Original Article

Remineralising potential of *Ocimum basilicum* varnish and fluoride varnish on initial enamel caries: An *in vitro* microscopic study

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Abstract

Background: The focus of caries research has switched to early identification and non-invasive treatment of carious lesions.

Aim: This study aimed to evaluate and compare the remineralising potential of *Ocimum (O.) basilicum* varnish and fluoride varnish on initial enamel caries.

Method: The authenticated *O. basilicum* seeds were procured from a repository, and the extract was prepared using the Soxhlet method, which was vortexed with Indian Pharmaceutical (IP)-graded chemicals to obtain varnish. Extracted premolar tooth samples were divided into three groups of 33 each after demineralisation with a pH of 4.5 for 48 hours at 37°C. Each group was subjected to remineralisation twice daily with respective agents for 4 minutes for 30 consecutive days. Each sample was ground-sectioned through an enamel window. The lesion depth was measured using a light microscope (Leica™ DM2500) and ImageJ software. The data were evaluated using analysis of variance (ANOVA) and post hoc analysis.

Results: The mean (± SD) pre-treatment lesion depth across the groups ranged from 242.11 ± 26.144 µm to 352.66 ± 34.531 µm. The highest lesion depth recovery rate of 45.938% was recorded for the fluoride varnish group, followed by 36.015% in the *O. basilicum* varnish group, which was statistically significant by Tukey's post hoc analysis ($p < 0.001$). The gingival fibroblast cells were viable by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.

Conclusion: The *O. basilicum* varnish demonstrated a homogenous layer of mineral deposition. However, the remineralising efficacy was slightly lesser than that of the fluoride varnish. Hence, the novel *O. basilicum*-based remineralisation agent appears to have potential as a non-invasive alternative to topical fluorides in the therapy of early caries lesions.

Keywords: Basil seeds, dental caries, fluoride, initial enamel lesion, *Ocimum basilicum*, remineralisation, varnish

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INTRODUCTION

Dental caries is an irreversible microbial disease of the calcified tissues of the teeth, characterised by demineralisation of the inorganic portion and destruction of the organic substance of the tooth, which often leads to cavitation.[1] It is a complex process where a multitude of factors influence and initiate the progression of the disease.[2] Bacterial activity initiates the production of organic acids, which penetrate the tooth through crystals.[3] This reduces the salivary pH below the 'critical pH' of 5.5, leading to an abnormal loss of minerals from the enamel surface or subsurface known as demineralisation.[4] This is the first step in the continuum of the dental caries process, which can eventually lead to cavitation. Therefore, the prevention and biomimetic treatment of initial caries in enamel have been one of the paramount challenges faced by dental professionals and public health communities.[5,6]

Remineralisation is the body's natural mending mechanism for non-cavitated subsurface carious lesions.[7] Fluoride is a preventative substance whose high cariostatic activity has captivated dental research.[8] The current concept indicates that fluorides function primarily through a topical mechanism by inhibition of demineralisation and enhancement of remineralisation and by inhibiting the enzymatic activity of cariogenic bacteria.[9,10] Despite its profound efficacy in preventing caries progression, it has certain limitations.[11] Fluoride does not completely eliminate caries, although excessive fluoride concentrations might be detrimental to the teeth.[12] Fluoride is often described as a double-edged sword as exposure to both high and low levels of fluoride can usher dental and skeletal harmful effects.[13]

Thus, contemporary research has focused on developing nontoxic, biocompatible and cost-effective anticariogenic agents that might be added to toothpaste, mouthwash and diet to reduce caries experience.[14] There is certainly a need for cutting-edge remineralisation technologies that can supplement fluoride, fill the void in its remineralising action and result in a more complete consolidation of carious lesions.[15] Therefore, it has been advocated to use medicinal plant extracts, which have a profound effect on caries prevention with minimum adverse effects.[16]

The primary benefits stated for medicinal plant therapeutic usage in many diseases are their safety, in addition to being affordable, effective and readily available.[17] Basil seeds are authenticated as *Ocimum (O.) basilicum* belonging to the family Lamiaceae, which is an annual plant.[18] Sweet basil seeds are a rich source of many polyphenolic flavonoids, especially orientin and vicenin; essential oils such as eugenol, citronellol, linalool, limonene, citral and terpineol; and high levels of beta carotene, lutein, zeaxanthin, vitamins and minerals.[19] Basil seeds contain a high amount of calcium of 2240 mg (244% of recommended daily dose (RDD)) and a phosphate concentration of 2630 mg (56% RDD), which is required for remineralisation.[20] A study was conducted by Kalra *et al*.[21] to test the antibacterial efficacy of essential oil extracts of different varieties of Ocimum (Tulsi) on common oral pathogens. The evidence obtained showed a significant inhibitory effect of essential oil on aerobic and anaerobic microorganisms. Similarly, an *in vitro* study by Wiwattanarattanabut *et al*.[22] concluded that cinnamon and sweet basil essential oils had impressive anticariogenic and antiplaque effects and may be proposed as alternative and effective supplements to promote oral health status. Moreover, a high concentration of calcium found in gum arabic, which is a natural polysaccharide exudate from Acacia senegal, is considered to have the ability to enhance remineralisation according to Onishi *et al*.[23] Gulcin Bilgin Gocmen *et al*.[16] evaluated the effectiveness of herbal medicaments such as ginger, rosemary and honey on remineralisation of initial enamel lesion. Shivakumar *et al*.[24] assessed the antimicrobial activities of *Tinospora cordifolia* and *Ocimum tenuiflorum* against *Streptococcus mutans* and *Candida albicans* to conventional medications such as chlorhexidine and nystatin. However, limited studies in the field of the remineralisation potential of *O. basilicum* seeds prove to be the lacunae as evidenced in the available data. Hence, the objective of this study was to evaluate and compare the remineralising potential of novel *O. basilicum* varnish and fluoride varnish on initial enamel caries by light microscopy method and using ImageJ software so that it might be used as an alternative to commercially marketed fluoride varnishes.

