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REMINERALIZATION AND ANTIBACTERIAL EFFICACY OF ASHWAGANDHA, MACA AND GINGER-BASED MOUTHWASHES VERSUS FLUORIDE-BASED MOUTHWASH ON INITIAL ENAMEL CARIES: AN IN-VITRO STUDY

Possy Moustafa Abd El Aziz*, Raghda Kamh** and Esraa Abdel Ghaffar El Gezawy***

ABSTRACT

Purpose: Non-invasive management of non-cavitated caries lesions by the medicinal use of plant extracts is the goal of modern dentistry; this study was accomplished to assess the remineralizing and antibacterial potential of some herbals (Ashwagandha, Maca, and Ginger) in comparison to Fluoride-based mouthwash on initial enamel carious lesions. **Materials and Methods:** In this study, fifty extracted premolars were allocated evenly into five groups of 10 teeth each. Group A: teeth treated with 0.5% Ashwagandha-based mouthwash (n=10); Group M: teeth treated with 0.5% Maca-based mouthwash (n=10); Group G: teeth treated with 0.5% Ginger-based mouthwash (n=10); Group F: teeth treated with Fluoride-based mouthwash as a positive control group (n=10); and Group C; negative control group: teeth not exposed to any treatment and stored in artificial saliva. All teeth were evaluated using the DIAGNOdent pen® at baseline, after demineralization, and after 14 days with different remineralizing materials. The antimicrobial properties of fluoride and plant extracts against *Streptococcus mutans* and Lactobacillus *acidophilus* were quantitatively evaluated by an antimicrobial susceptibility test. The data were statistically analyzed using an ANOVA test. **Results:** All experimental treated groups confirmed a reduction in DIAGNOdent pen® values. Group G showed the most significant decrease (-8.3±2.16), followed by Group M (-7.3±0.82), and Group A (-7.3±1.25), then Group F (-6.5±0.53). The lowest reduction was observed in the control group (-3.60.97). The distinction between groups was statistically significant (p=0.000). All used mouthwashes had antibacterial properties against Streptococcus mutans and Lactobacillus acidophilus bacteria, with no statistically significant difference. **Conclusion:** Herbal-based-mouthwashes of 0.5 % Ashwagandha, Maca, and Ginger are effective remineralizing agents on initial enamel carious lesions with antimicrobial activity against *Streptococcus mutans* and Lactobacillus *acidophilus*.

KEYWORDS: Ashwaganda, Maca, Ginger, initial enamel caries, remineralization, mouthwash.

* Assistant Professor, Conservative Dentistry Department, Faculty of Dentistry, Cairo University and Egyptian Russian University
** Associate Professor, Conservative Department, Faculty of Dentistry, Egyptian Russian University
*** Assistant Professor, Faculty of dentistry, Conservative Dentistry, Egyptian Russian University

INTRODUCTION

Dental caries is widely recognized as a chronic disease. The prevailing belief is that caries is primarily caused by cariogenic bacteria, which lead to demineralization, the breakdown of apatite crystals, and the depletion of calcium and phosphate ions from the tooth surface [1-2]. Early-stage enamel caries treatment involves non-invasive remineralization techniques within the minimally invasive dentistry framework. Consequently, several types of Fluoride, such as mouthwashes, dentifrices, gel, and varnish, were employed to treat early-stage tooth decay [3-4].

Fluoride is acknowledged as the most effective method for preventing tooth decay and promoting the restoration of minerals in teeth. However, excessive fluoride usage can lead to a condition known as dental fluorosis, which has detrimental effects on oral health. Additionally, using bactericides or antibacterial agents can lead to various adverse effects on the gastrointestinal system and may also contribute to the emergence of antibiotic-resistant bacterial strains. Hence, there is a significant need to utilize natural products rather than manufactured ones with antibacterial and remineralizing effects [5].

Recently, there has been a significant focus on natural products due to their advantageous effects on health. While numerous herbal plants have shown antibacterial properties, their effectiveness against cariogenic bacteria is currently being investigated. This study investigates the possible antibacterial and remineralizing effects of three unique herbal agents: Ashwagandha, Maca, and Ginger.

Ashwagandha, scientifically referred to as *Withania somnifera*, is a highly valued botanical species within traditional Indian medicinal practices. In vitro and in vivo studies have assessed its effectiveness and safety on various biological systems. Ashwagandha is a critical ingredient in over 100 herbal medicine formulations. It is rich in natural antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase. These antioxidants offer potential benefits, including antibacterial, anti-cancer, immunomodulating, and anti-inflammatory properties, which are believed to promote good health. [1,6].

