



Evaluation of the Antibacterial Activity and the Remineralizing Potentiality of Two Natural Extracts and Their Mixture (*In Vitro* Study)

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Abstract

AIM: This study aimed to assess the antibacterial activity against the cariogenic *Streptococcus mutans* (ATCC 25175) and the commensal *Actinomyces viscosus* (1300 EMCCN) oral bacteria and to determine the remineralizing effect of the previously demineralized bovine enamel lesion of ginger extract, eggshell extract as well as their mixture.

METHODS: The two different extracts; eggshell extract and ginger extract were prepared as well as their mixture in an equal volume. Their antibacterial activity against *S. mutans* and *A. viscosus* was assessed by well diffusion test and clear zones diameters measurement (mm). Chlorhexidine and Dimethyl sulfoxide solution were utilized as positive and negative control, respectively. Thirty-six anterior bovine teeth were subjected to artificial caries pH cycling regimen including alternate demineralization and remineralization for 5 days. Then they were randomly divided into three groups (G) (n = 12): G1: Ginger, G2: Eggshell, and G3: Mixture. Specimens of each group were separately immersed in 50 ml of the tested extract for 12 h seven successive days. Vickers Microhardness and Energy Dispersive X-ray (EDX) Analysis were assessed at baseline, after demineralization and remineralization.

RESULTS: Both extracts had antibacterial effect against the profile growth of *S. mutans*. However, the mixture showed the highest statistically significant mean inhibition zone diameters (mm), followed by ginger and eggshell extracts with no statistically significant difference between them. Regarding the remineralizing efficacy; G1:(Ginger) group showed the highest statistically significant mean microhardness followed by G3:(Mixture) group, then eggshell treated specimens; which showed the least statistically significant mean microhardness values. Regarding EDX analysis results, a statistically significant increase in F (wt%) after remineralization in both eggshell and ginger treated specimens.

CONCLUSION: Ginger as well as mixture extracts not only have antibacterial effect against cariogenic bacteria but also have a strong remineralizing ability of demineralized enamel.

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Introduction

Dental caries is considered a chronic disease throughout the whole world and still has a high prevalence in spite of having different developments in the field of dentistry focusing primarily on shifting to preventive dentistry [1]. It has been assumed that the main cause of caries is the cariogenic bacteria that cause demineralization and white spot lesions resulting in dissolution of the apatite crystals in addition to loss of the calcium and phosphate ions from the tooth surface [2].

There is a great demand to use natural products that offer antibacterial effect as well as remineralizing effect. Furthermore; using antibacterial agents have numerous negative feedback on the gastrointestinal system with elevated resistance to such chemicals. In addition, economically developing countries need cost-effective and biocompatible preventive methods [3].

Among natural products, ginger rhizome (*Zingiber officinale* Roscoe, and *Zingiberaceae*) natural

herbals with their antimicrobial activities as it has been stated that they have an antibacterial properties [2], [4]. In addition, there is no toxicity authorized by the United States Food and Drug Administration as 'generally recognized safe' natural planet. These herbs have a pungent oil that contain polyphenolic ketones which have a variety of pharmacological activities. Both antifungal and antimicrobial effects on oral cavity pathogens have been reported in many studies [3], [4], [5]. It has been stated that tooth paste containing ginger aids in remineralization of white spot lesions [6].

On the other hand, the outer crust of a chicken eggshell is a non-edible substance that is usually discarded. It is a porous natural substance that is been thoroughly investigated using scanning electron microscopy and microfocus X-ray scattering techniques. Chicken eggshell powder is a well-organized structure that is mainly composed of calcium carbonate (95%). It is known as a calcium rich source with a high degree of bioavailability [7]. Furthermore, it was found that the high pH of the eggshell powder when used in the form of a

solution along with its high bioavailability of the calcium content, provided it a great potentiality to enhance enamel tooth structure remineralization [8], [9]. On the other hand, it has been reported that chicken eggshell powder has an antibacterial effect [10].

Thus, it was of interest to use such natural materials to enhance remineralization as well as to modify the biofilm microbiota ecology.

