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The Anticariogenic Effect of Miswak (Silvadora Persica) and Grape Seed Extract

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ABSTRACT

Purpose: To evaluate the effect of Miswak and Grape seed extract (GSE) on enamel and dentine subjected to artificial acid challenge in-vitro. **Materials and Methods**: A total of thirty sound freshly extracted sound human permanent periodontally affected upper molars were used. 30 enamel specimens were cut from the buccal surface of the teeth and 30 dentin specimens were cut from the cervical root area. All specimens were polished to obtain a flat polished surface. Ethanolic miswak extract was prepared by percolation method. Solutions of 10% ethanolic miswak extract, 6.5% Grape seed extract and 0.05% sodium fluoride were prepared. Specimens were divided into two groups (n=30); enamel and dentin. Each group was further divided into three sub-groups (n=10) according to the material used. Group1; treated with 10% ethanolic miswak extract solution. Group2; treated with 6.5% GSE solution. Group3; treated with 0.05% sodium fluoride solution (positive control). Specimens were individually subjected to alternative treatment/pH cycling regimen for 7 days. Radiodensity (change in mineral content) for each enamel and dentine specimen was assessed before and after pH cycling using digital radiography. Data were statistically analyzed; the significance level was set at $p \leq 0.05$. **Results**: Miswak and GSE showed a high percent gain in enamel and dentine radiodensity compared to fluoride. **Conclusion**: Miswak and GSE are beneficial to both enamel and dentin through increasing their resistance to acid attacks.

KEYWORDS

Miswak, grape seed extract, fluoride, dentin, radiodensity.

INTRODUCTION

Dental caries is considered as a highly ubiquitous disease all over the world. It encounters a constant problem and a financial burden on public

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health. It results from the dynamic imbalance that exists in the oral environments resulting from pathogenic biofilm formation. Acids produced by Streptococcus mutans results in pH fluctuation causing, teeth demineralization [(1)].

Prevention of dental caries has been attempted through using fluoride for many years. It is considered one of the most prominent anti-cariogenic agents. Despite its benefits, excess fluoride could be toxic causing dental fluorosis. Emergence of natural products as anti-cariogenic agents has been popular in the last decade for their biological activities, safety, availability, absence of bacterial resistance, and affordability. Miswak (Silvadora persica) is a famous, traditionally used plant. It has a wide geographic distribution especially in the Middle East and Africa. It contains a variety of organic components that has anti-cariogenic, anticancerous, and antiulcer properties[(2)]. furthermore, it contains inorganic compounds like, calcium and phosphate[(3)]. Grape seed extract (GSE) is similarly a natural product from the seeds of red grapes. It is rich in proanthocyanidins, which acts as an antioxidant, antibacterial, and collagen cross-linking agent [(4)]. The crosslinking property may have a role in dentin remineralization. Therefore, this study aimed to investigate the anti-cariogenic effect of Miswak and GSE compared to fluoride through assessment of their effect on enamel and dentine radiodensity subjected to artificial acidic challenge in-vitro.

MATERIALS AND METHODS

Selection of teeth and specimen's preparation:

The research protocol was approved by the "Ethical Research Committee", Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt ***(REC 17-082).*** The study involved the use of 30 human upper molars extracted for periodontal considerations. The teeth were extracted from a pool of patients between 45 and 60 years of age and were utilized within one week from the extraction date. Selected teeth were intact, free from caries, no anomalies, no hypocalcification, no restorations or previous endodontic treatments, no morphological changes, or cracks. They were cleaned under running tap water to remove any blood or residual attached tissues. Calculus was removed using ultrasonic scaler (Cavitron). The crowns were separated from roots 3 mm apical to the (CEJ) using a low-speed double faced diamond disc size: S-22mm mounted to a low-speed angled hand piece. 30 enamel specimens (3.0x3.0 mm) were cut from the buccal surface of the teeth. Using the same contra and diamond discs, 30 dentin specimens (3.0x3.0 mm) were cut from the cervical root area. All specimens were polished to have a smooth flat surface.

Sample size calculation

This power analysis used radiodensity as the primary outcome. The effect sizes f = (0.668) was calculated according to the results of previous study[(5)]. Using alpha (α) level of (5%) and Beta (β) level of (20%) i.e. power = 80%; the minimum estimated sample size was a total of 7 specimens, and the sample size was raised to 10 specimens. Sample size calculation was done using G*Power Version 3.1.9.2.

