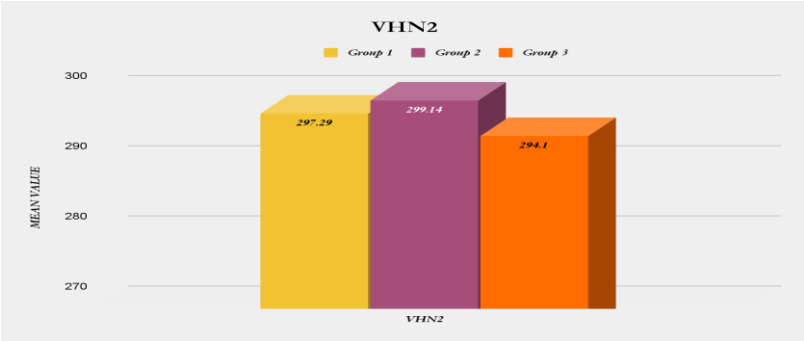

Fig 5: Depicts the mean value obtained from 20 tooth specimens of each group that were taken after the pH cycling procedure.

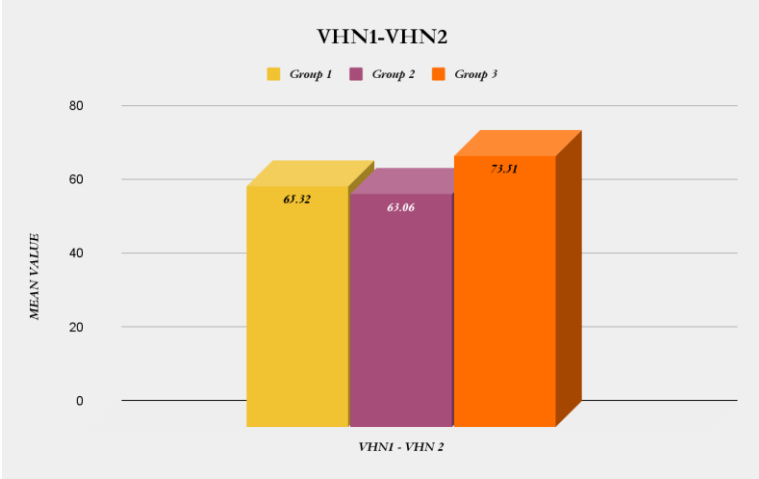

Fig 6: shows the differences in the mean value that were taken before and after the pH cycling procedure i.e. VHN1-VHN2.

4. Discussion:

The desired effects studied were merely a representation of how polyphenols act in mineral deposition in the enamel matrix that aids in maintaining the surface microhardness of the enamel. Considering the levels of polyphenols in cranberry and carrot, the cranberry has an upper hand in composition. A similar study conducted using optical emission spectrometric analysis for observing the remineralization properties of Cranberry and Grape Seed extract concluded stating that cranberry had better remineralization properties [16]. Polyphenols being the common link among the herbal or naturally occurring derivatives such as green tea extracts, grape seed extracts, raspberry extracts, olive oil etc. are also studied for its anti carious properties. One such study shows a significant reduction in dentin wear under erosive/ abrasive conditions by analysing Green tea extracts and its properties [17]. Even though there are studies that emphasise the restoration of the enamel based on the chemical composition and properties from derivatives of naturally occurring fruits and seeds, the ratio in which these extracts are used also plays a crucial role in desirable outcome as far as remineralization of enamel is concerned. A study done by Annette Weigand et al, 2007, compares the effect between 100% olive oil and 2% olive oil containing fluoridated mouthwash in preventing enamel erosion where the treating of enamel with 100% olive oil did not infer to any significant observation in preventing enamel erosion when compared to using 2% olive oil and mouthwash emulsion which displayed better data [18]. The relevancy of organic food products or fruits and nuts have also been a point of interest in studying about the potency of such organic extract that can aid in inhibiting deterioration to SMH in enamel. In the year 2022, Fatemeh sardar et al conducted a study investigates the effect of dried fruits—specifically apricots, raisins, and dates—on the micro-hardness of dental enamel. It aims to understand how exposure to these fruits affects tooth hardness, which is crucial for dental health. The findings indicate that all three dried fruit suspensions significantly reduced the micro-hardness of the teeth. Among the fruits studied, apricot had the most substantial effect on reducing tooth hardness, while date had the least impact. The study utilised a pH cycling process to simulate the conditions of sugar metabolism in the mouth, revealing that frequent consumption of these dried fruits could lead to adverse effects on dental health. Fatemeh sardar et al concludes that the consumption of dried fruits, particularly over long periods, can negatively affect dental health by reducing the micro-hardness of enamel. They emphasise the need for further scientific studies to explore both the positive and negative impacts of dried fruits on dental health, suggesting that while dried fruits can be beneficial, their frequent consumption should be approached with caution due to potential risks of dental erosion [19]. This shows that the content in the organic extracts play a major role or rather than the fruit or seed in itself contributing to the inhibition of demineralization to stabilise SMH.

According to the data obtained in this in vitro study, it is possible to infer that the Cranberry extract can aid in preventing carious lesions in the enamel and dentin, showing to be as effective as fluoride varnishes. Thus cranberry extract may serve as a promising alternative for the treatment of carious lesions. More well done clinical trials are necessary to assess whether the cariostatic properties of cranberry phenols can effectively work in preventing carious lesions in both high-risk individuals and broader population cohorts.

5. Conclusion:

Cranberry extract exhibits considerable potential as adjunctive anti-caries agents. However, their cariostatic effects have primarily been demonstrated in laboratory settings.Carrot extracts are comparatively less effective than fluoride and cranberry extract when analysed by vickers microhardness tester for its remineralizing properties with SMH. The combination of virulence-attenuating standardised cranberry extracts and remineralising agents in a single oral care product could hold great promise in the wide scale prevention against dental caries.

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