Maca is a widely recognized biennial herb with several physiological qualities, including anti-inflammatory, antioxidant activity, and control of immunological response, particularly in fermented plant extracts. The substance contains a variety of polysaccharides, minerals, amino acids, and phenolics, which indicate its pharmacological properties [7].

The Ginger rhizome is a frequently utilized natural herbal supplement recognized by the FDA and known for its lack of hazardous side effects. The herbs possess a potent oil that contains polyphenolic ketones, which exhibit diverse pharmacological properties. Ginger exhibits prominent innate antibacterial, antioxidant, and antifungal characteristics. However, there needs to be more research that evaluates the effectiveness of Ginger in remineralizing early-stage enamel caries [2].

Thus, using such natural agents to enhance remineralization and modify the biofilm microbiota ecology was interesting. The present in vitro study assessed the remineralization and antibacterial efficiency of Ashwagandha, Maca, and Ginger-based mouthwashes on initial enamel caries compared to Fluoride-based mouthwash.

The null hypothesis theorized that using herbal extract mouthwashes exhibits remineralization and antibacterial capabilities comparable to fluoride-based mouthwash in the context of the earliest enamel carious lesions.

MATERIAL AND METHODS

The study's materials are enumerated in (Table 1)

TABLE (1) Materials used in the study, composition, and manufacturer

Material	Composition	Manufacturer
Ashwagandha powder	100% Natural Ashwagandha Root Powder	Imtenan Health shop, Obour city, Egypt
Maca	Organic Maca Root	Oladole Natural, Indianpolis, IN, USA
Ginger	Fibers, protein, carbohydrates fat, lipids, protease, minerals, phenolic	Imtenan Health shop, Obour city, Egypt
Listerine total care	Eucalyptol 0.091%, Thymol 0.063%, Menthol 0.05%, Sodium Fluoride 220ppm, Zinc Chloride 0.09 %	Johnson & Johnson Consumer INC, Italy

METHODS

Study Design

Fifty extracted premolars were used in the study. Premolars were equally divided into five groups, according to the remineralizing agents used. Group A (n=10); teeth treated with 0.5% Ashwagandha-based mouthwash; Group M (n=10); teeth treated with 0.5% Maca-based mouthwash; Group G (n=10); teeth treated with 0.5% Ginger based mouthwash; Group F (n=10); teeth treated with Fluoride based mouthwash as a positive control group; and Group C (n=10); negative control group teeth were not exposed to any treatment and stored in artificial saliva. Using laser fluorescence (DIAGNOdent pen®), all teeth were evaluated at baseline, 48 hours after demineralization, and 14 days after remineralization. The antibacterial activity of the remineralizing agents against *Streptococcus mutants* and *Lactobacillus acidophilus* was measured quantitatively by an antimicrobial sensitivity test. The data were statistically analyzed using an ANOVA test.

Sample Size Calculation

A one-way analysis of variance (ANOVA) or a comparable non-parametric test will be employed to assess the impact of various treatments and controls on the remineralization of early enamel caries. According to a previous study by Rohym et al. [8], values after remineralization varied between (6.15±3.51), (7.78±4.58), (7.28±6.49), (11.75±1.25) and (8.82±5.54). Using G power statistical power Analysis program (version 3.1.9.4) for sample size determination [9], A total sample size (n=50; subdivided to 10 in each group) will be sufficient to detect a large effect size (f) = 0.54, with an actual power (1-β error) of 0.8 (80%) and a significance level (α error) 0.05 (5%) for two-sided hypothesis test.

Research Ethical Approval

Ethical approval was taken from Institutional Review Board Organization IORG0010866, Faculty of Oral & Dental Medicine, Ahram Canadian University—research number IRB0012891#84.

Sample Preparation

This study utilized fifty premolars that were extracted for orthodontic and surgical purposes. The premolars were disinfected in a 5.25% sodium hypochlorite solution for one hour. The crowns underwent manual scraping using a hand scaler and were subsequently rinsed with running tap water to eliminate every remaining tissue and debris. Finally, they were polished using a pumice paste that does not

contain fluoride. All teeth' surfaces, except a 2 mm by 2 mm window in the buccal and lingual middle third, were coated with an acid-resistant varnish [10]. A permanent marker was used to numerically code each tooth at the base for sample identification. Ten milliliters of fake saliva were placed in individual glass containers and kept at 37°C for each set of samples [11].