Experimental groups

Specimens were divided randomly into two groups (n=30); enamel group and dentin group. Every group was further divided into three sub-groups (n=10) according to the test material used. Group1; treated with 10% ethanolic miswak extract solution. Group2; treated with 6,5% GSE solution. Group3; treated with 0.05% sodium fluoride solution (as a positive control group). Every enamel and dentin specimen were kept in sterile labeled plastic Falcon tube and closed tightly with screw.

Preparation of Miswak extract

Commercial Miswak sticks were purchased (internal market of Makka Al Mokaramah, KSA) and air dried in a shaded area at room temperature for 1 month, then the sticks were sliced into discs

and grinded into a powder using a coffee grinder. The miswak extract was prepared by percolation method [14], by soaking Miswak powder in 3-liter of 95% ethanol by volume (95% ethanol: 5% water) at room temperature in a closed percolator for 72 hours. Fresh ethanol was added every 3 days and this procedure was repeated four times. The mark (miswak powder soaked in ethanol) was further extracted (soaked) by 60% ethanol by volume (40% water: 60% ethanol) for 6 days till mark exhaustion. The extract was filtered through filter paper into a glass flask.

Residual ethanol in the combined extract was evaporated and dried under reduced pressure in Rota vapor (Heidolph™ Hei-VAP Precision motor lift Rotary Evaporator, Germany) at 40°C till dryness. The extract (Fig.1) was kept in a closed glass container in the refrigerator until use. The extract was prepared in the National Institute of Research, Medicinal and aromatic plants research department, pharmaceutical and drug industries, Cairo, Egypt.

Figure (1): Ethanolic Miswak extract

Preparation of 10% ethanolic miswak extract solution:

One hundred gm of miswak dried extract was measured using sensitive balance and added to 1 L of distilled water, 8 gm glycerol as a surfactant and 8 gm propylene glycol as a co-surfactant. They were mixed in a vertical colloidal mill mixer (Vertical colloid mill, Karishma Pharma Machines, India) then the solution was transferred to a clean labeled glass bottle.

Preparation of 6.5% Grape seed extract solution:

GSE was purchased from (Bulk Powders, Vegan, UK.). Sixty five grams of GSE were weighted on a sensitive balance and mixed with 8 gm glycerol as a surfactant, 8 gm propylene glycol as co-surfactant and 1-liter distilled water in the mixer and transferred into a clean glass bottle and labelled.

Preparation of 0.05% sodium fluoride solution:

Sodium fluoride powder (0.5 gm by weight)) was measured on a sensitive balance. It was mixed with, 8 gm glycerol as a surfactant, 8 gm propylene glycol as co-surfactant and 1-liter distilled water in the mixer and transferred into a clean glass bottle and labelled. All solutions were freshly prepared in the first day of the study in Drug Manufacturing Unit (DMU), Faculty of Pharmacy, Cairo University, Egypt.

Preparation of Demineralizing and buffering solutions:

Demineralizing solution (50 mM acetate, 2.25 mM CaCl2 2H2O, 1.35 mM KH2PO4; 130 mm KCl pH=5.0) and buffering solution (20 mM HEPES, 2.25 mM CaCl22H2O, 1.35 mM KH2PO4; 130 mm KCl pH=7.0) were Prepared in analytical chemistry department, Faculty of Pharmacy, Cairo University, Egypt.

Treatment and pH cycling of the specimens:

Specimens were individually subjected to alternative treatment/pH cycling regimen for 7 days [6]. Every enamel and dentin specimen were immersed in 5 ml of the corresponding treatment solution in a tightly closed labeled falcon tube for 20 minutes then they were immersed in 5 ml of demineralizing solution for 3 hours, followed by immersion of specimens in 5 ml of the corresponding treatment again for 20 minutes. After that they were immersed in 5 ml of buffer solution for 20 hours.

All treatments were performed in an incubator at 37°C. Specimens in all groups were thoroughly washed with copious amount of distilled water in between treatments. All specimens were kept in the buffer solution in an incubator at 37°C in the last 2 days until analysis. Treatment solutions, demineralizing and buffering solutions were prepared in the first day of pH cycling. They were changed with fresh ones daily.