Methods

Preparation of the natural extracts

Preparation of ginger extract

Ginger was obtained from a local market; it was cleaned with distilled water to remove any dirt then it was peeled into thin slices. About 100 g of dried rhizomes were grounded until fine particles approached. The grounded rhizomes were then soaked in three liters of ethanol (99.9%) for 48 h at room temperature. Then, the supernatant was filtered through filter paper (150 mm, no.2). The supernatant was poured into rounded rotary evaporator flask using rotary evaporator (Rotavapor, WB 2000, Heidolph Instruments GmbH & Co KG, Walpersdorfer, Germany). The flask was partially immersed in a water bath with 60°C temperature and rotating at a fixed speed of 150 rpm for 2 h until the organic solvent was totally evaporated [11].

Preparation of eggshell extract

The eggshell powder was obtained by calcination in accordance with the World Intellectual Property Organization's protocol (WO/2004/105912: Method of generating eggshell powder) [12], [13]. Calcination was carried out to obtain pure powder free of pathogens and to boost the alkalinity of the powder. Twenty chicken eggs was obtained from a local market, after removal of their contents the eggshells was cleaned in distilled water then they were kept in hot water bath at 100°C for 10 min followed by removing the membrane. These eggshells were crushed using a hand blender to obtain fine powder particles. Then, the crushed shells were added into 700 ml boiling distilled water for 10 min. After that, eggshell powder was oven dried and re-crushed again into tiny pieces. One gram of this powder was dissolved into 20 ml of 4% aqueous acetic acid solution [13].

Preparation of mixture (eggshell/ginger extract)

An equal volume of ginger extract and eggshell extract was mixed in a beaker under continues stirring on a magnetic stirrer for 20 min.

Characterization

Determination of pH the prepared extracts

A digital pH meter was used to determine the pH of the prepared extracts (Ginger, Eggshell, and Mixture) (Jenway, Bibby Scientific Limited, Stafford-shire, UK). Before usage, the digital pH meter was calibrated with buffer solution at pH 7.0 and pH 10.0 at 25°C.

The measurements for each sample were done in triplicates [14].

Particle size analysis

Using a Zetasizer Nano ZS (Malvern Instruments Ltd, UK), the mean size and zeta potential of the prepared extracts were measured using photon correlation spectroscopy. Before measurement, the prepared samples were adequately diluted with distilled water. For each sample, three duplicates were obtained [15].†

Transmission electron microscopy (TEM) assessment

The prepared samples of ginger and eggshell extracts were investigated for their morphological structure using TEM (JEOL Co., JEM-2100, Japan). On a carbon-coated copper grid, one drop of the diluted extract was applied and allowed to dry. Then, one drop of phosphor-tungstic acid solution 1% (w/v) was mounted on the grid for 3 min. Photomicrographs were taken for both extracts at magnification 400×.

Antibacterial assessment of the prepared extracts

The antimicrobial activity of the two tested extracts as well as their mixture was evaluated against both cariogenic and commensal oral bacteria using well diffusion method [15].

Microorganism cultivation

One of the oral pathogenic bacteria, *Streptococcus mutans* ATCC 25175, and commensal bacteria, *Actinomyces viscosus* 1300 EMCCN, was employed to evaluate the inhibitory effects of the investigated extracts *in vitro*. Until cultivation for testing, the bacteria were kept on brain-heart infusion (BHI) agar plates (Acumedia Neogen do Brasil, Indaiatuba, SP, Brazil). Bacteria were cultured in liquid BHI for 12 h at 37°C with agitation at 150 rpm. Each microorganism's suspension was adjusted to 0.5 McFarland in phosphate buffered saline [16].

Well diffusion method

Twenty microliters of each tested strain were impaled on the surfaces of solid mitis salivaruis agar media for *S. mutans* and nutrient tryptone soya agar for *A. viscosus* using sterile cotton swabs. Then, wells were prepared by a cork borer in the agar layer and filled with 100 μ L of each tested extract and mixture of both extract. After that, plates containing *S. mutans* were cultured for 24 h at 37°C with 5% CO₂, while plates with *A. viscosus* were incubated without CO₂. The clear zones diameters were measured in millimeters after incubation [17], [18]. Chlorhexidine digluconate (CHX) solution in H₂O (Sigma-Aldrich, USA) and Dimethyl sulfoxide solution DMSO: 50 wt (Sigma-Aldrich, USA) were utilized as positive and negative control, respectively.