MATERIALS AND METHODOLOGY

The current investigation was an *in vitro* study that was carried out in accordance with Good Laboratory Practice (GLP)[25] principles and was approved by the Institutional Research and Ethics Committee (Ref No. 1453). *O. basilicum* seeds were authenticated by a recognised taxonomist. The herbarium specimen of the same has been deposited in the herbaria with accession number RMRC-1630. The coarse powder of *O. basilicum* seeds was procured from the Ayurveda pharmacy of the recognised repository (AYUSH Approved Central Research Facility and Drug Testing Laboratory for ASU Drugs).

Inclusion and exclusion criteria

Extracted human permanent maxillary and mandibular premolars, macroscopically caries-free or orthodontically extracted teeth were included in the study, whereas teeth having intrinsic stains, dental caries, and gross surface defects such as pits, fractures, fluorosis, developmental defects and enamel hypoplasia/hyperplasia were excluded from the study.

Sample size estimation

The sample size for the three groups was assessed using power and effect size by Cohen's statistical power analysis.[26] Power and effect size were estimated based on data procured from the pilot study by fixing the *P* value at 0.05 (alpha = 0.05, power = 80%). The effect size was assumed to be 0.5. Hence, the sample size was estimated to be 33 in each group.

Extract preparation using Soxhlet method

The extract was prepared using the Soxhlet method with 99% ethanol (Changshu Hongsheng Fine Chemicals Co. Ltd., China) as solvent. The powdered seeds (100 gms) were kept in a muslin cloth bag and placed in the body of the Soxhlet extractor (Sigma-Aldrich® Chemicals Pvt. Ltd., Bangalore, India), and ethanol (800 ml) was added to it. The isomantle (Vertex® Scientific and Lab Instruments Co., New Delhi, India) was used to gently heat ethanol, causing it to evaporate. This procedure used a total of 6 hours to complete 24 cycles at the boiling temperature of ethanol at 78^0 C (173^0 F). On completion of the procedure, ethanol was entirely evaporated with the help of a rotary evaporator (IKA™) at 40°C, to yield 20 mg of extract. The yield was resuspended in dimethyl sulphoxide (DMSO) (Qualigens, Thermo Fisher Scientific Pvt. Ltd., Mumbai, India) in 20 mg/ml ratio to obtain a stock solution.[27]

Phytochemical tests

The extract obtained was subjected to preliminary phytochemical screening for the qualitative detection of phytoconstituents of *O. basilicum* extract using standard procedures as described by Trease and Evans.[28-30]

Preparation of O. basilicum varnish

The extract was placed in a Branson Bath® Sonicator 1800 (Branson Ultrasonics, Danbury, CT) along with ethyl acetate (Sigma-Aldrich® Chemicals Pvt. Ltd., Bangalore, India) for about 30 minutes for the complete dissolution of the extract. After its dissolution, isoamyl propionate (Sigma-Aldrich® Chemicals Pvt. Ltd., Bangalore, India) and the colloidal solution (Vivid India® Chemicals Pvt. Ltd., New Delhi, India) were added. The contents were mixed in a MixMate® Vortex Agitator (Eppendorf, Sydney, Australia) at 1000 rpm (maximum setting) for about 30 seconds, and fumed silica (Research-Lab Fine Chem Industries, Mumbai, India**)** was added. The contents were then transferred to an amber-coloured sterile bottle and labelled. Physical properties of varnish such as colour matching, rate of evaporation, viscosity, pH of the varnish and film-forming ability were evaluated.

Assessment of cytotoxicity

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) (HiMedia® Laboratories, Pvt. Ltd., Mumbai, India) reagent was prepared using 5 mg in 1 ml of phosphate-buffered saline (PBS—pH 7.4) (HiMedia® Laboratories, Pvt. Ltd., Mumbai, India), and a cytotoxicity assay was performed on adult human gingival fibroblasts (HGFs) that were harvested from healthy gingival tissue of a mandibular premolar region.[31] *In vitro* growth inhibition effect of *O. basilicum* varnish was assessed by enzyme-linked immunosorbent assay (ELISA) reader (Epoch, BioTek® Instruments, Inc., USA) by the determination of conversion of MTT into 'formazan blue' by living cells. The 50 µl of 4000 cells/ml cell suspension was seeded into each well in a 96-well microtitre plate (NEST-Biotechnology, Jiangsu, China), and the final volume was made up to 150 µl by adding Dulbecco's modified Eagle's medium (DMEM) (Gibco™ Life Technologies, Bangalore, India). 100 µl of the *O. basilicum* varnish and fluoride varnish was added to the separate wells and incubated for 24 hrs, in the presence of 5% CO_2, at 37°C in a CO_2 incubator (New Brunswick™ Galaxy® 170 R, Eppendorf, Germany). After 24 hrs, 20 µl of 5 mg/ml MTT reagent was added to the wells. The supernatant was carefully removed without disturbing the precipitated formazan crystals, and 100 µl of DMSO was added to dissolve the crystals formed. The optical density (OD) was measured at a wavelength of 492 nm.