Imaging and Radiographic evaluation of samples

The radiodensity of each sample was measured using standardized reproducible digital periapical radiographs for each sample at baseline and after the alternative application of pH cycling and treatments (Fig.2). An image plate size 2 was used to transfer the images of the samples to the digital scanner then to the computer. Each specimen was placed in the rubber base block so the polished surface facing the X-ray cone. The distance between the cone and the specimen was standardized at 4 cm using a ruler. X-ray machine (Kodac 2200, France) with 70KV, 0.5 mA for 0.04 seconds was used for imaging. The image plate was scanned by Durr Vista Scan (Durr Dental Bietigheim, Bissingen, Germany) and images were saved in the computer. The saved images of each sample were interpreted to evaluate and record the pixel grey measurement (mineral content change) using image software (Image J 1.43U, National Institute of Health, USA).A standardized square area of (2.6 X 2.6 mm) on the center of the specimen image were selected and the software shows the radiodensity of 50 to 56 points on the specimen's surface**.** The mean of those values was calculated to determine the radiodensity of the specimen.

Statistical analysis

Data were presented as means and standard deviations (SD). Kruskal-Wallis test was used for comparison of the mean values and percent change in radiodensity between the three groups. Wilcoxon signed-rank test was used for comparison of pre- and post-operative values within each group. The significance level was set at $P \leq 0.05$.

RESULTS

Comparisons of radiodensity before and after the acid challenge for the different treatments are shown in table (1). Results showed that for both enamel and dentin, all treatments were successful in preventing significant loss of radiodensity after the acid challenge. Interestingly, the miswak group achieved an increase in radiodensity even with the acid challenge. Regarding enamel radiodensity mean values, in miswak group there was a significant increase in radiodensity mean values postoperative compared to preoperative values (P-value = 0.022, Effect size = 2.106). While GSE group showed a non-significant increase in radiodensity mean values compared to preoperative values. (P-value = 0.646, Effect size = 0.293), while there was a non-significant decrease

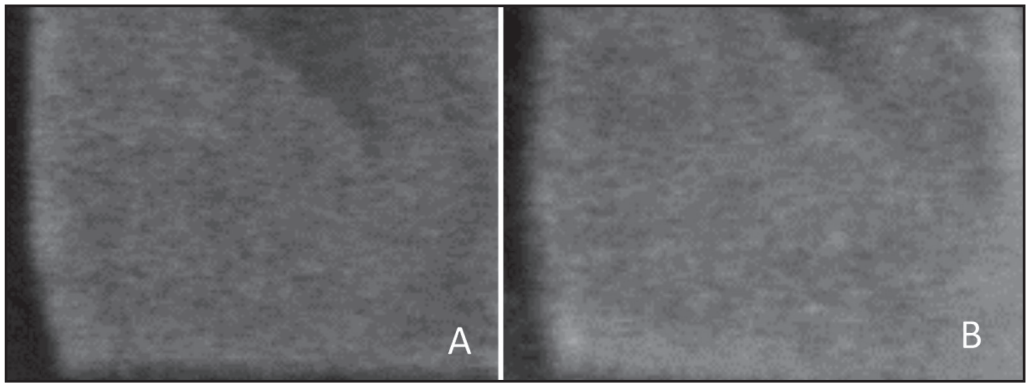

Figure (2): Digital radiographs of dentin specimen before (A) and after (B) the alternative application of pH cycling and treatments.

in radiodensity mean values in fluoride group compared to preoperative values (P-value = 0.508, Effect size = 0.429).

Regarding Changes in dentin radiodensity mean values within each group, there was a significant increase in radiodensity mean values of Miswak group postoperative compared to preoperative values (P-value = 0.013, Effect size = 2.574). GSE group showed a nonsignificant increase in dentin radiodensity mean values postoperatively compared to preoperative values (P-value = 0.114, Effect size = 1.154). There was no statistically significant change in mean radiodensity values of fluoride group postoperatively compared to preoperative values (P-value = 0.386, Effect size = 0.569).

The percent change of enamel and dentin radiodensity in the three groups are shown in table (2) and (Fig.3). Results revealed that, regarding the percent change of enamel radiodensity, miswak group showed the highest percent change (20.85% ± 35.23), followed by GSE group, that showed a percent change of (10.69% ± 56.47) then, fluoride group showed the least percent change of enamel radiodensity (0.38% ± 45.9). For percent change of dentin radiodensity, GSE group showed the highest percent change (59.26% ± 115.34), followed by miswak group that showed a percent change of (37.10% ± 44.85) then, fluoride group showed the least percent change of dentin radiodensity (15.73% ± 52.12).

