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The Effect of Siwak and Brushing on Cariogenic Bacteria and Surface Characteristics of Enamel and Two Brands of resin Composite (An in vitro study)*

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ABSTRACT

Purpose: The aim of this study was to evaluate the effect of siwak extract and toothbrush using two brands of toothpaste one containing siwak extract and one without on the cariogenic bacteria and surface characteristic of enamel and two types of composite resin. **Materials and Methods:** A total of 90 specimens were used in the present study. The specimens were divided into three groups according to the type of material used (n=30); Enamel specimens, Feltk Z350 specimens, and Tetric-N-Ceram Specimens. Each group was further subdivided into 3 subgroups according to the method of treatment (n=10); specimens treated with Siwak sticks, specimens treated with toothbrush and toothpaste containing Siwak, and specimens treated with toothbrush and toothpaste not containing Siwak. All specimens were stained by coffee and tea stains, then surface treatment was done using electrical toothbrushing machine. Color measurements, surface roughness for all specimens and wear resistance for composite resin specimens were done after staining and then after surface treatment. Antimicrobial activity was done using minimum inhibitory concentration (MIC) method. **Results:** Antimicrobial activity of toothpaste containing siwak showed the lowest significant (MIC) on all the tested microorganisms. There was a statistically significant differences in the ΔE values and ΔW values of the treated subgroups. Moreover, significant differences in Ra values were observed between the control and stained specimens, as well as between subgroups treated with Siwak sticks, toothpaste containing Siwak, and toothpaste not containing Siwak. **Conclusions:** Siwak and toothpaste containing siwak are effective in removing the stains from enamel and resin composite surface without increase surface roughness or causing wear of the resin composite, however, the impact of the toothpaste containing siwak was more effective than siwak extract on the tested microorganisms.

- Extracted from thesis "The Effect of Siwak and Brushing on Cariogenic Bacteria and Surface Characteristics of Enamel and Two Brands of resin Composite (An in vitro study)

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INTRODUCTION

Plants are natural source of antibacterial agents. Plant-derived medicines have been a part of our traditional health care system; the antimicrobial properties of plant derived compounds are well documented. Herbal medicines are more effective and less harmful, as they have negligible side effects, they exhibit low mammalian toxicity and can be handled easily [1]. *Salvadora persica L.* of the family *Salvadoraceae* an ever green Shrub, 4-6 m tall with a short trunk, white bark and smooth green leaves. In medicines Salvadora *persica* has potent activity for dental complaints, the use of Siwak as an oral tool, as well as the biological effects of *S. persica* extracts are reviewed [2].

"Siwak" tree, for the roots and twinges of this tree have been used for teeth cleaning since ancient times. It is one of the most commonly used medicinal plants for oral hygiene among global Muslim community. Moreover, the tree has been used by many Islamic communities as toothbrushes, has been scientifically proven to be very useful in the prevention of tooth decay, even when used without any other tooth-cleaning methods [3].

A variety of chemical components have been identified in *S.Persica* extracts. Some of these biologically active chemical constituents as sodium chloride, potassium chloride, salvadorea, alkaloids, oleic and linoleic acids have been suggested to contribute to the cleansing efficacy of Siwak by leaching out in saliva, for their anti-fungal properties[4]. In recent years, human pathogenic microorganism have developed resistance in response to the use of commercial antimicrobial drugs commonly used, in addition they are expensive and undesirable side effects of certain antibiotics are present, this forced scientists to look for new antimicrobial substances, such as medical plants [5].

Therefore, this study was conducted to evaluate the antimicrobial activity of Siwak extract against some oral pathogenic microorganism like *Streptococcus mutans*, *Staphylococcus aureus,*, that cause most predominant infections in oral cavity. The effects of Siwak containing toothpaste on surface characteristic of Enamel and Composite restorative materials was also studied.

MATERIALS AND METHODS

Siwak stick, siwak powder, two commercial brands of toothpastes one containing siwak and one without and two types of nanocomposite restorative materials. A total of 90 specimens were used in the present study (Table 1).