Artificial Non-cavitated Formation of Initial Enamel Lesion

For 48 hours, all specimens were submerged in a demineralizing solution (10 mL for every specimen). The pH of 4.4 was maintained by the demineralizing solution, which contained 2.2 mM calcium chloride, 2.2 mM potassium dihydrogen phosphate, 0.05 M acetic acid, and 1 M potassium hydroxide (KOH). To replicate the circumstances of the mouth cavity, samples were first cleaned with distilled water and then preserved in fake saliva. To avoid depletion, the demineralizing solution was replenished every 12 hours [10].

Elemental analysis of Ashwagandha, Maca, and Ginger by use of Inductively coupled plasma optical emission spectrometry (ICP-OES)

The levels of Ca and P were determined using an optical emission spectrometer, ICP-OES 5800 (Spectro Analytical Instruments, Agilent, USA). The wavelengths for Ca and P were 396.847 and 177.434 nm, respectively. Each determination was performed in triplicate, and the analytical procedure was validated [13].

Preparation of 0.5% Ashwagandha, Maca, and Ginger-based mouthwash

After weighing each resulting powder to 0.5g, 100 ml of sterile, warm distilled water was combined for five minutes in a sterile, sealed glass flask. After filtering particles with a muslin cloth, the extract was again strained and purified using Whatman No. 1 filter paper. Next, an airtight container was used to keep the purified extract [11].

Enamel Surface treatment with remineralizing mouthwashes

The samples in the experimental groups (A, M, G, and F) were immersed in 20 ml of the respective solutions (0.5% Ashwagandha, 0.5% Maca, 0.5% Ginger, and Fluoride mouthwash) at a temperature of 37°C for a duration of five minutes. This process was repeated twice a day for a period of 14 days. Following a distilled water wash, the samples were placed again in the artificial saliva container. This contained 500 milliliters of distilled water, 20 grams of potassium chloride, 0.843 grams of sodium chloride, 0.051 grams of magnesium chloride, carboxymethyl cellulose, 20 milliliters of tricalcium phosphate, and 0.05 milliliters of sodium hydroxide to maintain a pH of 6.8. For 14 days, the teeth belonging to Group C were kept in artificial saliva without exposure to any experimental remineralizing substance. It was replaced once a day to reduce the possibility that the artificial saliva might become saturated and impede the course of treatment [11,14].

Laser Fluorescence Testing (DIAGNOdent pen)

DIAGNOdent pen (KaVo, Bilberach/Riss, Germany) was used at baseline, after initial enamel demineralization, and after 14 days of remineralization. The mean value was measured and documented for each specimen. DIAGNOdent depends on the absorption of diode laser light, using organic and inorganic materials gathered at the probe tip to induce infrared fluorescence and finally demonstrating a digital number between 0 and 99. According to the manufacturer's instructions, the scoring system is as follows: Caries is not present in a score of 0–4; it is limited to the outside half of the enamel thickness in scores 5–10; it is restricted to the inner half of the enamel thickness in scores 11–20, and it is diffused throughout the dentin in scores ≥ 21 [15].

Antimicrobial Susceptibility Test

The susceptibility tests were conducted under the guidelines set by the National Committee for Clinical Laboratory Standards (NCCLS, 1993). The Well diffusion approach was employed to conduct screening experiments to measure the inhibition zone (Hindler et al., 1994). The inoculum suspension was prepared by transferring colonies grown overnight on an agar plate into Mueller-Hinton broth (or malt broth for fungi). An aseptic brush was submerged in the suspension to introduce microorganisms into Mueller-Hinton agar plates (fungi employing malt agar plates). The chemicals were dissolved in dimethyl sulfoxide (DMSO) at varying concentrations (10, 5, and 2.5 mg/ml). The diameter of the inhibitory zone surrounding each well was measured after 24 hours at 37 degrees Celsius. The controls utilizing dimethyl sulfoxide (DMSO) were satisfactorily conducted [12].

Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 20 was utilized for managing data and statistical analysis. Numerical data were gathered using mean, standard deviation, confidence intervals, median, and range. The normality of the data was assessed by examining the data distribution and doing Kolmogorov-Smirnov and Shapiro-Wilk tests. Based on the normal distribution of most data, the ANOVA test, followed by Bonferroni's post hoc test, was used to compare groups, while the paired t-test was used to make intra (within) group comparisons. The amount of difference tended to show a non-parametric distribution; therefore, the Kruskal-Wallis test was used to compare groups.

The amount of difference was calculated as:(value after-value before). All P-values are two-sided. P-values ≤0.05 were considered significant.