Remineralization assessment

Teeth selection and preparation

By adopting a power of 80% to detect a standardized effect size in microhardness $d = 0.65$ (medium-sized standardized effect size), and the level of significance 95% ($\alpha = 0.05$), the minimum required sample size was found to be 12 specimens per group. (Number of group = 3) sample size was $12 \times 3 = 36$ specimens for microhardness. A total of 36 bovine anterior teeth were selected for this study. Removal of any residual tissues and debris was done by teeth scraping with hand scaler then washing under running tap water. At the level of the cemento-enamel junction, the roots of the cleaned teeth were sliced with a double side cutting low speed disc. The pulpal tissues were removed with barded broaches, and the pulp chambers were sealed with pink wax [19], [20]. Except for a tiny window of 4 mm \times 4 mm in the middle third of the crown, the labial surface of the crowns was painted with acid resistant varnish (Nail polish, Barielle, NY, USA) to standardize the area of microhardness and Energy dispersive X-ray (EDX) measurement.

Formation of artificial carious enamel lesions

Following baseline microhardness and EDX testing, all specimens were put through a 5 consecutive days pH cycling routine that included alternate demineralization for 3 h in a demineralizing solution, followed by remineralization for 21 h in artificial saliva.

At each change of medium, specimens were washed in distilled water. Every 24 h, the demineralization solution and artificial saliva were replaced [21].

The artificial saliva had the following composition: 1.5 mmolL⁻¹ Ca [NO₃]2.4H₂O, 0.9 mmolL⁻¹ NaH₂PO₄.2H₂O, 150 mmolL⁻¹ KCl, 0.1 molL⁻¹ Tris buffer, and 0.03 ppm F. The saliva pH will be adjusted and maintained at 6.57. While the demineralizing solution had the following composition: 2.2 mM calcium chloride (CaCl₂ 2H₂O) and 2.2 mM monosodium phosphate (NaH₂PO₄ 7H₂O), 0.05 mM lactic acid; pH was

adjusted to 4.5 with 50% sodium hydroxide (NaOH). All the demineralized specimens were subjected for Vickers microhardness (VHN) and EDX testing.

Study design

The demineralized specimens were randomly assigned into three groups (n = 12) according to the used extract as follow; G1: (Ginger), G2: (Eggshell), and G3: (Mixture). Specimens of each group were separately immersed in 50 ml of the tested extract for 12 h and after washing they were kept in artificial saliva for another 12 h. This process was repeated for seven successive days using freshly prepared extracts every day. VHN test and EDX Analyzer were assessed at baseline, after demineralization and remineralization.

Surface microhardness assessment

Using rounded sectional Teflon molds of 4 cm diameter, with their treated labial sides facing upward, the prepared crown specimens were centered and inserted horizontally in auto-polymerized acrylic resin blocks. After setting of the acrylic resin for 1 h, the Digital VHN tester (NEXUS 4000TM, INNOVTEST, model number 4503, The Netherlands) was used to assess enamel surface microhardness at 20 \times magnification while a load of 200 g was applied for 15 s [12], [22], [23], [24].

EDX analysis

Three specimens from each group were chosen randomly and evaluated using an Environmental EDX Analysis (EDX, Model Quanta 250, FEI business, Netherlands) to determine the amount of Calcium, Phosphorus, and Fluoride ions. The specimens were positioned within the chamber on aluminum stubs with the labial enamel sides facing upward. The contents of calcium, phosphorus and fluoride ions were then calculated in weight percent [25], [26], [27].

Statistical analysis

In each test, the mean and standard deviation values were computed for each group. The data were examined for normality using the Kolmogorov–Smirnov and Shapiro–Wilk tests and the data revealed a parametric (normal) distribution.

Measurement was repeated to compare more than two groups in linked samples, the ANOVA test was utilized. To compare two groups in related samples the Paired sample t-test was employed. In non-related samples, to compare more than two groups one-way ANOVA test was utilized, followed by the Tukey *post hoc* test. The level of significance was fixed at $p \leq 0.05$. IBM® SPSS® Statistics Version 20 for Windows was used for statistical analysis.

Results

Results of the natural extracts characterization

pH results for the prepared extracts

The pH results were 5.8, 7.2, and 6.2 for ginger extract, eggshell extract, and mixture, respectively.

Particle size distribution and zeta potential

The average size distribution of the prepared extracts and the mixture was found to be in the range of 5.6 ± 4.1 up to 11.25 ± 6.25 μm . The eggshell extract showed significantly lower size distribution than that observed with either the ginger extract or the mixture extract ($p < 0.05$).