Table (1) *The mean, standard deviation (SD) values and results of Wilcoxon signed-rank test for comparison between enamel and dentine radiodensity pre-and post-operatively with different materials:*

Substrate	**Enamel**						**Dentine**					
Time \ **Material**	**Fluoride (n = 10) M1S1**		**Grape seed extract (n = 10) M2S1**		**Miswak (n = 10) M3S1**		**Fluoride (n = 10) M1S2**		**Grape seed extract (n = 10) M2S2**		**Miswak (n = 10) M3S2**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pre-operative	92.6	39.4	72.9	15.3	66.9	6.3	48.3	12.9	55.3	18.3	46.4	16.7
Post-operative	82.7	21.8	75.9	22.5	85.9	22.2	51.3	12.1	76.2	32.5	62.5	28.7
P-value	0.508		0.646		0.022*		0.386		0.114		0.013*	
Effect size (d)	0.429		0.293		2.106		0.569		1.154		2.574	

**: Significant at P ≤ 0.05*

Table (2) *The mean, standard deviation (SD) values and results of Kruskal-Wallis test for comparison of percent change of enamel and dentin radiodensity between the three groups:*

	Miswak (n = 10)		**GSE (n = 10)**		**Fluoride (n = 10)**		**P-value**	**Effect size**
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**		
Enamel	20.85%	35.23	10.69%	56.47	0.38%	45.9	.314	.082
Dentin	37.10%	44.85	59.26%	115.34	15.73%	52.12	.381	.069

*Significant at P ≤ 0.05, *significant*

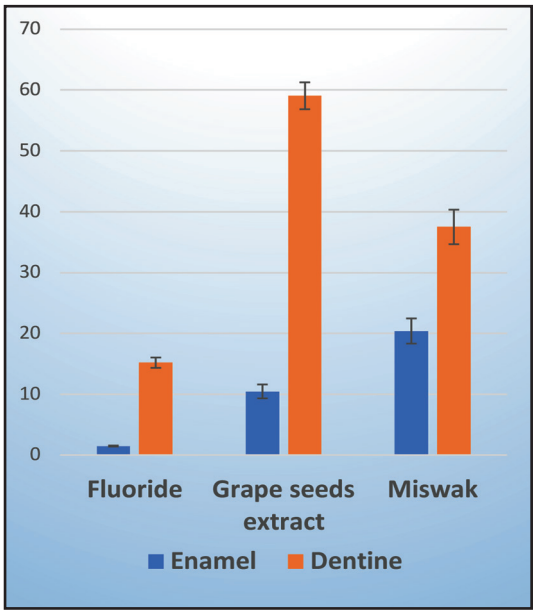

Figure (3): Bar chart representing mean and standard error values for percent change of enamel and dentine radiodensity of the three groups.

DISCUSSION

Prevention of dental caries was attempted through several arms, including the use of antimicrobial agents, or enhancement of acid resistance. One of the most efficient ways to prevent caries is by using fluoridated dental products. However, it may cause some adverse effects like fluorosis and toxicity in some cases [7]. Using plant extracts and natural products in the prevention of dental caries increased in the past decade as an emerging trend replacing fluoride. The main advantages of herbal substitutes are low toxicity, lack of microbial resistance, increased shelf life, and cost-effectiveness [8].

Miswak is a part of (Arak tree) used by the Prophet Muhammad (Peace be upon him). It is traditionally used in the middle east and most of the Muslim communities around the world as an anticariogenic natural toothbrush substitute long time ago. it contains a wide variety of (polar and non-polar) compounds: flavonoids, tannins, minerals, and volatile oils that possesses many medicinal values [14]. Grape Seed extract (GSE) is a natural extract of Vitis vinifera seeds. It consists of Proanthocyanidins (PAS) that have been used as, antioxidant, anti-inflammatory, crosslinking agent, anti-bacterial and anti-cancer substances [4].

This study was conducted for investigation of the effect of Miswak and GSE on enamel and dentine subjected to artificial acid challenge, through the assessment of their radiodensity. The null hypothesis was that Miswak and GSE have no influence on enamel or dentine subjected to artificial acid challenge.