RESULTS

Elemental analysis of Ashwagandha, Ginger, and mama powders using Inductively coupled plasma optical emission spectrometry (ICP-OES) results:

Elemental analysis confirmed the concentration of Ca and P in 100 mg of Ashwagandha, Maca, and Ginger powder (table 2). Results revealed that Maca had the highest Ca content (71892.49 ppm), followed by Ashwagandha (34251.65 ppm) and Ginger (15834.92 ppm). Regarding the P concentration, Ashwagandha had the highest content (12736.53 ppm), followed by Maca (3758.88 ppm) and Ginger (826.71 ppm).

TABLE (2) Concentration of Ca and P in Ashwagandha, Maca and Ginger powder

Label	Ca (396.847 nm)	P (177.434 nm)
blank	0.00 (ppm)	0.00 (ppm)
Standard	5.00 (ppm)	5.00 (ppm)
Ashwagandha	34251.65 (ppm)	12736.53 (ppm)
Maca	71892.49 (ppm)	3758.88 (ppm)
Ginger	15834.92 (ppm)	826.71 (ppm)

Laser Fluorescence Testing (DIAGNOdent pen) results

Results of laser fluorescence testing of Ashwagandha, Maca, and Ginger at baseline, after demineralization, and after two weeks of remineralization are summarized in Tables (3 and 4), Figures (1 and 2).

Intergroup comparison (Table 3, Figure 1)

Baseline: a statistically significantly higher value (p=0.000) was recorded in Group C (5.9±0.88), Ginger group (5.5±1.27), and Fluoride group (4.7±1.64) in comparison to the Ashwagandha (3.1±0.88) and Maca group (2.9±0.88).

After demineralization, a significantly higher value (p=0.000) was recorded in the control group (12.4±1.17), followed by the Ginger group (11.2±2.49) and Fluoride group (10.9±1.45) in comparison to the Ashwagandha (9.7±0.82). In contrast, the Maca group recorded the lowest value (8.5±0.53).

After two weeks of remineralization: after two weeks of remineralization, a significantly higher value (p=0.000) was documented in the control group (8.8±0.42). The Fluoride group was recorded (4.4±1.17), followed by the Ginger group (2.9±0.74) and the Ashwagandha group (2.4±1.17). In contrast, the Maca group (1.2±0.42) recorded a value significantly lower than all other groups.

TABLE (3) Descriptive statistics of remineralization potential (Diagnodent) and comparison between groups regarding the recorded values (ANOVA test) and the amount of difference (Kruskal Wallis test)

		Mean	Std. Dev	Median	95% Confidence Interval		Min	Max	P value
					Lower Bound	Upper Bound			
Baseline	Ashwagandha	3.10 [b]	.88	3.00	2.47	3.73	2.00	4.00	.000*
	Maca	2.90 [b]	.88	3.00	2.27	3.53	2.00	4.00	
	Ginger	5.50 [a]	1.27	5.50	4.59	6.41	4.00	7.00	
	Fluoride	4.70[a]	1.64	4.00	3.53	5.87	3.00	7.00	
	Control	5.90 [a]	.88	6.00	5.27	6.53	5.00	7.00	
After Demin	Ashwagandha	9.70[b,c]	.82	9.50	9.11	10.29	9.00	11.00	.000*
	Maca	8.50[c]	.53	8.50	8.12	8.88	8.00	9.00	
	Ginger	11.20[a,b]	2.49	11.50	9.42	12.98	8.00	14.00	
	Fluoride	10.90[a,b]	1.45	10.00	9.86	11.94	10.00	13.00	
	Control	12.40[a]	1.17	12.00	11.56	13.24	11.00	14.00	
After 2weeks remin	Ashwagandha	2.40[c]	1.17	2.50	1.56	3.24	1.00	4.00	.000*
	Maca	1.20[d]	.42	1.00	.90	1.50	1.00	2.00	
	Ginger	2.90[c]	.74	3.00	2.37	3.43	2.00	4.00	
	Fluoride	4.40[b]	1.17	4.00	3.56	5.24	3.00	6.00	
	Control	8.80[a]	.42	9.00	8.50	9.10	8.00	9.00	
Difference between Demin & Remin	Ashwagandha	-7.30[b,c]	1.25	-7.00	-8.20	-6.40	-9	-6	
	Maca	-7.30 [b,c]	.82	-7.50	-7.89	-6.71	-8	-6	.000*
	Ginger	-8.30[c]	2.16	-8.00	-9.85	-6.75	-11	-6	
	Fluoride	-6.50[b]	.53	-6.50	-6.88	-6.12	-7	-6	
	Control	-3.60[a]	.97	-3.00	-4.29	-2.91	-5	-3	

Significance level p≤0.05, *significant, ns=non-significant

Post hoc test: Within the same comparison, means sharing the same superscript letter is not significantly different.