Table 1. *The materials, compositions and manufacturers of the materials employed in this study.*

Brand Name	Composition	Manufacturer
Miswak Toothpaste	Siwak extract, Calcium carbonates, Sorbitol, Treated water, Silica, sodium lauryl sulphate, Flavour, Sodium carboxyl methyl cellulose, Sodium silicate, Sodium benzoate, Glycerin, Sodium saccharin.	Natural LLC, Dubai, U.A.E Product of United Arab Emirates
Close Up white now Toothpaste	Sodium fluoride, Zinc sulfate, Sorbitol, Aqua(Water), Hydrated silica, Sodium Lauryl sulfate, Flavor, Cellulose Gum, Sodium bicarbonate, Mica, Sodium saccharin, lecithin, limonene, glycerin.	Personal Care Company (S.A.E)
Filtek Z350	**Matrix:** Bis-GMA, UDMA,TEGDMA, Bis-EMA esins **Filler:** Non-agglomerated 20 nm silica filler, Non-agglomerated 4 to 11 nm zirconia filler, Aggregated zirconia/silica cluster filler (comprised of 20 nm silica and 4 to 11 nm zirconia particles) in the size range of 0.6 to 1.4 microns, Filler loading (78wt%)	3M ESPE St. Paul, Minn
Tetric N Ceram	**Matrix:** Bis-GMA, UDMA, TEGDMA, Bis-EMA esins **Filler:** Barium, glass, ytterbium,trifluoride, mixed oxide, silicon dioxide, Prepolymers ,Nanofillers, Filler loading (80 wt%)	Ivoclar, Vivadet, Liechtenstiem

1. Preparation of specimens:

1.1. Teeth specimens:

Forty freshly extracted bovine maxillary central incisors were obtained. Only the coronal segments of the teeth were used, the roots were separated using diamond disk (3M ™, St Paul, MN) using water coolant. The crowns were cut at the cemento-enamel junction (CEJ) using a diamond saw (Micro slice 2, Metals Research Ltd., Cambridge, UK) with a low speed straight hand piece (Allowable max. speed 40,000 rpm weight 48g.NSK, Japan) using water coolant, then the specimens were embedded in self-cure acrylic resin (GC Pattern Resin, GC Co., Chicago, USA and stored in a distilled water.

1.2. Resin Composite Specimens:

The resin composite was packed into a specially fabricated cylindrical spitted Teflon mold, with 6mm diameter and 2mm height, with the upper and lower surfaces covered with Mylar strips (Mylar, Henry Schein, Melville, NY, USA). The specimens were Light activated from both sides for 20 sec. using LED light curing (LED.F with rechargeable Li ion battery as power supply and light source of high power LED blue light with wave length 420-480nm.Woodpecker) .Following light-curing, the specimens were removed from the mold, then were stored in 37°C distilled water for 24 hours, and then fixed in Teflon mold to facilitate their surface treatment.

2. Grouping of specimens:

The specimens were divided into three groups according to the type of material used (A) (n=30); Enamel specimens (A1), Feltk Z350 specimens (A2), Tetric-N-Ceram Specimens (A3). Each group was further subdivided into 3subgroups according to the method of surface treatment (B) (n=10); specimens treated with Siwak sticks (B1), specimens treated with toothbrush and toothpaste containing Siwak (B2), specimens treated with toothbrush and toothpaste not containing Siwak (B3). All specimens were stained by coffee and tea solutions. Color measurements, surface roughness for all specimens and wear resistance for composite resin specimens were done after staining and then after surface treatment. Surface treatment (Brushing) of enamel surface and composite specimens was done either by Siwak (as natural tooth brush) or synthetic tooth brush supported to Tooth brushing machine.

3. Antimicrobial assessment:

3.1. Preparation of Salvadora persica extracts:

A specimen of the most commonly used chewing sticks from Siwak trees was purchased from a local market. The fresh Siwak was cut into small pieces and allowed to dry at room temperature for couple of days. Then it was ground to powder. Successive 10 g quantity was put into sterile screw-capped bottle to which 100 ml of ethanol was added**.** The extract was allowed to soak for 48 hours at 4°C and then centrifuged at 2000 rpm for 15 minutes. The supernatant was passed through rotary evaporator. Finally, the extract was stored in sterile screw-capped vials in the refrigerator to be used within one week for studying anti-bacterial activity. However, the chewing sticks were used for the surface treatment of both enamel and composite specimens**.**