In this study, 10% ethanolic miswak extract solution was used. The Phyto-Chemical analysis reported that ethanolic miswak extract contains a wide variety of chemicals that ranges in polarity from polar to non-polar compounds [14]. Miswak was reported to release calcium and fluoride that, inhibit enamel demineralization [9]. GSE mouthwash (6.5%) was prepared for its reported anticariogenic properties [5]. GSE, is a rich source of polyphenolic compounds that contains 95% oligomeric proanthocyanidins (PAS) (monomeric catechin and epicatechin and gallic acid) that have a remineralizing properties. It was considered as a dentin crosslinking agent. Sodium fluoride (0.05%= 250 ppm F) mouthwash was prepared and used as a positive control group. It was recommended to include a (250 ppm F) to obtain a "dose-response group "Gold standard" for its anticariogenic properties [10]. Non active ingredients (surfactants) were included in our solution formulations; Glycerol and Propylene glycol. They are binding and emulsifying agents that prevent the solid and liquid substances from separation [11].

In the present study, regarding the enamel radiodensity, results revealed that, miswak showed the highest percent increase in enamel radiodensity, followed by GSE. Fluoride showed non-significant decrease in enamel radiodensity. For percent change of dentin radiodensity, GSE showed the highest percent increase in dentin radiodensity, followed by miswak then, fluoride showed the least percent increase of dentin radiodensity.

Miswak increased enamel radiodensity, this was supported by previous studies [12, 13]. Which showed that, miswak precipitate a polysaccharides layer on enamel surface protecting it and increasing its resistance to acid challenges. Also, 10% miswak extract increased enamel microhardness, they attributed this to miswak release of calcium and phosphate ions. Calcium and phosphate release was supported by the Phyto-Chemical analysis reported in another study [14]. In addition, it was reported that miswak possess a small amount of fluorides (1.0μg/gm) that increases enamel resistance to acid attacks [15].

Also, GSE increased enamel radiodensity, which is in the same line with previous study [7] Which also reported that, GSE increased the enamel microhardness. GSE effect on the enamel is not fully explained. It may be attributed to the effect of gallic acid, which is a major constituent of the GSE, that facilitates the minerals deposition on enamel surface [16]. In addition, it has remineralizing properties through their catechin compound, which is a free radical scavenger needed for calcium absorption to enamel surface [17]. Also, spherical particles of calcium and phosphate were observed under scanning electron microscope deposited on enamel surface treated with GSE [18].

Moreover, in this study miswak increased dentin radiodensity, which coincide with previous study [19]. Which found that miswak contains flavonoids, salvadorine, cyanogenic glycosides, lignans, saponins, alkaloids, tannins, linoleic acid, stearic acid and salvadourea. Some of these compounds have active sites that bond with same sites in collagen proteins with hydrogen bonds. These hydrogen bonds protect cleavage sites in the collagen protein molecules from the attack of Collagenase enzymes. Also, hydrogen bonds could occur with Collagenase enzyme, deforming its molecular shape, and subsequently deactivation of these enzymes. In addition, flavonoids in miswak can form a complex or bind to zinc ions in zinc-containing metalloproteinases (Collagenase and MMPs), preventing their degradation activity.

On the other hand, GSE showed the highest percentage increase in dentine radiodensity, this was in accordance with other studies [7, 20-22]. Which showed that, 6.5% GSE solution increased dentin mechanical properties, microhardness and tensile strength. This may be attributed to the effect of PAS on enhancing collagen stability against proteolytic degradation, inhibition of MMPs enzyme activity and increasing the quantity of cross-linked collagen. GSE tannins play an important role in collagen cross linking through; strengthening of collagen bonds and make collagen bond difficult to be hydrolyzed.

CONCLUSION

Miswak and GSE are considered promising anticariogenic tools that are comparable to fluoride.

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RECOMMENDATION

Findings of this study recommend the use of miswak and GSE as promising anticariogenic agents.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Abdel Tawab HA, Niazy MA, El Elsharkawy DA. Antibacterial Effect of Ginger, Green Tea and Pomegranate versus Chlorhexidine using Stevia and Sucrose Sugar. ADJ-for Girls 2020;7:329-6.
2. Nordin A, Bin Saim A, Ramli R, Abdul Hamid A, Nasri NW, Idrus RB. Miswak and oral health: An evidence-based review. Saudi J. Biol. Sci. 2020;1801–10.