Zeta potential results were -21.54 , -18.76 , and -20.08 , for ginger extract, eggshell extract, and the prepared mixture, respectively.

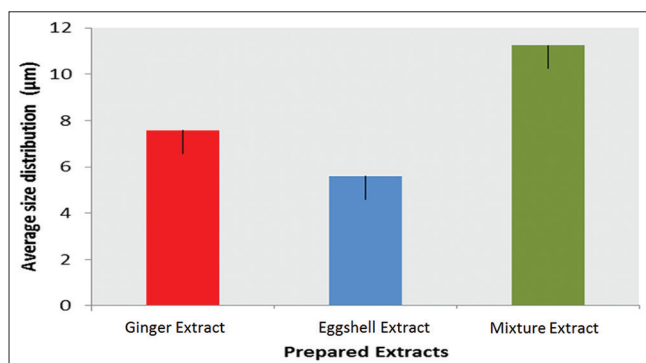


Figure 1: Size distribution of the prepared extracts

TEM results

Figure 2 showed that the structure of ginger extract showed larger size and less aggregation than eggshell extract.

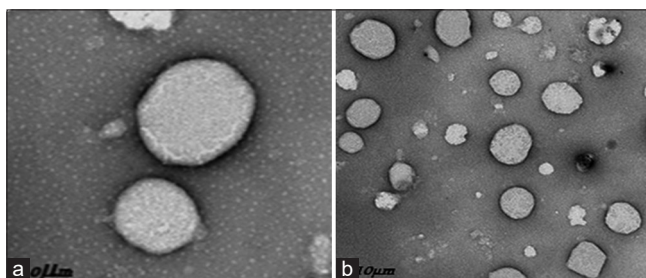


Figure 2: TEM morphological examination for (a): Ginger extract and (b) eggshell extract

Antibacterial assessment

Antibacterial assessment against *S. mutans* cariogenic bacteria

Mean inhibition zone diameters (mm) for different tested extracts against *S. mutans* were presented in Table 1 and Figure 3. Results revealed that all prepared extracts had antibacterial effect against *S. mutans*.

Table 1: Mean inhibition zone diameters (mm) for different tested extracts and the positive (CHX) and negative (DMSO) controls against *Streptococcus mutans*

CHX (+ve control)	DMSO (-ve control)	Ginger extract	Eggshell extract	Mixture	p-value
25.6 (0.7) ^a	0	18.8 (0.6) ^b	17.4 (1.1) ^b	26.8 (0.8) ^a	< 0.001*

*: Significant at $p \leq 0.05$, Different superscripts are statistically significantly different

There was no statistically significant difference between mixture extract and CHX (positive group); in terms of mean inhibition zone diameters as both had the maximum statistically significant mean inhibition zone diameters (mm) followed by ginger and eggshell extracts

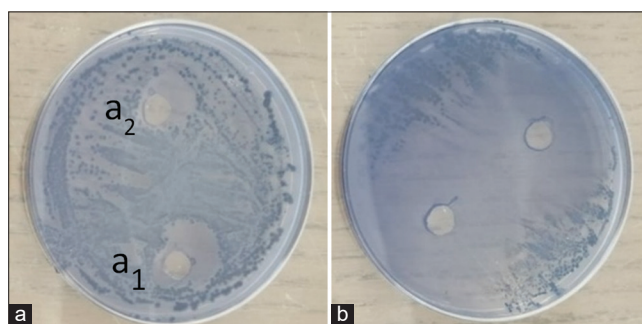


Figure 3: Antibacterial efficacy of the tested extracts against *Streptococcus mutans*. a: (a1; Eggshell extract, a2; Ginger extract) and (b): Mixture of extracts

where there was no statistically significant difference between them.

Antibacterial assessment against *A. viscosus* commensal bacteria

Mean inhibition zone diameters (mm) for different tested extracts against *A. viscosus* are presented in table (2) and Figure 4. Results revealed that the positive control as well as ginger extract had antibacterial potential against *A. viscosus*, where there was a statistically significant difference between them. While -ve control, eggshell extract, and the mixture have no antibacterial efficiency toward *A. viscosus* the commensal oral bacteria.