3.2. Micro dilution assay:

The antimicrobial activity was examined under strict aseptic conditions. The bacterial strains used in this test were; Streptococcus mutans, Streptococcus pyogens, Staphylococcus aureus and Lactobacillus acidophilus. Sensitive and quick (Micro titer plate) method was used to determine the Minimal inhibitory concentration (MIC) of the Siwak extract and toothpastes against the tested bacteria. MIC for the Salvadora persica extract was determined based on a micro dilution method in 96 multi-well micro titer plates. This technique used serial dilution of the Siwak extract and toothpastes in a number of test tubes followed by the tested organism to determine the MIC for the test organism using turbidity as an indication of growth. Color change was then assessed visually; any color change from purple to pink or colorless was recorded as positive. The lowest concentration at which color change occurred was taken as the MIC value

4. Color measurement

The color of the specimens was measured by spectrophotometer (Pocket Spee-Color QA Pro, Pocket Spee- Technologist Inc., Denver, Colo, USA). During baseline measurements, three measurements were performed for each specimen, the mean of the readings was calculated. The magnitude of the total color difference ΔE was calculated from the equation:

$$\Delta E = (\Delta L2+\Delta a2+\Delta b2)^{1/2}$$

Where L* (lightness), a* (red-green), b* (blue-yellow).

5. Roughness measurements

The surface roughness of the specimens was examined by using Atomic Force Microscope (AFM)**, (**Auto probe cp-research head, Thermo microscope). A scan for an area of10*10 micron was done with number of data points 256*256, scanning rate 1 Hz. AFM was operated in contact mode using nonconductive silicon nitride probe (MLCT-MT-A,,Bruker), using pro-scan 1.8 software for controlling the scan parameters and IP 2.1software for image analysis.

6. Wear measurements:

Wear measurement of composite specimens was done by weight loss technique using electron sensitive balance system (analytical Balance: BL210, Cap: 210gm d.:0.1mg, Ser.No.:36681-SartoriusAG,Germany).The specimens were dried and weighted in a digital analytical balance with 0.0001gm accuracy after application of staining and before surface treatment by toothbrushing machine and considered as an initial weight of the specimens(W1). Then after brushing the specimens were dried and weighted again using digital analytical balance with 0.0001 gm. accuracy and considered as final weight (W2).The loss of weight (ΔW) for each specimen was calculated by the difference between weight before and after wear testing from the following equation.

Wear = Volume loss = (final weight – original weight)

$$\Delta W = W1 - W2$$

7. Statistical analysis

Two-Way ANOVA was used to compare between the different materials within each treatment, and interaction between variables for mean color parameters and Roughness (nm) and Weight loss (gm) before and after. Independent t-test was used to compare between before and after mean roughness for the different tested variables. Tukey's post-hoc test was used for pair-wise comparison between the mean values when ANOVA test was significant. Statistical analysis was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 22 for Windows.

RESULTS

Antimicrobial assessment

The Minimum inhibitory concentration (MIC) determined by (Tube Dilution Method) of different treatment against oral pathogenic bacteria which were used in the study (*Streptococcus mutans, Streptococcus pyogens, Staphylococcus aureus, lactobacillus*) are shown in Table (2). Where the toothpaste containing siwak showed the lowest MIC value against all the tested bacteria.

Table 2. *Minimum inhibitory concentration (MIC) of different treatments against the tested oral pathogenic bacteria*

Oral pathogenic bacteria	Treatment'MIC of different		
	Siwak Extract	Toothpaste containing siwak extract	Toothpaste not containing siwak extract
Streptococcus mutans	3.125	0.39	0.78
Streptococcus pyogens	6.25	0.78	1.56
Staphylococcus aureus	Negative	0.1	3.125
Lactobacillus	6.25	0.39	1.56

Color measurement

The mean ΔE values for the different groups are represented in Figure (1). Regarding Enamel group, the subgroup treated by siwak sticks (B1) showed the highest significant ΔE value (22.86± 3.07), followed by subgroup treated by toothpaste not containing siwak (B3) (16.77±2.65), meanwhile the subgroup which was treated by toothpaste containing siwak (B2) showed the lowest ΔE value (9.72± 2.31). However, In Feltk Z 350 group and Tetric N Ceram group, the subgroup treated by toothpaste not containing siwak (B3) showed the highest ΔE value (9.86±0.76, 18.61±1.44 respectively), followed by subgroup treated by siwak sticks (B1) (6.09±1.21, 11.35± 0.56), whereas, the subgroups treated by toothpaste containing siwak (B2) showed the lowest ΔE value (5.94±0.65,7.16 ± 0.66). There was a statistically significant effect of the different methods of surface treatment on ΔE values of the tested materials.