Preparation of demineralising solution

2 mM calcium chloride (SDFCL Sd Fine Chem Limited., Tamil Nadu, India), 2.2 mM monosodium phosphate (HiMedia® Laboratories, Pvt. Ltd., Mumbai, India) and 0.5 M acetic acid (Molychem® Pvt. Ltd., Mumbai, India) were mixed with 1 litre of distilled water by an electromagnetic stirrer (REMI Sales and Engineering Ltd., Mumbai, India) at 800 rpm, and pH was adjusted at 4.4 with 1 M potassium hydroxide (HiMedia® Laboratories, Pvt. Ltd., Mumbai, India) by pH metre.

Randomisation and subsurface lesions

Enamel specimens derived from extracted teeth were used in this study after screening for any macroscopically visible caries or surface defects and stored in 10% formalin

solution (Sigma-Aldrich® Chemicals Pvt. Ltd., Bangalore, India). Each enamel surface was marked with a square area of 4×4 mm to mimic an enamel window buccally. The remainder of the enamel specimen was coated with acid-resistant nail varnish. The enamel specimens were divided randomly into three groups mainly fluoride varnish (group 1), *O. basilicum* varnish (group 2) and placebo (group 3). After immersing enamel specimens in demineralising solution for the 72-hour cycle, artificial subsurface carious lesions were created. Each group was subjected to remineralisation twice daily with their respective agents for 4 minutes, once in the morning and later in the afternoon for 30 consecutive days using an applicator tip (pH cycling). The samples were stored in artificial saliva (Biochemazone™ Alberta, Canada) with adjusted pH of 7.0 to simulate the normal pH of the oral environment.

Enamel specimens and evaluation of remineralisation potential

Each sample was sectioned longitudinally in the labio-lingual direction through the window using a diamond disc (San-I® Grinding Wheel Products Co., Ltd., Changhua Hsien, Taiwan) to produce approximately 700- to 1000-μm-thick sections. These sections were further ground on silicon carbide combination stone (CUMI® Carborundum Universal Limited, Tamil Nadu, India) to produce 150 to 250 μm paper-thin ground sections [Figure 1]. The light microscope (Leica™ DM 2500) was standardised for the visualisation of ground sections by an expert. A trained examiner calibrated the ImageJ software to record and transfer the lesion depth into an electronic database and checked for data entry consistency. The images were captured on screen by a charge-coupled device (CCD) shading camcorder (Leica™ DFC320) attached to the microscope. These images were scaled using ImageJ software for measuring lesion depth in enamel. The images were captured and measured by the same examiner who evaluated microscopic slides in a standardised closed environment by viewing for approximately 20 seconds at a distance of approximately 3 metres from the examiner.

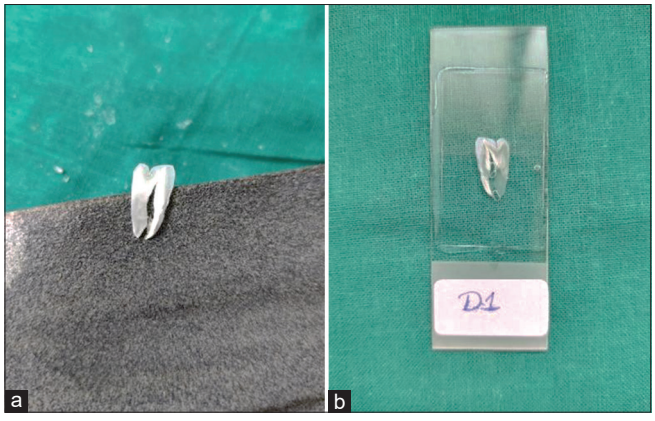

Figure 1: a) Ground sectioning of the tooth samples b) Representative mounted ground section in the demineralising group

Statistical analysis

Data obtained were entered in Microsoft Excel 2020 and analysed using the IBM Corp. Released 2012. IBM SPSS® Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp. The data were subjected to the normality (Shapiro–Wilk) test, and they were found to fall under the normal distribution. The mean and standard deviation (SD) of the lesion depth from each group were obtained. The values were tabulated and statistically analysed using the one-way analysis of variance (ANOVA). Comparison of each group with the control group was analysed using 'Student's *t*-test'. The comparison of mean lesion depths between groups was conducted using Tukey's post hoc analysis. $P \leq 0.05$ was considered to be significant.