Table 2: Mean inhibition zone diameters (mm) for different tested extracts and the positive (CHX) and negative (DMSO) controls against *Actinomyces viscosus*

CHX (+ve control)	DMSO (-ve control)	Ginger extract	Eggshell extract	Mixture	p-value
32.6 (1.1) ^a	0	18.7 (0.8) ^b	0	0	< 0.001*

*: Significant at $p \leq 0.05$, Different superscripts are statistically significantly different

Remineralization assessment results

VHN test results

Table (3) showed the mean, standard deviation (SD) values of the microhardness results of different groups. Results revealed that there was no statistically significant difference between eggshell, ginger, and mixture groups at baseline and after demineralization, at ($p=0.490$) and ($p=0.154$) respectively (Figure 5). On the other hand, there was a statistically significant difference between the eggshell, ginger, and mixture groups following remineralization treatment ($p<0.001$). At ($p<0.001$), and ($p=0.001$), respectively,

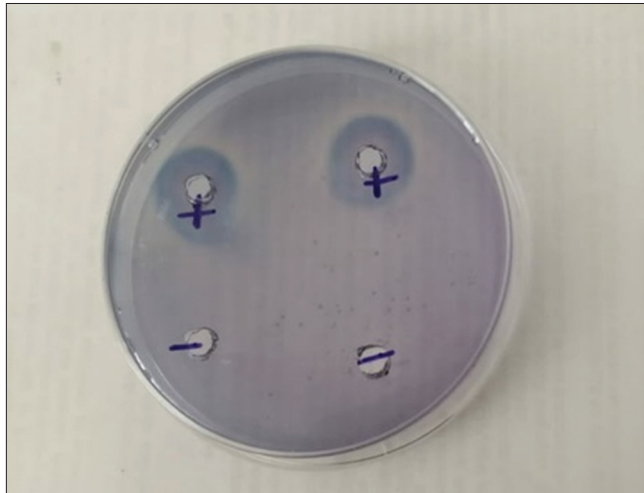


Figure 4: Antibacterial activity exhibited by positive control (CHX) and negative control (DMSO) against Streptococcus mutans

a statistically significant difference was identified between eggshell treated specimens and each of the ginger and mixture groups. In addition, there

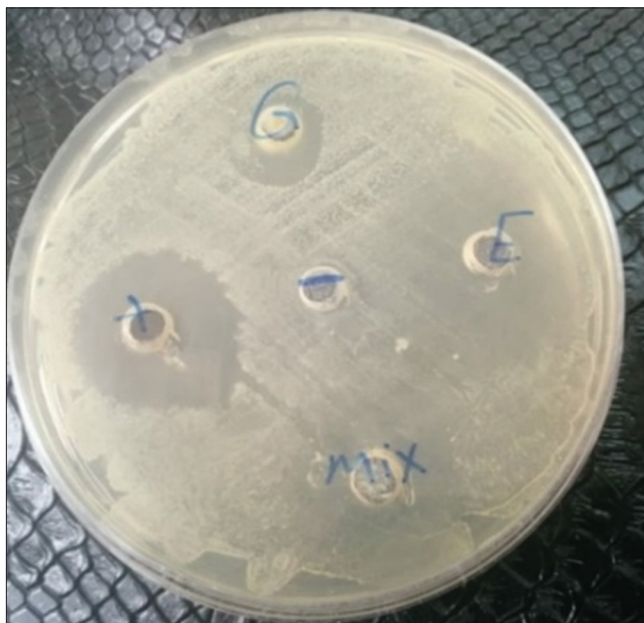


Figure 5: Antibacterial efficacy of the tested extracts against Actinomyces viscosus (G; ginger, E; eggshell, +ve control, and mixture of two extracts)

was a statistically significant difference between the ginger and mixture groups ($p=0.001$). Where ginger treated specimens showed, the highest statistically significant mean microhardness, followed by mixture treated specimens. While, eggshell treated specimens showed the least statistically significant mean microhardness values.

