EFFECTIVE EVALUATION OF RE-MINERALIZING POTENTIAL OF *OCIMUM BASILICUM* VARNISH AND FLUORIDE VARNISH ON INITIAL ENAMEL CARIES: AN IN-VITRO STUDY

By

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KLE Academy of Higher Education & Research, Belagavi

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LIST OF ABBREVIATION

Sl. No.	Abbreviation	Expanded form
1.	WHO	World Health Organisation
2.	OBL	*Ocimum basilicum Linn*
3.	OB	*Ocimum basilicum*
4.	EOOB	*Ocimum basilicum essential oil*
5.	DPPH	Diphenylpicrylhydrazyl
6.	EOs	Essential Oils
7.	HPLC-MS	High-performance liquid chromatography coupled to mass spectrometry
8.	MIC	Minimum Inhibitory Concentration
9.	GAE	Gallic Acid Equivalents
10.	QE	Quercetin Equivalent
11.	EEs	Ethanolic extracts
12.	HPLC	High-performance liquid chromatography
13.	RDD	Recommended Daily Dose
14.	GC-MS	Gas chromatography–mass spectrometry
15.	NDs	Neurological disorders

16.	BAG	Bioactive Glass
17.	HA	Hydroxyapatite
18.	SEM	Scanning Electron Microscope
19.	DM	Digital Microscopy
20.	VHM	Vickers Hardness Number
21.	ppm	parts per million
22.	DW	Double distilled water
23.	DMSO	Dimethyl sulfoxide
24.	GLP	Good Laboratory Practice
25.	ICMR-NITM	Indian Council of Medical Research National Institute of Traditional Medicine
26.	BSRC	Basic science research Centre
27.	CAP 2000+ Viscometer	Cone and plate 2000+ viscometer
28.	HGF	Human Primary Gingival Fibroblasts
29.	DMEM	Dulbecco's modified Eagle's medium
30.	FBS	Fetal Bovien Serum
31.	CO2	Carbon dioxide
32.	MTT	methyl-thiazol-tetrazolium

33.	OD	Optical Density
34.	CPP-ACPF	Casein phosphopeptide-amorphous calcium phosphate fluoride
35.	FV	Fluoride varnish
36.	IP	Indian pharmaceutical
37.	CCD	Charge coupled device
38.	SPSS	Statistical Package for Social Sciences
39.	IC_{50}	Inhibitory concentration for 50% of plaques
40.	CC_{50}	50% cytotoxic concentration
41.	Cal	Calibration
42.	FeCl	Ferric Chloride
43.	NaOH	Sodium hydroxide
44.	KOH	Potassium hydroxide
45.	KCl	Potassium chloride
46.	NaH2PO4	Sodium monobasic phosphate
47.	MgCl2$6H2O	Magnesium chloride hexahydrate
48.	CaCl2$2H2O	Calcium chloride dihydrate
49.	KH2PO4	potassium dihydrogen phosphate

50.	K2HPO4	Dipotassium hydrogen phosphate
51.	Ca	Calcium
52.	P	Phosphorus
53.	SD	Standard deviation
54.	SE	Standard error
55.	Sig.	Significance
56.	ANOVA	Analysis Of Variance
57.	pH	Potential of Hydrogen
58.	mg	milligram
59.	ml	millilitre
60.	µg	microgram
61.	SI	Selectivity index
62.	mM	millimole
63.	mm	millimeter

ABSTRACT

BACKGROUND: The focus of caries research has lately switched to the development of approaches for early identification and non-invasive treatment of caries lesions. These can be a major advancement in the clinical management of the disease.

AIM: To evaluate and compare the re-mineralizing potential of *Ocimum basilicum* varnish and fluoride varnish.

METHOD: *Ocimum basilicum* seeds extract was prepared using Soxhlet method which was vortexed with IP graded chemicals to obtain varnish. 99 Extracted Premolar teeth samples collected and stored in 10% formalin solution. Each enamel surface was marked with an area 4x4mm on the middle third of the buccal surface and covering the remaining surface with two coats of acid resistant nail varnish. The study samples were then subjected to demineralization by immersing in artificially prepared demineralizing solution with a final pH of 4.5 for 48 hours at 37ºC. Teeth were divided in groups using computerized table of random numbers with 33 samples each. Each group was subjected for re-mineralization twice daily with respective agents for 4 minutes for 30 consecutive days using applicator tip. Each tooth was longitudinally sectioned by cutting through the centre of the enamel window to make a ground-section of approx. 0.2-0.4 mm thickness. Each ground-section obtained was analyzed for depth of the lesion by capturing image with camera attached to light microscope (LeicaTM DM 2500). The lesion depth was measured from the surface of the tooth to the maximum depth using the Image J software (Java-based image processing program). The data were evaluated using ANOVA and post hoc analysis.