3. Khalil WH, Sukkar MY and Gismalla BG, Comparing Salivary and Dental Plaque Content of Sodium, Calcium and Phosphate after Sucrose Ingestion between Salvadora persica Sticks and Toothbrush Users. Arch. Curr. Res. Int. 2017;8:1-6.
4. Gupta M, Dey S, Marbaniang D, Pal P, Ray S, and Mazumder B. Grape seed extract: having a potential health benefits. J Food Sci Technol.2020;57:1205–15.
5. Ismail SA, Niazy MA, and El-Sharkawy DA. Effect of Two Different Collagen Cross Linking Agents versus Topical Fluoride on Management of Root Caries (In Vitro Study). ADJ-for Girls. 2017;4:161-6.
6. Vieira AE, Delbem AC, Sassaki KT, Rodrigues E, Cury JA, Cunha RF. Fluoride Dose Response in pH Cycling Models Using Bovine Enamel. Caries Res 2005;39:514–20.
7. Silva AP, Goncalves RS, Borges AF, Bedran Russo AK, Shinohara MS. Effectiveness of plant derived proanthocyanidins on demineralization on enamel and dentine under artificial cariogenic challenge. J Appl Oral Sci. 2015; 23:302-9.
8. Elumalai M, Indiran MA, Rathinavelu PK, Doraikannan SS, Prabhakar J. Grape seed extract as a potential remineralizing agent, A structured review. Drug Invent. Today. 2018; 10:3116-28.
9. Mortazavi S, Aslani A, Babaee M, and Hajiahmadi M, Persica Chewing Gum Effects on Saliva Fluoride Concentration and Flow Rate: A Triple-Blind Randomized Clinical Trial. Contemp Clin Dent. 2019;10:117–22.
10. Buzalaf MA, Hannas AR, Magalhães AC, Rios D, Honório HM and Delbem AC. pH-cycling models for in vitro evaluation of the efficacy of fluoridated dentifrices for caries control: strengths and limitations. J Appl Oral Sci. 2010;18:316-34.
11. Vrani E, La~evi A, Mehmedagi A, Uzunovi A. Formulation ingredients for toothpastes and mouthwashes. Bosn. J Basic Med Sci. 2004;4: 51-8.
12. Wassel MO, Sherief DI. Ion release and enamel remineralizing potential of miswak, propolis and chitosan nano- particles based dental varnishes. Pediatr. Dent. J. 2019;29:1-10.
13. Bawazeer TM, Alsoufi MS, Katowah D, Alharbi WS. Effect of Aqueous Extracts of Salvadora persica "Miswak" on the Acid Eroded Enamel Surface at Nano-Mechanical Scale. MSA. 2016;7:754-71.
14. Abhary M, Al Hazmi AA. Antibacterial activity of Miswak (Salvadora Persica L.) extracts on oral hygiene. J. Tibah Univ. Sci. 2016;10:513-20.
15. Halawany HS. A review on miswak (Silvadora persica) and its effect on various aspects of oral health. Saudi Dent J. 2012;24:63–9.
16. Cheng L, Li J, Hao Y and Zhou X. Effect of compounds of Galla chinensis on remineralization of enamel surface in vitro. Arch Oral Biol. 2010;55:435-40.
17. Elumalai M, Indiran MA, Rathinavelu PK, Doraikannan SS, Prabhakar J. Grape seed extract as a potential remineralizing agent, A structured review. Drug Invent Today. 2018;10:3116-28.
18. Amin RA, Awad SA and Abd El Sattar EA. Evaluation of remineralization of Grape Seed Extract Versus Sodium Fluoride on Demineralized primary Anterior Teeth: an In Vitro Study. E.D.J. 2019;65:1977-84.
19. Khunkar S, Hariri I, Alsayed E, Linjawi A, Khunkar S, Islam S, Bakhsh TA. Inhibitory effect of salvadora persica extract (Miswak) on collagen degradation in demineralized dentin: In vitro study. J. Dent. Sci. 2021;16: 208-13.
20. Alrafee SA, Niazy MA, and El yassaky MA. Remineralization potential of Grape seed versus Amorphous Calcium phosphate nanoparticles on sound and caries affected dentin. ADJ-for Girls,2017;4:231-7.
21. Yassin A and Safy R. Grape seed extract and dentine remineralization. EDJ. 2018;64:1-8.
22. Delimont MN and Carlson BN. Prevention of dental caries by grape seed extract supplementation: A systematic review. J. Nutr. Health. 2020;26:43–52.