RESULTS

The present *in vitro* study was designed to evaluate and compare *O. basilicum* varnish on the remineralising potential of artificially created initial enamel caries. Lesion depths were recorded in all group samples using a light microscope and analysed with ImageJ software. Upon application of remineralising agents, the light microscopy photomicrograph revealed a coating of fine mineral deposition, coupled with adequate replenishing of the porosities and voids that occurred from the previously formed carious lesions. A homogenous, uniform surface of remineralisation was observed in the samples of *O. basilicum* varnish as compared to test groups of fluoride varnish application [Figure 2].

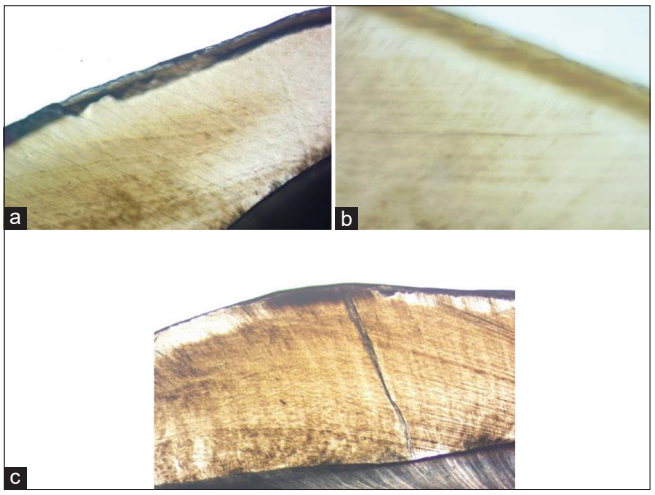

Figure 2: Light microscope 4X magnification representative sample lesion depth. a) Fluoride varnish. b) *Ocimum basilicum* varnish. c) Placebo

The mean (± SD) pre-treatment lesion depth across the groups ranged from 242.11 ± 26.144 µm to 352.66 ± 34.531 µm. Comparisons between pre-test lesion depths in all groups were statistically insignificant ($p = 0.380$) [Table 1]. In group 1 positive control group (fluoride varnish), the average post-lesion depth was 148.62 µm with a range of 124.59 µm to 166.61 µm. In the group 2 test group (*O. basilicum* varnish), the average post-lesion depth was 180.52 µm with a range of 158.12 µm to 218.48 µm, and in the group 3 negative control group (placebo), the average post-lesion depth was 282.45 µm with a range of 216.32 µm to 348.30 µm. ANOVA revealed statistically significant differences among the three groups ($p < 0.001$) [Table 2]. The mean comparison of pre- and post-treatment lesion depths is depicted in Figure 3.

The paired *t*-test showed a significant decrease in lesion depths after the specific treatment regimen. The reduction in mean lesion depth after pH cycling was maximum for group 1, followed by group 2 ($p < 0.001$) indicative of effective remineralisation post-pH cycling. However, there was a considerable increase in mean lesion depth in group 3 with $P = 0.028$ [Figure 4]. Inter-group analysis within the groups for lesion depths revealed a statistically significant difference by Tukey's post hoc analysis ($p < 0.001$). The mean intra-group lesion depth in group 1 positive control was higher as compared to the group 2 test group. However, the mean difference in group 3 was negative and found to be statistically significant by ANOVA ($p < 0.001$) [Table 3]. The percentage of mean lesion depth recovery was calculated for all the experimental groups. The highest recovery rate of 45.948% was recorded for the group 1 positive control group, followed by 36.015% in the group 2 test group. However, the lesion depths in group 3 were increased by 3.5% [Table 4].

The MTT assay results obtained for the *O. basilicum* varnish and the fluoride varnish control revealed that it was non-toxic to gingival fibroblast cells as the viability of cells was maintained [Figure 5]. It was observed that the

Table 1: Comparison of mean pre-treatment lesion depth across test groups

Groups	Mean	SD	SE	95% confidence interval for mean		*F*	*P*
				Lower	Upper		
Group 1	278.112	29.625	5.157	267.607	288.617	0.978	0.380
Group 2	284.280	26.144	4.551	275.009	293.550		
Group 3	273.911	34.531	6.011	225.01	352.66		

All values are expressed as mean±standard deviation (SD) in parentheses; statistical test used: ANOVA; group 1: fluoride varnish group (positive control), group 2: *Ocimum basilicum* varnish (test), group 3: placebo group (negative control); level of significance: **P*≤0.05 is considered statistically significant, ***P*≤0.001 is considered highly significant

Table 2: Comparison of mean post-treatment lesion depth across test groups

Groups	Mean	SD	SE	95% confidence interval for mean		*F*	*P*
				Lower	Upper		
Group 1	148.62	9.626	1.675	145.204	152.031	381.297	<0.001*
Group 2	180.52	11.876	2.067	176.307	184.729		
Group 3	282.45	32.172	5.60	271.043	293.858		

All values are expressed as mean±standard deviation (SD) in parentheses; statistical test used: ANOVA; group 1: fluoride varnish group (positive control), group 2: *Ocimum basilicum* varnish (test), group 3: placebo group (negative control); level of significance: **P*≤0.05 is considered statistically significant, ***P*≤0.001 is considered highly significant