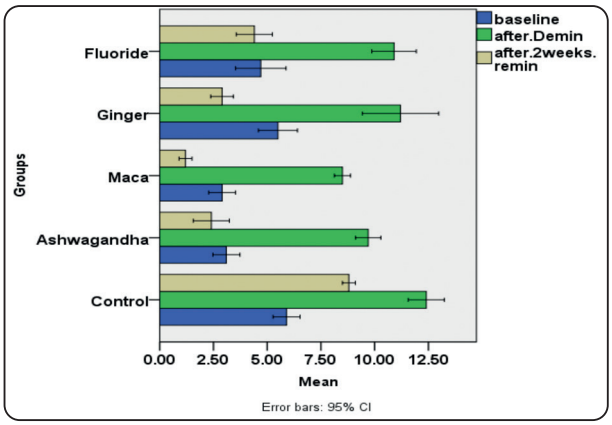

Fig. (1): The bar chart explains the mean value of remineralization potential at different observation times

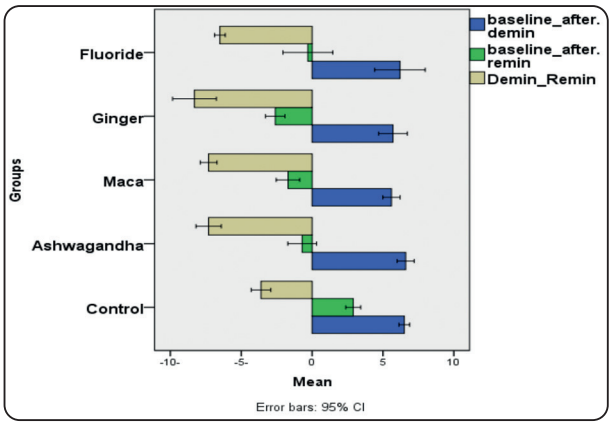

Fig. (2): The bar chart explains the mean amount of difference in remineralization potential of all groups

Amount of Difference between demineralization and after remineralization

The difference showed a decrease in all groups. The Ginger group showed the most significant decrease (-8.3±2.16), followed by the Maca group (-7.3±0.82), and the Ashwagandha group (-7.3±1.25), then the Fluoride group (-6.5±0.53). The least decrease was noted in the control group (-3.6±0.97). The difference between groups was statistically significant (p=0.000).

Intragroup comparison between demineralization and remineralization:

A statistically significant decrease in Diagnodent values after remineralization in comparison to demineralization was noted in all groups: control, Maca and Ginger groups (P = 0.004), Ashwagandha group (P = 0.003), and Fluoride group (P = 0.005) Table (4), Figure (1).

Antimicrobial Susceptibility Test results:

Antibacterial assessment against Streptococcus mutans bacteria

Table (5) and Figure (3a, b, c, d) display the measured diameters (in mm) of inhibition zones for several tested extracts against Streptococcus

TABLE (4): Descriptive statistics of remineralization potential (Diagnodent) and comparison between groups regarding the recorded values (ANOVA test) and within the same group (paired t-test)

	After Demin		AfterTwo weeks remain		P value of difference within the same group
	Mean ± SD	Median	Mean ± SD	Median	Demin. Versus remin.
Ashwagandha	9.70 ±.82	9.50	2.40±1.17	2.50	.003*
Maca	8.50±.53	8.50	1.20±.42	1.00	.004*
Ginger	11.20±2.49	11.50	2.90±.74	3.00	.004*
Fluoride	10.90±1.45	10.00	4.40±1.17	4.00	.005*
Control	12.40±1.17	12.00	8.80±.42	9.00	.004*
P value (between groups)	.000*		.000*		

Significance level p≤0.05, *significant, ns=non-significant

mutans. Results showed that all solutions used had antibacterial potential as opposed to Streptococcus mutans bacteria, with no statistically significant difference between them. Ginger-based mouthwash noted the highest inhibition zone with a mean value (32.33±0.58), followed by Ashwagandha (29.67±0.58) and Maca (29.33±0.58). At the same time, Fluoride mouthwash recorded the lowest values (30±0.00).