Roughness measurement

The mean Ra values for the different groups are represented in Figure (2). The subgroup treated by toothpaste not containing siwak (B3) showed the highest Ra values for the three groups; Enamel group, Feltk Z 350 group and Tetric N Ceram group respectively (133.5±21.9, 187.37± 29.47, 92.2±16.73). However lowest Ra values were recorded for the subgroups treated by siwak sticks (B1) for the three groups respectively (86.45±49.43, 60.12± 8.55, 72.02± 33.58). Regardless to different surface treatment, totally it was found that Feltk Z350 recorded statistically non-significant higher roughness mean value (91.37±28.65) than Tetric N Ceram (65.58±15.73) ($P<0.05$). Figure (3 a-c) show the micro morphological 3D images illustrating the changes in the surface roughness of the tested materials as a function of different surface treatment.

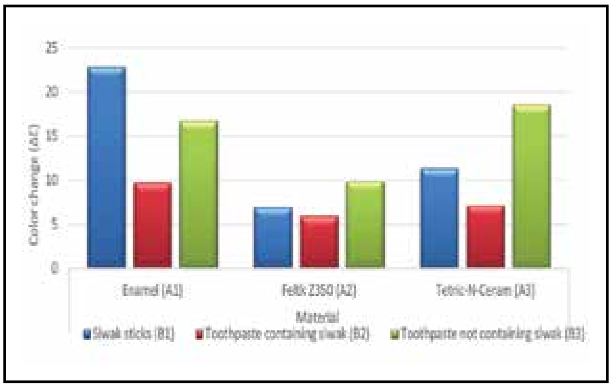

Fig. (1) Histogram showing the difference in ΔE value between the investigated subgroups as a function of different methods of surface treatment

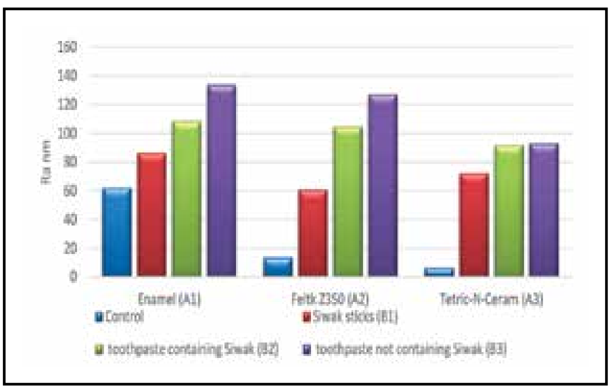

Fig. (2) Histogram showing the Ra values of the investigated subgroups as a function of different methods of surface treatment