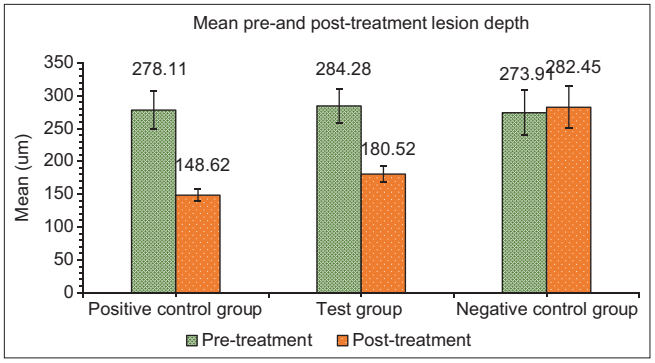

Figure 3: Mean comparison between groups with respect to lesion depth

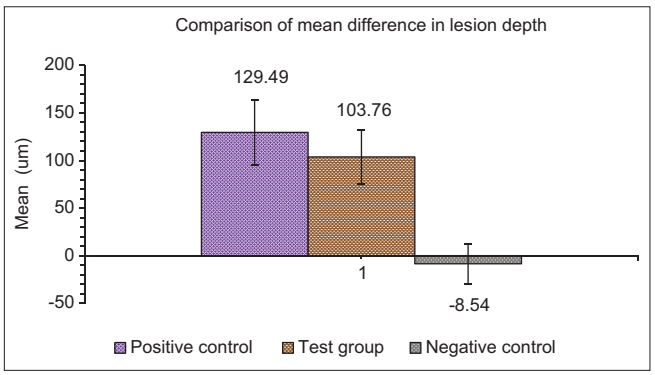

Figure 4: Comparison of intra-lesion mean difference between pre- and post-treatment groups

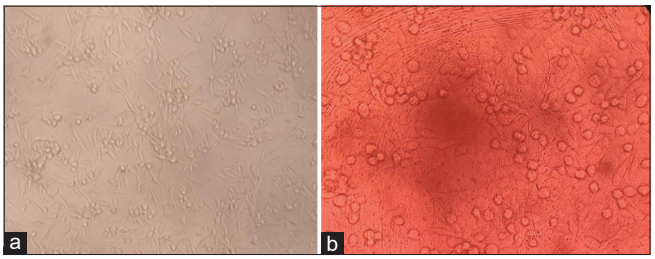

Figure 5: Photomicrograph showing viable gingival fibroblast cells in the log phase of growth for MTT assay; (a) spindle shape appearance and (b) epithelioid-shaped fibroblasts appearance

viability of the cells was more towards *O. basilicum* varnish as compared to the control group by 20%. The half-maximal inhibitory concentration (IC50) (concentration at which the drug induces up to 50% cell death) obtained for *O. basilicum* varnish was 47 lg/ml and that obtained for fluoride varnish was below 36 lg/ml. The statistically significant difference observed between *O. basilicum* varnish and fluoride varnish indicated that *O. basilicum* varnish provided better cytocompatibility than fluoride varnish and showed no toxic effects on fibroblasts [Table 5].

Table 3: Inter-group comparison of mean difference in lesion depth

(I) Factor		Mean difference (I-J)	Std. error	Sig.	95% confidence interval	
					Lower bound	Upper bound
Group 1	2	25.73260*	6.964	0.001*	9.1534	42.3118
	3	138.03424*	6.964	<0.001*	121.4551	154.6134
Group 2	1	-25.73260*	6.964	0.001*	-42.3118	-9.1534
	3	112.30164*	6.964	<0.001*	95.7225	128.8808
Group 3	1	-138.03424*	6.964	<0.001*	-154.6134	-121.4551
	2	-112.30164*	6.964	<0.001*	-128.8808	-95.7225

All values are expressed as mean±standard deviation (SD) in parentheses; statistical test used: Tukey's post hoc analysis; group 1: fluoride varnish group (positive control), group 2: *Ocimum basilicum* varnish (test), group 3: placebo group (negative control); level of significance: *$P \leq 0.05$ is considered statistically significant, **$P \leq 0.001$ is considered highly significant

Table 4: Mean percentage change in lesion depth

Groups	Mean	SD	SE	95% confidence interval for mean		*F*	*P*
				Lower	Upper		
Group 1	45.938	6.873	1.196	43.501	48.376	408.256	<0.001*
Group 2	36.015	6.938	1.207	33.555	38.475		
Group 3	-3.552	8.418	1.465	-6.537	-0.567		

All values are expressed as percentage in parentheses; statistical test used: ANOVA; group 1: fluoride varnish group (positive control), group 2: *Ocimum basilicum* varnish (test), group 3: placebo group (negative control); level of significance: *$P \leq 0.05$ is considered statistically significant, **$P \leq 0.001$ is considered highly significant

Table 5: Mean optical densities of surviving cells of study groups at a wavelength of 570 nm

Conc.	OD (nm)	Mean	Percentage viability (%)	Results as observed	IC50 conc. (μg)
NC	0.164 0.439 0.259	0.287	100	No lysis	Nil
OBV	0.269 0.245 0.31	0.275	95.592	No lysis	47 lg/ml
FV	0.154 0.264 0.233	0.243	72.822	No lysis	36 lg/ml

NC=negative control; OBE=*Ocimum basilicum* varnish; FV=fluoride varnish; OD=optical density; IC50=half-maximal inhibitory concentration; nm=nanometre; μg=microgram