Table 3: The mean, standard deviation (SD) values of microhardness (VHN) of different groups

Variables	Microhardness (VHN)						p-value
	Eggshell		Ginger		Mixture		
	Mean	S.D	Mean	S.D	Mean	S.D	
Baseline	285.20 ^{Aa}	8.88	304.15 ^{Aa}	22.21	287.38 ^{Aa}	33.14	0.490 ns
After demineralization	227.70 ^{Ba}	7.27	250.40 ^{Ba}	17.54	235.55 ^{Ba}	18.06	0.154 ns
After remineralization	152.13 ^{Cc}	18.63	342.70 ^{Ab}	33.10	250.48 ^{Ca}	19.87	< 0.001*
p-value	0.001*		0.010*		0.016*		

*, significant ($p < 0.05$), ns; non-significant ($p > 0.05$); Means with different upper-case letters in the same column indicate statistically significance difference, means with different lower case letters in the same row indicate statistically significance difference

Moreover, there was a statistically significant difference between Baseline, after demineralization and after remineralization groups for each of the eggshell, ginger, and Mixture groups at ($p=0.001$), ($p=0.010$), and ($p=0.016$), respectively, when comparing each individual enamel treatment within each group. Demineralized groups showed the least statistically significant mean microhardness values. Only for ginger treated specimens, there was no statistically significant difference between baseline and after remineralization groups, at ($p=0.067$).

Table 4: The mean, standard deviation (SD) values of Calcium (Ca) (wt%) of different groups

Variables	Ca (wt%)						p-value
	Eggshell		Ginger		Mixture		
	Mean	S.D	Mean	S.D	Mean	S.D	
Baseline	64.44	0.45	64.24	0.48	64.55	0.99	0.862ns
After demineralization	60.71	1.90	59.64	3.23	60.58	3.29	0.885ns
After remineralization	64.51	1.00	65.67	0.51	65.25	0.95	0.309ns
p-value	0.110ns		0.099ns		0.128ns		

ns: non-significant ($p > 0.05$)

EDX analysis results

Mean and standard deviation (SD) for Ca, P, and F (wt%) results for tested groups are shown in tables (4, 5, and 6). Results revealed that there was no statistically significant difference in Ca, P (wt%) values for different groups. A statistically significant increase in F (wt%) after remineralization in both eggshell and ginger treated specimens as shown in tables 6.

Table 5: The mean, standard deviation (SD) values of phosphorus (P) (wt%) of different groups

Variables	P (wt%)						p-value
	Eggshell		Ginger		Mixture		
	Mean	S.D	Mean	S.D	Mean	S.D	
Baseline	28.37	0.82	28.24	0.21	28.84	0.68	0.550 ns
After demineralization	29.10	0.63	28.11	1.03	29.07	0.42	0.252 ns
After remineralization	28.09	0.67	27.79	0.45	28.21	0.49	0.641 ns
p-value	0.296 ns		0.634 ns		0.337 ns		

ns: non-significant ($p > 0.05$)

Table 6: The mean, standard deviation (SD) values of fluoride (F) (wt%) of different groups

Variables	F (wt%)						p-value
	Eggshell		Ginger		Mixture		
	Mean	S.D	Mean	S.D	Mean	S.D	

Baseline	1.70 ^A	0.09	1.83 ^A	0.24	1.72	0.25	0.706 ns
After demineralization	1.24 ^A	0.21	1.13 ^B	0.20	1.06	0.23	0.616 ns
After remineralization	2.19 ^B	0.08	2.94 ^{AB}	0.93	2.65	0.61	0.414 ns
p-value	0.004*		0.016*		0.071 ns		

*; significant ($p < 0.05$), ns: non-significant ($p > 0.05$); Means with different upper case letters in the same column indicate statistically significance difference

Discussion

Nowadays, the tendency toward using natural and biocompatible products has increased to avoid health problems. Hence, researchers have been trying to incorporate natural ingredients in dental product. There were plenty of studies confirmed that plants have many favorable properties such as antibacterial, antioxidant, and anti-inflammatory [3]. However, limited number of researches studied the effects of such natural extracts on cariogenic oral bacteria. Moreover, there was no research studied its effect on commensal oral bacteria. Hence, it was of value to study the antibacterial ability as well as the remineralization efficiency of naturally prepared extracts.

In this *in vitro* study, characterization of the prepared extract was done by measuring pH and particle size. The acidic pH of ginger extract (5.8) might favor the removal of the superficial layer on enamel surface that inhibit penetration of remineralizing particles. This was in accordance with Ambarkova *et al.* [22] and Liang *et al.* [28] and who proved that remineralization can occur even in acidic solution. On the other hand, plenty of studies stated the importance of raising the local pH for successful remineralization [29], [30], [31]. In accordance with such studies our research revealed that the pH of eggshell extract and mixture preparation were 7.2 and 6.8, respectively. This neutral pH might induce fast penetration of remineralizing particles inside the demineralized tooth surface.