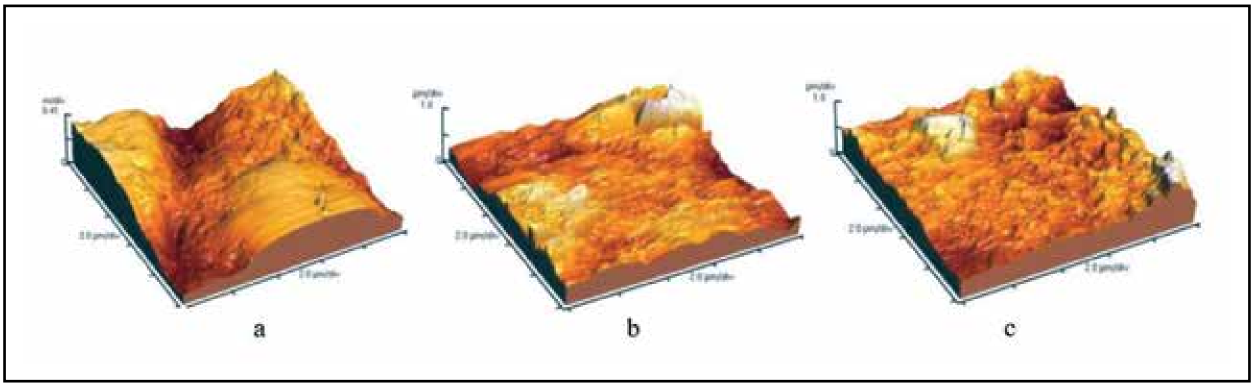

Fig. (3) a) Enamel specimen after treated with toothpaste not containing siwak, b) Feltk Z350 specimen after treated by toothpaste not containing siwak, c) Tetric N Ceram after treated by toothpaste not containing siwak.

Wear assessment

The mean **Δ**W values for the different groups are represented in Figure (4). Regarding Feltk Z350 and Tetric N Ceram groups, the subgroups treated by toothpaste not containing siwak (B3) showed the highest significant **Δ**W values (0.0152± 0.0014, 0.0203± 0.0028 respectively), followed by subgroups treated by toothpaste containing siwak (B2) (0.0110± 0.008, 0.0190± 0.0001respectively). However, the subgroups treated by siwak sticks (B1) showed the lowest significant **Δ**W values (0.0100±0.0026, 0.0147± 0.0020 respectively).The difference between treated subgroups was statistically significant as indicated by one way ANOVA (at p=0.001)**.**

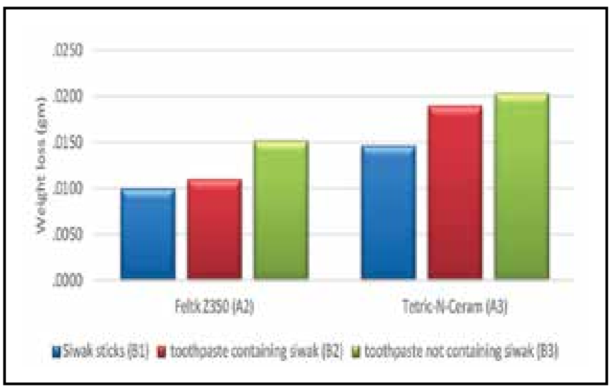

Fig. (4) Histogram showing the mean weight loss (gm) as a function of different surface treatment

DISCUSSION

Siwak (*Salvadora persica*) is one of the most commonly used medicinal plants for oral hygiene among global Muslim community [3].The Siwak is a natural toothbrush made from the twigs of the tree (ARAK).It has been reported that various components of Salvadora persica have beneficial biological properties, including significant antibacterial and antifungal activity[6,7,8].Furthermore, extracts from Siwak are reported to be effective against some periodontal pathogens and other bacteria that are important during development of dental plaque[8,9]. All specimens were stained by coffee and tea stains as they are the most common clinical cause for the extrinsic stains. The Enamel specimens were cut from bovine teeth as they are analogue in mineral content and composition to human teeth and easier to be collected. The Enamel surface was selected in the present study as during using siwak sticks or brushing, the toothbrush comes in contact with either enamel surface or restorative filling materials. Recently nanocomposite restorative filling materials are widely used for their esthetic appearance. Both the nanocomposite i.e. (nanofilled and nanohybrid) (Feltk Z350, Tetric N Ceram) which were used in the study possess similar resin matrix composition but differ in their filler particle type, size and distribution.

Antimicrobial activity was examined using Tube dilution method, this method is preferred than conventional plate or inhibition zone method as it is less time consuming, less technically demanding procedure, and more accurate method [10].The results of the present study demonstrated that the toothpaste containing siwak showed the lowest MIC value against all the tested bacteria as compared to siwak extract and toothpaste not containing siwak. This was in agreement with several studies [11,12] which found that Siwak extract can be used in mouth rinses and tooth pastes because of its antibacterial effect. This result could be due to the biologically active compounds contained in the toothpaste such as trimethylamine, salvadorine chlorides, fluoride, silica, sulphur, vitamin C and small quantities of tannins, saponins flavonoids and sterols with sufficient concentration to be more effective compared with Siwak extracts and toothpaste not containing siwak. This is in addition to the bioactive constituents present in the siwak extract itself. According to results of this study the most susceptible bacteria to Siwak extract was *Streptococcus mutans,* while no effect on *staphylococcus aureus* was reported. However, this result was in disagreement with a study[13]which tested the antibacterial activity of Salvadora persica against some oral aerobic and anaerobic bacteria and reported that the extract of siwak sticks had a drastic effect on the growth of *Staphylococcus aureus* and that the inhibitory effect of siwak was less on *Streptococcus mutans*. This may be due to the use of the roots of siwak instead of the stems, different concentrations of extract in the study, different types of yeast strains, isolations area and different assay method.