TABLE (5) Mean inhibition zone diameters (mm) for different tested extracts Ashwagandha, Maca, Ginger, and the positive control Fluoride against *Streptococcus mutans*

	Inhibitions zones on Strept. mutans		
	Mean (mm)	SD	SE
Ashwagandha	29.67	0.58	0.19
Maca	29.33	0.58	0.19
Ginger	32.33	0.58	0.19
Fluoride	30	0.00	0.00
P value	< .00001		

Significance level p≤0.05

**** 100 ul Samples and Control were inoculated each well at 10mg/ml concentration.***

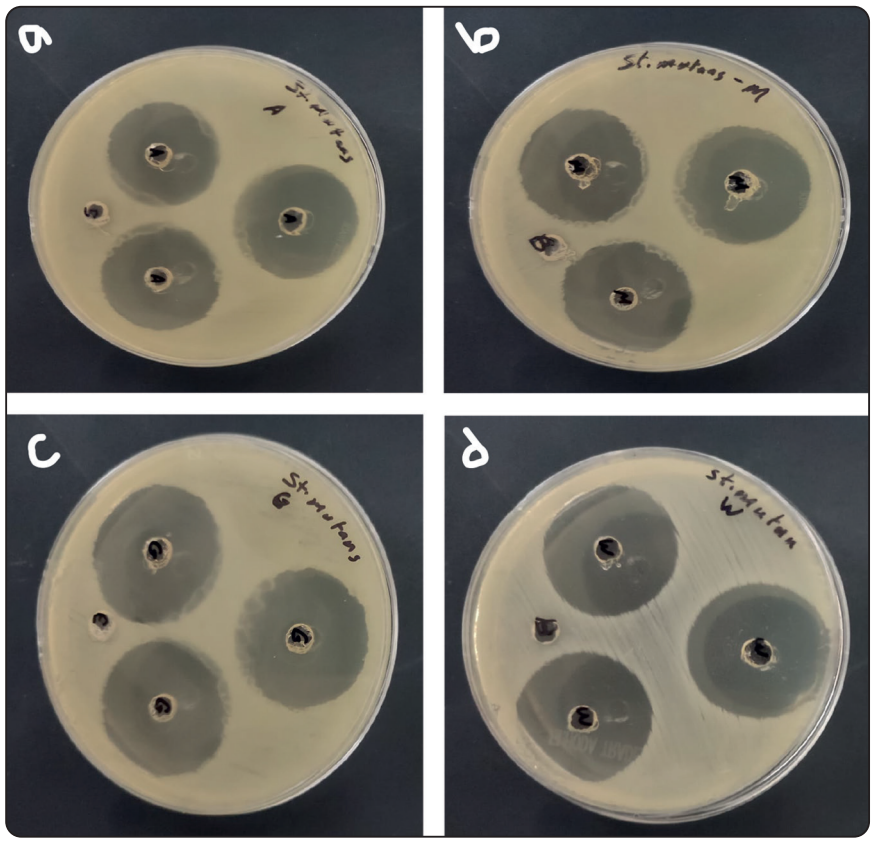

Fig. (3) Inhibition zone diameters (mm) for different tested extracts (a) Ashwagandha, (b) Maca, (c) Ginger, and (d) Fluoride against *Streptococcus mutans*

Antibacterial assessment against Lactobacillus acidophilus bacteria

Table (6) and figure (4 a,b,c,d) display the measured diameters (in mm) of inhibitory zones for several tested extracts against Lactobacillus acidophilus. Results revealed that all solutions used had antibacterial potential against *Lactobacillus acidophilus* bacteria, with no statistically significant difference between them. Ginger-based mouthwash noted the highest inhibition zone with a mean value (39.67±0.58), followed by Ashwagandha (36.33±0.58) and Maca (34.83±0.29). In contrast, Fluoride mouthwash recorded the lowest values (36±0.00).

TABLE (6) Mean inhibition zone diameters (mm) for different tested extracts Ashwagandha, Maca, Ginger, and the positive control Fluoride against *Lactobacillus acidophilus*

	Inhibition zones on Lactobacillus acidophilus		
	Mean (mm)	SD	SE
Ashwagandha	36.33	0.58	0.19
Maca	34.83	0.29	0.09
Ginger	39.67	0.58	0.19
Fluoride	36	0.00	0.00
P-value		< .00001	