RESULTS: The mean (± SD) pre-treatment lesion depth across the groups ranged from 242.11 ± 26.144μm to 352.66 ± 34.531μm. Comparisons between pre-test lesion depths in all groups were statistically insignificant (p = 0.380). The reduction in mean lesion depth after pH cycling was maximum for group 1, followed by group 2 (p<0.001) indicative of effective remineralization post pH cycling. However, there was considerable increase in mean lesion depth in group 3 with p=0.028. The highest lesion depth recovery rate of 45.938% was recorded for fluoride varnish group, followed by 36.015% in *Ocimum basilicum* varnish group. However, the lesion depths in placebo group were increased by 3.5%. Inter group analysis for lesion depths revealed statistically significant difference by Tukey's post hoc analysis (p<0.001). The MTT assay results obtained for the *O. basilicum* varnish and the fluoride varnish control revealed it to be non-toxic to gingival fibroblast cells as the viability of cells was maintained.

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

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INTRODUCTION

"An ounce of prevention is worth a pound of cure." — Benjamin Franklin

"Dental caries is an irreversible microbial disease of the calcified tissues of the teeth, characterized by demineralization 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 multitude of factors influence and initiate the progression of the disease.[2] The caries signs range from the earliest molecular alterations in the apatite crystals of the tooth, to a visible white-spot lesion.[3] A continual imbalance in pathological and physiological factors results in the dissolution of apatite crystals.[4] Uniqueness of dental caries disease lies within enamel which is both acellular and avascular, and hence lacks the ability to repair itself through a cellular process.[5] Therefore, the prevention and biomimetic treatment of initial caries in enamel, has been one of the paramount challenges faced by dental professionals and public health communities.[6]

The cariogenic bacteria essential for the disease progression, namely the *streptococcus mutans* and *lactobacilli* species, produce organic acids during the fermentation of carbohydrates.[7] This reduces the salivary pH below the "critical pH" of 5.5, leading to abnormal loss of minerals from the enamel surface or subsurface known as demineralization.[1,8] Bacterial activity initiates with the production of organic acids, which penetrates the tooth through crystals. When the acid dissolves through a susceptible area on the surface of a crystal, calcium and phosphate ions leaches into the neighboring aqueous phase between the crystals.[9] If electronegative fluoride ions are in an adequate concentration prior to or during demineralization, it

can adsorb at the crystal surface and markedly inhibit the process of demineralization.[10]

Fluoride is a preventative substance whose high cariostatic activity has captivated dental research.[6] The current concept indicates that fluorides function primarily through a topical mechanism by inhibition of demineralization and enhancement of re-mineralization as well as by inhibiting the enzymatic activity of cariogenic bacteria.[11] Fluoride levels in oral fluids can be found at low levels for several hours after brushing, which has been shown to have a significant impact on enamel remineralization.[12,13] Despite its profound efficacy in preventing caries progression, it has certain limitations.[14] Fluoride does not completely eliminate caries, although excessive fluoride concentrations might be detrimental to the teeth.[15] Furthermore, calcium and phosphate ion availability may restrict fluoride retention and net remineralisation.[16] Nonetheless, concerns have recently been raised about the large range of prescription and over-the-counter fluoride medications currently being promoted in every nation, which has boosted fluoride consumption to potentially hazardous levels.[17] 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 on the neurological system, cardiovascular system, gastrointestinal disorder, genetic damage and reproductive effects.[18] However, the recent fluoride classification as a chemical neurotoxicant could raise safety concerns among the general public regarding the use of high concentration fluoride products.[19]

Preventing caries extension by remineralization is extremely desirable and is one of the cornerstones of minimal invasive dentistry.[20] The buffering function of saliva allows calcium and phosphate ions to concentrate onto the tooth and produce

new minerals, facilitating remineralisation. Re-mineralization is the body's natural mending mechanism for non-cavitated subsurface carious lesions.[21] Despite the fact that fluoride remains the cornerstone of current non-invasive dental caries control, there are new and developing approaches that can be employed as fluoride substitutes. Thus, contemporary research has focused on developing nontoxic, biocompatible, and cost-effective anticariogenic agents that might be added to toothpaste, mouthwash, and diet in order to reduce caries experience.[6] There is certainly a need for cutting-edge remineralization technologies that can supplement fluoride, fill the void in its remineralizing action, and result in a more complete consolidation of carious lesions. Therefore, it has been advocated to use medicinal plant extracts which have a profound effect on caries prevention with minimum adverse effects.