The color results of the present study demonstrated that toothbrushing either by siwak sticks or toothbrush with different toothpastes result in color change in both enamel and composite restorative materials. This was in accordance with results of other studies [14] which showed that some extrinsic stains can be removed partially or totally by means of toothbrushing with dentifrice. Moreover, others [15] have suggested that unlike in-vitro conditions, actual staining in the oral cavity would be influenced by the intermittent nature of stain exposure, the dilution of staining media by saliva and other fluids, and the polishing of restorations through toothbrushing. The enamel specimens which were brushed with siwak sticks showed the highest ΔE value , followed by those brushed with toothpaste not containing siwak, then those brushed with toothpaste containing siwak .This was in agreement with studies [16,17] which reported that the Siwak improves the value of tooth color as measured by a spectrophotometer by a mechanical effect, because it contains silica grains and sodium bicarbonate which act as abrasive agents which remove stains and provide whiteness of the teeth. Moreover, Siwak has a chemical effect, because it contains chloride. Both Feltk Z350 and Tetric N-Ceram specimens which were brushed with toothpaste not containing siwak showed the highest ΔE value, followed by those brushed with siwak sticks and the lowest values were for those brushed with toothpaste containing siwak. This could be due to the use of whitening dentifrices with tooth brushing which could act to decrease plaque and surface deposits on composite as well as helping in removing stains and discolorations. These findings are similar to those [18] which suggested that ingredients of the toothpastes used include range of components such as sodium bicarbonate, hydrated silica, sodium tripolyphosphate and other ingredients which have the ability to influence substratum surfaces, could affect the surface characteristics of composite materials. The whitening ingredients in close up toothpaste are special silica abrasives that prevent formation of stains and remove stains from the surface.

The surfaces of enamel or any restorative materials in the oral cavity are subjected to a variety of factors that can influence surface quality, oral hygiene procedures such as toothbrushing which can increase the Ra values of the enamel or restorative materials, thus promoting bacterial growth and staining [19]. The results of the current study showed that there was significant increase in the mean Ra values after application of the staining agent in all the specimens as compared to their values before application of staining agents. In the enamel, Feltk Z350, and Tetric N Ceram groups the Ra values were higher as compared to those before staining. These results could be explained as the particles of the coffee and tea pigmentation originated from mechanism of adsorption of colorant on the surface and absorption in the subsurface layer of tooth structure or composite restoration [20]. Also micro cracks and micro voids located at the interface between the filler and the matrix are the most likely to allow penetration pathways for stain and roughness of the surface [21].

Regarding the surface roughness of the enamel, Feltk Z350, and Tetric N Ceram values after different surface treatments, the specimens which were brushed with toothpaste not containing siwak showed the significantly highest Ra values, followed by those which were brushed with toothpaste containing siwak, meanwhile the lowest values were for those which were brushed with siwak sticks. These results are similar to those [22] which found that toothbrushing alone did not have the capability to promote a significant increase in roughness, but brushing with toothpaste affect surface texture due to the retention of the abrasive agents .This was also in accordance with a study [23] which proved that dentifrices produced changes in the Ra values of the resin composite after brushing, that larger abrasive particles means more abrasion from the toothpaste. Silica is more abrasive than calcium carbonate when their particles are the same size [24].The presence of abrasives in the composition of toothpastes could be responsible for brushing-related abrasion, the toothpastes containing siwak which were used in the present study contains abrasive particles such as alumina, silica, calcium carbonate. In addition to the previous ingredients toothpastes not containing siwak contains also sodium bicarbonate which could account for changes in the roughness values of all the tested materials. However, others [19] showed that there was highest increase in Ra values of hybrid composite after stimulated brushing, but whitening toothpastes do not necessarily exhibit higher abrasivity [25] and that the Ra values for most of the composite materials increased between 1 and 6 hours of brushing only which indicate that the surface became rougher without using dentifrices [25].