Significance level p≤0.05

**** 100 ul Samples and Control were inoculated in each well at 10mg/ml concentration***

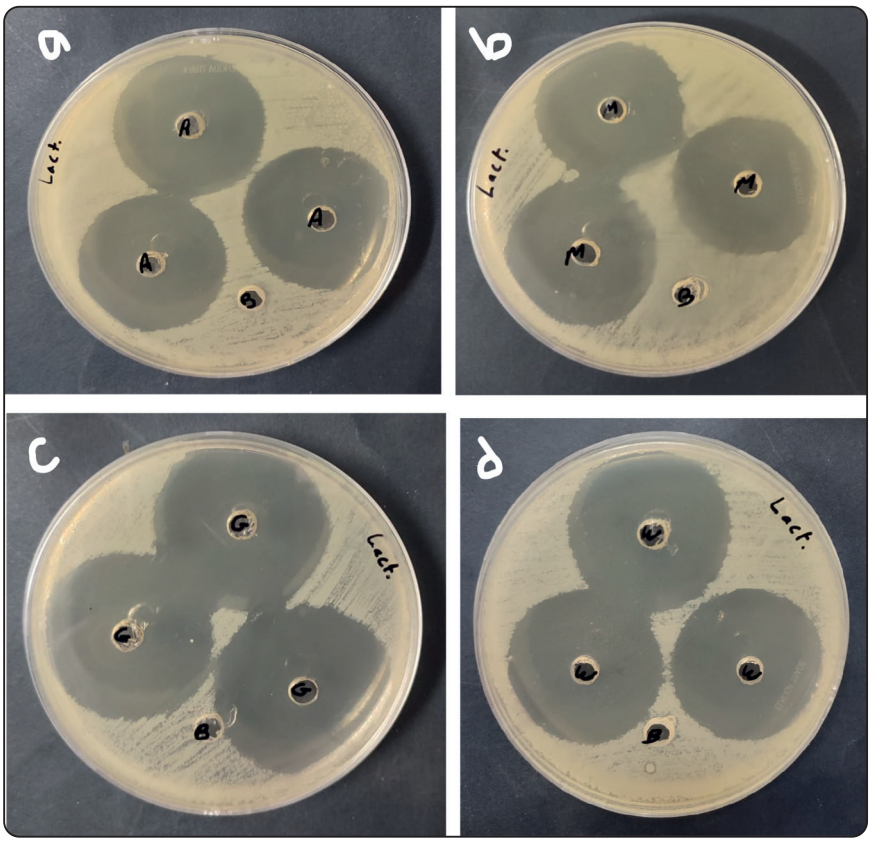

Fig. (4) Inhibition zone diameters (mm) for different tested extracts (a) Ashwagandha, (b) Maca, (c) Ginger, and (d) Fluoride against *Lactobacillus acidophilus*

DISCUSSION

Dental caries is a continuous process in which the tooth's hard structure is affected by cariogenic bacteria in the oral cavity, leading to demineralization and remineralization. Tooth caries are initially indicated by an opaque lesion that appears milky white in opacity due to porosity in the enamel under the surface. Early stages of tooth decay can be stopped and reversed with effective plaque management and remineralization treatment [8]. Hence, there is a significant demand for natural therapeutic drugs that provide remineralizing and antimicrobial properties while minimizing adverse effects. Furthermore, economically developing nations require affordable and compatible preventive measures [1]. Therefore, this study addresses the issue of early-stage tooth decay and explores the use of natural herbal mouthwashes as a non-invasive treatment for these lesions. This in-vitro study assessed the remineralizing and antibacterial properties of mouthwashes containing Ashwagandha, Maca, and Ginger compared to a mouthwash containing Fluoride.

This study tested a mouthwash containing sodium fluoride as a control agent. Sodium fluoride is often used to manage early caries lesions due to its remineralization effectiveness and antibacterial properties [15-18]. While Fluoride has been proven effective in preventing first carious lesions, excessive usage of Fluoride can have hazardous and detrimental effects. The American Association of Poison Control (AAPC) has confirmed that the primary cause of toxicity is ingesting toothpaste, followed by consuming mouthwash and Fluoride supplements. [8,19].

Ashwagandha, Maca, and Ginger have widely recognized medicinal properties as potent anti-inflammatory and antioxidant agents. Additionally, they serve as valuable sources of essential minerals, amino acids, and phenolics. The elemental analysis of Ashwagandha, Maca, and Ginger powders using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) showed a high concentration of calcium (Ca) and phosphorus (P). These findings align with the results of laser fluorescence in this study, which confirmed the ability of these herbal extracts to remineralize early enamel carious lesions.

The DIAGNOdent pen® has become known as a dependable and non-intrusive diagnostic instrument for detecting early enamel caries and monitoring the impact of preventive measures, owing to its superior sensitiveness [8,20]. The specimens underwent laser fluorescence analysis using a DIAGNOdent pen at three stages: baseline, demineralization, and remineralization. The remineralization was achieved using Ashwagandha, Maca, Ginger, and Fluoride mouthwash (positive control), whereas some specimens were left untreated (negative control).