As shown in Figure 1, the average size distribution of the prepared extracts and their mixture were small enough to allow easy penetration of the small remineralizing particles into the demineralized enamel surface.

On the other hand, Muzzalupo *et al.* stated that zeta potential considered as an important indicator which reflects how much stable and free of agglomeration will be in the prepared system [31]. Our results were -21.54, -18.76, and -20.08, for ginger, eggshell, and the prepared mixture, respectively. As zeta potential increases, it refers to the state of stable solution free from aggregation [32].

Regarding the antibacterial assessment test, the routinely used agar diffusion method was utilized. This method involves placing the prepared extract on an agar plate inoculated with certain oral bacteria. To produce the zone of inhibition which denote its antibacterial activity, the extract needs to leach a soluble antibacterial agent. If the amount of eluted

agents was not enough the zone of inhibition will not be produced. *S. mutans* plays an important role in dental biofilm formation and in the etiology of caries initiation and propagation [10].

The current study proved that all of the prepared extracts had strong antibacterial effect against *S. mutans* as shown in table (1) and Figure 3. The antibacterial activity of ginger against *S. mutans* might be due to its valuable phytoconstituents which had a good binding affinity to the surface protein antigen of *S. mutant*, in addition to the hydrophobic interactions with the protein active site that increase the inhibitory effect against *S. mutans* surface protein antigen [2], [14]. On the other hand, the high concentration of bio-available calcium ions and the alkalinity of the eggshell extract were the main cause of its antibacterial activity [8], [10], [33].

To the best of our knowledge, no previous research studied the antibacterial effect of remineralizing agents against commensal oral bacteria. Philip *et al.* [34] stated that actinomyces were considered as anaerobic, nonvirulent commensal bacteria that were normally found in the oropharynx and gastrointestinal tract as a resident microbial flora. *Actinomyces viscosus* was one of the clinically relevant species. This organism required either a disruption of the natural barriers or a change in the typical microbial flora to cause infection.

According to Duggal *et al.*, most of the microorganisms implicated in dental caries etiology were indigenous organisms and survive in a dynamic relationship with the host [35]. Disease was not always the end result of this association, but it might ensue when the balance was greatly disturbed.

Our findings revealed that only ginger extract could inhibit the commensal oral bacteria *A. viscosus*. While, both eggshell extract and the mixture preparation had no antibacterial efficiency against the tested commensal oral bacteria.

Regarding VHN test results, (table 3), ginger extract, showed the highest statistically significant mean microhardness values which almost exceed the base line results, with no statistical significance. This might be due to high fluoride content of ginger [3], [36]. That was confirmed by the EDX results which showed a significant increase in F wt% of demineralized enamel after Ginger remineralization (table 6).

On the other hand, eggshell extract treated specimens, showed a statistically significant decrease in the mean microhardness of the enamel surface after remineralization (table 3). This was in contrary to other studies that proved that eggshell solution was capable of increasing the microhardness of demineralized enamel specimens in a statistically significant way due to its high calcium and phosphorous content that should favor the remineralization process for initial caries lesions [12], [37]. This unexpected result for the eggshell extract in the current study might be attributed to the method of extract preparation, as 4% acetic acid

was used for its preparation, which might cause further tooth demineralization.

In the current study, the mixture of eggshell and ginger was efficient as a remineralizing solution. The efficient remineralizing role of ginger and eggshell mixture could not be differentiated whether it was a result of ginger high fluoride content, or it seems that there could be certain boosting effect on initial lesions. The acidic pH of the eggshell solution might enhance the release of fluoride from ginger at the time of application resulting in higher remineralization when compared to the eggshell treated specimens.

Result of EDX revealed non statistical significance in Ca and P wt% between all tested groups. Although Ca wt% was increased in all groups after remineralization and exceed that of demineralization that increase was not statistically significant. That might be due that the remineralization protocol utilized in the current study was only for 7 days, in contract to other studies that found significant increase in the ions content after 15 and 30 days of remineralization [38].

Conclusions

Within limitations of this current study, it could be concluded that:

- 1- The mixture of ginger and eggshell extracts was capable of modifying the oral biofilm by having a strong antibacterial effect against *S. mutans* cariogenic oral bacteria and had no negative effect on *A. viscosus* commensal oral bacteria.
- 2- Ginger extract as well as its mixture with eggshell had a strong remineralizing ability of the demineralized artificial enamel lesions.

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