Currently, the modern approach in medicine is turning to utilizing ingredients derived from nature.[22] World Health Organization (WHO) reported that herbal medicine is currently used by 80 percent of the world's population for some component of primary health care.[23] Natural products with potent pharmacological or biological activities play a very important role in medicine. Secondary metabolites and essential oils with therapeutic value abound in medicinal plants. The primary benefits stated for medicinal plant therapeutic usage in many diseases are their safety, in addition to being affordable, effective, and readily available.[24]

Among the plants renowned for their medicinal usefulness, genus *Ocimum* plants are rich in phenolic compounds and have high therapeutic prospects. Basil seeds are authenticated as "*Ocimum basilicum*" belonging to the family '*Lamiaceae'* which is an annual plant popularly known as "Sweet basil" and is used in both Unani and Ayurvedic systems of medicine.[25] Basil is a well-known herb, regarded for its

affluent and spicy, gently peppery flavour with hints of mint and clove, and has been frequently used as a food component to flavour confectionery, baked goods, and meat items.[26] Basil seeds are commonly used as an emollient and a source of nutritional fiber in beverages and ice desserts. The seeds are used to diminish body heat and nervous debility.[27,28]

Basil seed is black in colour and oval in shape with mean dimensions of 3.11± 0.29mm (length), 1.82±0.26mm (width) and 1.34 ±0.19mm (height). It is used both as a culinary and an ornamental herb. Basil, widely known as the "King of Herbs," includes a high concentration of phytochemicals that have substantial nutritional, antioxidant, and health benefits. This plant may be found all over the world, particularly in the tropical regions of Asia, Africa and central as well as south America. Sabja seeds, falooda seeds, and tukamaria seeds are some other names for sweet basil seeds. Basil seeds possess a generous percentage of carbohydrates (42%), fats (25%) and proteins (20%) and are a magnificent source of fiber. Usage of seeds are superior to leaves in growth performance as the former contain a much higher content of protein and lipid relative to leaves. Sweet basil seeds contain a high concentration of polyphenolic flavonoids, particularly Orientin and Vicenin; essential oils such as eugenol, citronellol, linalool, limonene, citral, and terpineol; vitamin A, vitamin C, and vitamin K; and minerals such as potassium, manganese, copper, calcium, magnesium, and folates. Active compounds found in basil seeds are planteose, mucilage, and polysaccharides. Furthermore, secondary metabolites of plants including phenolic and flavonoid have been shown to exhibit pharmacological properties such as antioxidant, antibacterial, antiviral, anti-diabetic, anti-inflammatory, antiallergic, anticancer, neurodegenerative and vasodilatory effects. Furthermore, several types of research have been undertaken to demonstrate that

sweet basil seeds offer numerous advantages such as weight reduction, good skin, cooling impact, acidity prevention, anti-inflammatory, anticancer, and so on. [28,29]

Nowadays, traditional medicinal practices form an intrinsic aspect of complementary or alternative medicine. Plant-derived phytochemicals having therapeutic qualities might be exploited in medication development as a single therapeutic agent or in combination formulations. The approach used is mostly determined by the solubility and volatility of the compounds to be separated. Basil seeds contain a high amount of calcium 2240mg (244% of recommended daily dose {RDD}) and Phosphate concentration of 2630mg (56% RDD) which is required for re-mineralization.[29] There exist numerous experiments previously carried out to test the remineralizing efficacy of various herbal products, individually and in combination, on extracted permanent teeth, utilizing various de- and remineralization techniques. Of this, only a few have been reported to remineralize carious lesions effectively. Limited studies in the field of the remineralization potential of *Ocimum basilicum* seeds prove to be the lacunae as evidenced in the available data. Hence, the rationale of this in vitro study was to evaluate and compare *Ocimum basilicum* varnish on the remineralizing potential of initial enamel caries so that it might be used as an alternative to commercially marketed fluoride varnishes.

AIM AND OBJECTIVES

AIM OF THE STUDY:

To evaluate and compare the re-mineralizing potential of *Ocimum basilicum* varnish and fluoride varnish on initial enamel caries

OBJECTIVES OF THE INVENTION:

1. To assess the re-mineralizing potential of *Ocimum basilicum* varnish by light microscopy method and measurement using Image J software.

2. To assess the re-mineralizing potential of fluoride varnish by light microscopy method and measurement using Image J software.

3. To compare and evaluate the re-mineralizing potential of *Ocimum basilicum* varnish with fluoride varnish and placebo by light microscopy method and measurement using Image J software.

NULL HYPOTHESIS:

There will be no difference in re-mineralizing potential between fluoride varnish and *Ocimum basilicum* varnish on initial enamel caries after application twice daily consecutively for 30 days.

ALTERNATIVE HYPOTHESIS:

There will be a difference in re-mineralizing potential between fluoride varnish and *Ocimum basilicum* varnish on initial enamel caries after application twice daily consecutively for 30 days.