Cariogenic bacteria such as S. mutans and Lactobacilli play a significant role in developing dental biofilm and the onset and spread of dental caries [19]. The antimicrobial assessment test was conducted utilizing the agar diffusion method. The agar well diffusion method is commonly employed to assess the antibacterial efficacy of plant or microbial extracts. This procedure involves placing the produced extracts onto an agar plate and subsequently inoculating them with specific oral bacteria. The presence of a zone of inhibition indicates the antibacterial activity of the substance. For this extract to have antibacterial properties, it must release an antibacterial agent. If the amount of this agent released is insufficient, a zone of inhibition will not be observed.

Laser fluorescence outcomes presented a statistically significant difference between the demineralized and treated enamel with Ashwagandha, Maca, Ginger, and Fluoride mouthwash after two weeks. Ginger showed the highest statistically significant remineralizing capacity, almost exceeding the baseline results. This study agreed with other studies that revealed this remineralization ability due to the

high Fluoride, calcium, and phosphorous content [8] [16-19] [21]. Additionally, using a (0.5%) concentration of ginger extract was the reason for this study's highest remineralizing capacity. At this concentration, ginger provides the maximum quantity of free calcium ions that can be deposited on the tooth surface; directly above this concentration, there may be a reduction in this quantity of free calcium due to increased concentrations of elements other than calcium [15].

Outcomes presented a statistically non-significant difference between Maca and Ashwagandha regarding remineralization and a statistically significant higher remineralizing capacity than Fluoride-based mouthwash. Ashwagandha [22-23] and Maca root [7] are well known to have plentiful biological active ingredients, including minerals; this was confirmed by elemental analysis, which revealed high Ca and P content.

The remineralization action of Fluoride mouth rinses referred to an increase in the Fluoride concentration in the oral cavity during rinsing and retention of varying levels of Fluoride in the oral cavity, forming fluorapatite and calcium Fluoride [24]. The sodium Fluoride mouthwash-treated group exhibited less remineralization potential when contrasted to the Ginger, Ashwagandha, and Maca groups. This agreed with a study [8] that mentioned this result as the in vitro study conditions lacking oral soft tissues that serve as reservoirs for Fluoride. Because fluoride is occupied on a wide surface area of soft tissue, such as the tongue, in an in vivo setting, remineralization may be impacted differently than in our in vitro investigation. The availability of active remineralizing agents may be increased. [8] [25-26].

Certain bacteria, including Streptococcus mutans and other non-streptococcus strains, such as Lactobacillus acidophilus, generally cause dental caries. These bacteria create acid, which lowers plaque's pH to a crucial level. *Streptococcus mutans* is responsible for the onset of dental caries, while *Lactobacillus acidophilus* contributes to the advancement of dental caries. [5].

This study demonstrated that all prepared extracts and sodium Fluoride mouthwash had a strong antibacterial effect against Streptococcus mutants and Lactobacilli. Sodium Fluoride mouthwash can kill cariogenic bacteria such as Streptococcus mutants and lactobacilli by hindering their metabolic activities and forming lactic acid. Fluoride ions inhibit glycolytic and other enzymes participating in bacterial metabolism. It alters the permeability of cell membranes, causing the cytoplasmic pH to drop and a reduction in acidity, which is typically linked to tooth decay [27-28].

Ginger is a medicinal plant with an antioxidant and antibacterial function due to the phenols that interfere with the growth of Gram-positive and Gram-negative bacteria. This is achieved by their deep attraction to the surface protein antigen of Streptococcus mutants and their ability to interact with the hydrophobic region of the protein's active site, enhancing their ability to block the surface protein antigen of Streptococcus mutants. It also binds to the active site of metabolic enzymes, leading to a decrease in the enzyme. Besides, the antibacterial property of Ginger is referred to as the high concentration of Fluoride in it [16,19,21,29,30].

Maca root has numerous biologically active properties and antioxidant, immunomodulatory, anti-cancer, and antibacterial special effects because it contains many polysaccharides, amino acids, and phenolics [7]. Similarly, Ashwagandha, which contains alkaloids and steroidal compounds, possesses antioxidant, anxiolytic, anti-inflammatory, and significant antimicrobial activities [23] [31]. These substances can attach and precipitate macromolecules, like bacterial enzymes that influence the metabolism of bacteria. The null hypothesis was rejected as the herbal-based mouthwashes revealed a statistically significant remineralizing potential compared to fluoride-based mouthwash.

CONCLUSION

Within the limits of this investigation, it was determined that:

- Herbal-based-mouthwashes of 0.5 % Ashwagandha, Maca, and Ginger are effective remineralizing agents on initial enamel carious lesions with antimicrobial activity against Streptococcus mutants and Lactobacillus *acidophilus*.
- The previously mentioned herbal-based mouthwashes could be a cheap and safe substitute for fluoride-based mouthwash.

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