DISCUSSION

The notion of employing medicinal plants to treat human ailments is not new, and their use in many developing countries is still in vogue.[32] The current approach in medicine is shifting towards using ingredients derived from nature. Recognising medicinal plants becomes vital and requires development since medications generated from natural materials are thought to be generally safe and economical.[33] Due to financial constraints, developing nations require biocompatible, efficacious and cost-effective preventative measures. Therefore, it has been advocated to employ medicinal plant extracts, which have a significant impact on caries prevention.[34]

O. basilicum Linn. is a medicated herb that has traditionally been used to treat a variety of health conditions.[35] The present study's investigation pertains to the aspects of oral health care. The requisites of suitable physiochemical conditions for mineral dissolution determine the onset of dental caries.[36] The diagnosis of early stages of carious lesions, as well as the use of non-invasive remineralisation treatment for these lesions, has the potential to represent a substantial development in the clinical management of the disease.[1] The bioactivity of *O. basilicum* seeds has projected the great importance of functional foods. The mechanism of these antimicrobial effects has been proposed in a number of *in vitro* studies.[37-39] It has been established that the preservation of the supersaturated state of calcium around the tooth surface decreases demineralisation and promotes remineralisation.[40] It is believed that *O. basilicum* forms a substantial calcium and phosphate reservoir inside the core plaque to limit mineral loss during a cariogenic event. Hence, the use of *O. basilicum* varnish can be indicated in early caries/white spot lesions to prevent further caries progression.[24]

To comprehend the impact of such herbal agents on carious processes, experimental models based on the construction of lesions in *in vitro* systems might be used.[41] These *in vitro* systems are the spearhead of caries research, because they are less costly and take less time than conventional testing methods.[6] This study used an *in vitro* pH cycling model to assess the remineralisation effects of three treatment agents namely fluoride varnish, *O. basilicum* varnish and placebo on artificial carious lesions of extracted sound permanent human teeth. Another major advantage of the former system is the ability to perform single-variable experiments in a controlled environment.[6,42] There is substantial evidence that the pH cycling model is reliable for evaluating lesion development and mineral changes in artificial enamel carious lesions

because it closely mimics the *in vivo* circumstances that contribute to caries.[43]

In the interest of standardisation, all of the specimens in the current study were subjected to 30 days of pH cycling. This helps to mimic the real-life situations to a greater extent that occur *in vivo* according to evidence obtained by Buzalaf *et al.*[43] and Itthagarun *et al.*[41] 'pH cycling' refers to *in vitro* experimental techniques that include exposing substrates, enamel or dentin, to alternating solutions of remineralisation and demineralisation.[44] These combination tests are intended to simulate the dynamic changes in mineral saturation and pH that occur throughout the caries process.[45] In the current investigation, the light microscope was devised to be the appropriate approach for measuring lesion depth supported by Sadoon *et al*.,[46] who reported a significant degree of discrimination between demineralised and normal areas of tooth samples using a light microscopy.

In the current study, it was observed that there was surface precipitation on enamel with fluoride varnish application by way of nonhomogeneous surface deposition in contrast to the study by Aziznezhad *et al.,*[47] stating fluoride varnish increases the surface hardness and reduces the dissolution of enamel in acid.[48] The explanation for this might be the short contact period of fluoride varnish with enamel in our investigation. A certain plateau effect in remineralisation has been described *in vitro* when high-dose fluoride administration did not result in considerably increased remineralisation. This might be because diffusion pathways in the surface layer of incipient carious lesions are blocked.[47,48] Fluoride varnish and *O. basilicum* varnish group samples revealed a significant reduction in lesion depth. This could be because of the subsurface remineralisation of the white spot lesion, thereby proving *O. basilicum* varnish to have a preventative role in lesion formation and lesion progression.

Limitation

Inevitably, the limitations of this *in vitro* study include difficulty to precisely simulate the biological aspects of caries and the multitude of intraoral conditions that contribute to dental caries and the role of enzymes is not accounted. Other confounding factors involve the possibility of experimental errors and dissimilarities in the micro-structure of the enamel between specimens.

Recommendations

It is recommended that further controlled *in vivo* studies and extensive clinical trials in various local delivery systems be evaluated to ascertain the true clinical efficacy of *O. basilicum* varnish. It is critical to assess the effectiveness of lesions on various surfaces (e.g. occlusal, where most lesions develop) and with validated outcomes for remineralisation. Reduction in outcome reporting and publication bias is crucial in the validation of novel agents for remineralisation.

CONCLUSION

Establishing steady systems capable of delivering bioavailable calcium and phosphate unswervingly to the lesion or the surrounding biofilm encourages remineralisation. The fluoride varnish group and *O. basilicum* varnish group showed increased remineralisation capacity than the negative control group, as evidenced by higher mean remineralisation value. *O. basilicum* varnish group showed homogenous remineralisation of enamel subsurface lesions *in situ* significantly slowing the progression of the lesion. Hence, the new *O. basilicum*-based remineralisation agent appears to have potential as a non-invasive alternative to topical fluorides in the therapy of early carious lesions.