Regarding the surface roughness of the nanocomposite materials used in the study, totally the Feltk Z350 specimens showed higher insignificant Ra value (91.37±28.65) after different dentifrices brushing treatment as compared to those obtained in the Tetric N Ceram specimens (65.58±15.73).These results are in accordance with a study [26] which compared the surface roughness of nanofilled and nanohybrid composites before and after toothbrush-dentifrice abrasion and found that Tetric Evo-Ceram(nanohybrid composite) exhibited the lowest surface roughness at all times than Filtek Supreme XT (nanofilled composite). However ,these findings disagree with the study [27] which found that Filtek Z350 showed lower surface roughness as well as higher abrasion resistance as compared to Tetric N Ceram. Furthermore, others [28] demonstrated that Nanofilled composites present lower roughness values and better polishing characteristics than do hybrid composites, that Filtek Z350 specimens retained the similar roughness values before and after erosion and abrasion challenge. This could be due to the biomechanical degradation resistance and high abrasion resistance of nanofilled composite which basically related to its chemical composition.

Usually after consuming beverages and foods, people brush their teeth to prevent caries development; exerting mechanical forces on enamel and restorative material surface. Regarding the weight loss values of the nanocomposite specimens used in the study after different surface treatments, the results showed that the specimens treated by toothpaste not containing siwak showed the highest significant ΔW values, followed by toothpaste containing siwak, and the lowest ΔW values were for those treated by siwak sticks. This was in agreement with the studies [29,18] which found that the ingredients of the toothpastes include a range of component such as sodium bicarbonate, hydrated silica, sodium tripolyphosphate and other ingredients have the ability to influence substratum surfaces, which could affect the surface characteristics of composite materials. Also these findings are in accordance with others [30] who stated that there are dentifrices with low, medium, high abrasivity. However, when minerals are combined with abrasives such as calcium carbonate, sodium pyrophosphate, titanium oxide, sodium phosphate, it is considered a high abrasive dentifrice. It is worthy to mention that the main causative factor of abrasivity is not limited to the type of abrasive present in the dentifrice, but the physical characteristics of the mineral as the size and the shape of the particles. Silica, for example, when used in fine particles with regular shapes, preserves its mild abrasivity mineral characteristic, but when coarse and irregular particles are incorporated, it is highly abrasive. Thus, the type of the abrasive agents present in the dentifrice has been sufficient to characterize its abrasivity to the tooth structures and composite resins [31].

Concerning filler particles of the nanocomposite specimens which were used in the present study, totally it was found that Tetric N Ceram specimens recorded statistically the higher significant weight loss value (0.018±0.002), than Feltk Z350 specimens (0.0125±0.002). These results are in accordance with several studies [27,32,33] which found that that Tetric N Ceram (nanofilled composite) showed lower wear resistance than Filtek Z350 (nanohybrid composite) and that the greater the size of filler particles, the greater the amount of material lost which influence the chemical degradation and abrasion on the surface properties of the nanorestorative materials. The difference constitution of the organic matrices and higher filler loading could explain the difference in the behavior of the two materials. Regarding to the filler particles, the nanofilled resin composite materials is formulated by a combination of nanosized particles with the nanoclusters formulations [34]. The higher filler loading with smaller particle size provides a reduction in the interstitial spacing, which effectively protects the softer matrix, reduces the incidence of filler exfoliation, enhance the material's overall resistance to abrasion [35], when the nanocomposite undergoes toothbrush abrasion, only nanosized particles are plucked away, leaving the surfaces with defects smaller than light wave length [36].

CONCLUSIONS

Under the limitation of this study, it was found that:

- The impact of the toothpaste containing siwak was more effective than siwak extract on the tested microorganisms.
- The type of dentifrices affects surface roughness of the nanocomposite.
- Siwak extract and toothpaste containing siwak are effective in removing the stains from enamel and resin composite surfaces.
- Wear of the resin composite depends on the ingredients of the dentifrices.
- Toothpaste containing whiting agents caused weight loss of the tested resin composites than those with siwak extract.

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