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Ethics statement

Ethical clearance was obtained from the Institutional Research and Ethics Committee (IRB number: EC/NEW/2021/2435). The study was conducted in accordance with the World Medical Association Declaration of Helsinki. Informed consent was obtained from the parents of the participating subjects after giving them the full details of the study.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Chaudhury T, Ananthakrishna S, Kumari RV, Kour S, Syam A. Comparative study of remineralization potential of three different remineralizing agents on demineralized enamel using light fluorescence

and confocal fluorescence microscope: An *in vitro* study. Int J Prev Clin Dent Res 2018;5:45-9.
2. Sivapathasundharam B, Raghu AR. Dental caries. In: Rajendran B, Sivapathasundharam B, editors. Shafer's textbook of oral pathology. 5th ed. Elsevier Publishers; 2006. p. 567-658.
3. Winston AE, Bhaskar SN. Caries prevention in the 21st century. J Am Dent Assoc 1998;129:1579-87.
4. Rajan R, Krishnan R, Bhaskaran B, Kumar SV. A polarized light microscopic study to comparatively evaluate four remineralizing agents on enamel viz CPP-ACPF, ReminPro, SHY-NM and colgate strong teeth. Int J Clin Pediatr Dent 2015;8:42-7.
5. Abou Neel EA, Aljabo A, Strange A, Ibrahim S, Coathup M, Young AM, *et al*. Demineralization-remineralization dynamics in teeth and bone. Int J Nanomed 2016;11:4743-63.
6. Manchery N, John J, Nagappan N, Subbiah GK, Premnath P. Remineralization potential of dentifrice containing nanohydroxyapatite on artificial carious lesions of enamel: A comparative *in vitro* study. Dent Res J (Isfahan) 2019;16:310-7.
7. Sulistianingsih S, Irmaleny I, Hidayat OT. The remineralization potential of cocoa (Theobroma cacao) bean extract to increase the enamel micro hardness. Padjadjaran J Dent 2017;29:107-12.
8. Loesche WJ. Microbiology of dental decay and periodontal disease. In: Baron S, editor. Medical Microbiology. 4th ed. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. p. 1068-194
9. Reynolds EC. Calcium phosphate-based remineralization systems: Scientific evidence. Aust Dent J 2008;53:268-73.
10. Ten Cate JM. Current concepts on the theories of the mechanism of action of fluoride. Acta Odontol Scand 1999;57:325-9.
11. Vale GC, Cruz PF, Bohn AC, de Moura MS. Salivary fluoride levels after use of high-fluoride dentifrice. ScientificWorldJournal 2015;2015:302717.
12. Soares R, De Ataide ID, Fernandes M, Lambor R. Assessment of enamel remineralisation after treatment with four different remineralising agents: A scanning electron microscopy (SEM) study. J Clin Diagn Res 2017;11:ZC136-41.
13. Kanduti D, Sterbenk P, Artnik B. Fluoride: A review of use and effects on health. Mater Sociomed 2016;28:133-7.
14. Everett ET. Fluoride's effects on the formation of teeth and bones, and the influence of genetics. J Dent Res 2011;90:552-60.
15. Philip N. State of the art enamel remineralization systems: The next frontier in caries management. Caries Res 2019;53:284-95.
16. Gocmen GB, Yanikoglu F, Tagtekin D, Stookey GK, Schemehorn BR, Hayran O. Effectiveness of some herbals on initial enamel caries lesion. Asian Pac J Trop Biomed 2016;6:846-50.
17. Bilal A, Jahan N, Ahmed A, Bilal SN, Habib S, Hajra S. Phytochemical and pharmacological studies on ocimum basilicum linn-a review. Int J Curr Res Rev 2012;4:73-83.
18. Javanmardi J, Khalighi A, Kashi A, Bais HP, Vivanco JM. Chemical characterization of basil (Ocimum basilicum L.) found in local accessions and used in traditional medicines in iran. J Agric Food Chem 2002;50:5878-83.
19. Parikh NH, Kothari CS. Phytochemical analysis and total phenolic and flavonoid contents determination of methanolic extract of ocimum basilicum L seed. Int J Pharm Tech Res 2016;9:215-9.
20. Bucktowar K, Bucktowar M, Bholoa LD. A review on sweet basil seeds: ocimum basilicum. World J Pharm Pharm Sci 2016;5:554-67.
21. Kalra K, Vasthare R, Shenoy PA, Vishwanath S, Singhal DK. Antibacterial efficacy of essential oil of two different varieties of ocimum (tulsi) on oral microbiota-an invitro study. Indian J Public Health Res Dev 2019;10:188-93.
22. Wiwattanarattanabut K, Choonharuangdej S, Srithavaj T. *In vitro* anti-cariogenic plaque effects of essential oils extracted from culinary herbs. J Clin Diagn Res 2017;11:DC30-5.
23. Onishi T, Umemura S, Yanagawa M, Matsumura M, Sasaki Y, Ogasawara T, *et al*. Remineralization effects of gum arabic on caries-like enamel lesions. Arch Oral Biol 2008;53:257-60.
24. Shivakumar VH, Tegginamani AS, Zain NM. Antimicrobial efficiency of tinospora cordifolia and ocimum tenuiflorum against streptococcus mutans and candida albicans. J Oral Maxillofac Pathol 2022;26:470-5.
25. Kendall G, Bai R, Blazewicz J, De Causmaecker P, Gendreau M, John R, *et al*. Good laboratory practice for optimization research. J Oper Res Soc 2016;67:676-89.
26. Cohen J. Statistical power analysis for the behavioral sciences. In: Cohen J, editor. Statistical power analysis for the behavioral sciences. 2nd ed. New York: Routledge; 2013. p. 1-17.
27. Pai Khot AJ, Ankola AV, Dodamani S, Sankeshwari RM, Kumar RS, Santhosh VN. Assessment of potential antimicrobial activity of ocimum basilicum extract and chlorhexidine against socransky's complex pathogens of oral cavity: An *in vitro* study. J Indian Soc Periodontol 2023. [Ahead of print] doi: 10.4103/jisp.jisp_406_22.
28. Evans WC. Trease and Evans pharmacognosy bailliere tindall. London; 1989. p. 216-7.
29. Rezzoug M, Bakchiche B, Gherib A, Roberta A, Kilinçarslan Ö, Mammadov, *et al*. Chemical composition and bioactivity of essential oils and ethanolic extracts of ocimum basilicum L. and thymus algeriensis Boiss. & Reut. from the algerian saharan atlas. BMC Complement Altern Med 2019;19:146.
30. Marwat SK, Khan MS, Ghulam S, Anwar N, Mustafa G, Usman K. Phytochemical constituents and pharmacological activities of sweet basil-ocimum basilicum L. (Lamiaceae). Asian J Chem 2011;23:3773-82.
31. Patil R, Kale AD, Mane DR, Patil D. Isolation, culture and characterization of primary cell lines of human buccal mucosal fibroblasts: A combination of explant enzamytic technique. J Oral Maxillofac Pathol 2020;24:68-75.
32. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol 2014;4:177.
33. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules 2016;21:559.
34. Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. Afr J Tradit Complement Altern Med 2013;10:210-29.
35. Aminian AR, Mohebbati R, Boskabady MH. The effect of ocimum basilicum L. and its main ingredients on respiratory disorders: An experimental, preclinical, and clinical review. Front Pharmacol 2022;12:805391.
36. Advani S, Sogi S, Hugar S, Indushekar KR, Kiran K, Hallikerimath S, *et al*. Remineralization effects of two pediatric dentifrices and one regular dentifrice on artificial carious lesion in primary teeth: An *in vitro* study. J Int Soc Prev Community Dent 2014;4:96102.
37. Gajendiran A, Thangaraman V, Thangamani S, Ravi D, Abraham J. Antimicrobial, antioxidant and anticancer screening of ocimum basilicum seeds. Bull Pharm Res 2016;6:114-9.
38. Ahmed R, Mohammed A, Hatem Z. The effects of ethanolic extract of seed sweet basil (Ocimum Basilicum) against different gram negative and positive bacteria and fungi. Asian Acad Res J Multidiscip 2016;3:201-6.
39. Adam ZA, Omer AA. Antibacterial activity of ocimum basilicum (Rehan) leaf extract against bacterial pathogens in Sudan. Am J Res Commun 2015;3:94-9.
40. Amaechi BT, AbdulAzees PA, Alshareif DO, Shehata MA, Lima PPCS, Abdollahi A, *et al*. Comparative efficacy of a hydroxyapatite and a fluoride toothpaste for prevention and remineralization of dental caries in children. BDJ Open 2019;5:18.
41. Itthagarun A, Wei SH, Wefel JS. The effect of different commercial dentifrices on enamel lesion progression: An *in vitro* pH-cycling study. Int Dent J 2000;50:21-8.
42. Fontana M, Buller TL, Dunipace AJ, Stookey GK, Gregory RL. An *in vitro* microbial-caries model used to study the efficacy of antibodies to streptococcus mutans surface proteins in preventing dental caries. Clin Diagn Lab Immunol 2000;7:49-54.
43. Buzalaf MA, Hannas AR, Magalhães AC, Rios D, Honório HM, 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.
44. Featherstone JD, Shariati M, Brugler S, Fu J, White DJ. Effect of an anticalculus dentifrice on lesion progression under pH cycling conditions in vitro. Caries Res 1988;22:337-41.
45. Queiroz CS, Hara AT, Paes Leme AF, Cury JA. pH-cycling models to evaluate the effect of low fluoride dentifrice on enamel de- and remineralization. Braz Dent 2008;19:21-7.
46. Sadoon NY, Fathy SM, Osman MF. Effect of using biomimetic analogs on dentin remineralization with bioactive cements. Braz Dent J 2020;31:44-51.
47. Aziznezhad M, Alaghemand H, Shahande Z, Pasdar N, Bijani A, Eslami A, *et al*. Comparison of the effect of resin infiltrant, fluoride varnish, and nano-hydroxy apatite paste on surface hardness and streptococcus mutans adhesion to artificial enamel lesions. Electron Physician 2017;9:3934-42.
48. Altenburger MJ, Schirrmeister JF, Wrbas KT, Klasser M, Hellwig E. Fluoride uptake and remineralisation of enamel lesions after weekly application of differently concentrated fluoride gels. Caries Res 2008;42:312-8.