REVIEW OF LITERATURE

Studies on antimicrobial activity, antiplaque activity of Ocimum basilicum

1. An invitro study by **Ikram et al 2021**., concluded that Ethyl acetate fraction and crude ethanolic extract from leaves of Ocimum basilicum showed good antibacterial effectiveness against ESBLs and carbapenem resistant organisms than other fractions. A total of 80 multidrug resistant gram-negative rods were included. Agar dilution method was performed to determine minimum inhibitory concentration of crude ethanolic extract and different fractions i.e., n-hexane, chloroform and ethyl acetate of Ocimum basilicum leaves against multidrug resistant gram-negative rods i.e., extended spectrum beta lactamases and carbapenemase producers. Multi-inoculator was used for inoculation. This finding may also promote the effective use of O. basilicum herb and its components in modern medicine.
2. An invitro study by **Kalra et al., 2019** concluded that the essential oil of two varieties of Tulsi showed good antimicrobial activity against the common anaerobic and aerobic organisms of the oral cavity. Commercially available essential oil of two varieties of Ocimum i.e. Ocimum sanctum and Ocimum basilicum were procured and checked for their antibacterial activity in-vitro. Five common oral pathogens were selected (two aerobic and three anaerobic). The activity of oils was compared with chlorhexidine. The zone of inhibition produced by Ocimum sanctum oil was maximum for Porphyromonas gingivalis (55 mm) followed by Prevotella intermedia (48 mm). The zone produced was much wider than that of chlorhexidine. For Fusobacterium nucleatum, the zone was equivalent to control. For aerobic bacteria, Ocimum sanctum, showed

almost equal efficacy as of chlorhexidine but the effect produced by Ocimum basilicum oil was lesser than control. The activity was more pronounced against anaerobes and was found to be better than chlorhexidine.

3. **Dr Ramprasad Vasthare in 2019** conducted research to test in-vitro the antibacterial efficacy of commercially available essential oil extracts of different varieties of Ocimum (Tulsi) on common oral pathogens wherein the organisms were incubated on respective culture media. Agar well diffusion method was used to check their activity. The oils were tested undiluted form and 1 in 10 dilutions. The activity of oils was compared with chlorhexidine. The essential oil of two varieties of Tulsi showed good antimicrobial activity against the common anaerobic and aerobic organisms of the oral cavity. The activity was more pronounced against anaerobes and was found to be better than chlorhexidine. Ocimum sanctum oil produced a wider zone of inhibition as compared to Ocimum basilicum for all the test strains.
4. An invitro study by **Wiwattanarattanabut et al., 2017** concluded that the Cinnamon and sweet basil essential oils had impressive anti-cariogenic and antiplaque effects and may be proposed as alternative and effective supplements to promote oral health status. Essential oils extracted from sweet basil (Ocimum basilicum), cinnamon bark (Cinnamomum zeylanicum), sweet fennel (Foeniculum vulgare), kaffir lime (Citrus hystrix), black pepper (Piper nigrum), peppermint (Mentha piperita), and spearmint (Mentha spicata) were primarily examined for their antimicrobial activities against the cariogenic bacteria (Streptococcus mutans KPSK2 and Lactobacillus casei) using the agar disk diffusion and broth microdilution methods, respectively. All selected essential

oils showed different degrees of antimicrobial activity against the planktonic form of both cariogenic bacteria.

5. **Ahmed et al., 2016** studied the effects of ethanolic extract of seed sweet basil (Ocimum basilicum) against different bacteria such as E. coli, S. aureus, S. epidermidis, P. aerugionsa and the fungi Candida. albicans. At a concentration of 100 mg/ml, Ocimum basilicum caused a marked increase in zone of inhibition (mm) on this bacteria and fungi growth. Inhibition zones sizes were different and increased according to concentration of extract and again the growth was completely inhibited in the highest concentration. A similar outcome was observed using 24 hours incubation period of bacterial growth. Furthermore, Ocimum basilicum had dependent concentration effect on this bacteria inhibition; it extended the diameter of zone inhibition.
6. **Gajendiran et al., 2016** studied the antimicrobial, antioxidant and anticancer screening of Ocimum basilicum seeds. basil (Ocimum basilicum) seeds were used as the raw material for evaluation of their bioactive compounds. Active components of the seeds were extracted using Soxhlet apparatus with two different solvents petroleum ether and methanol. Basil seeds extract exhibited strong antibacterial activity against nine pathogenic bacteria. The strongest inhibitory activity of basil seeds extract was observed against Pseudomonas aeruginosa, Escherichia coli, Shigella dysenteriae and Klebsiella pneumoniae. As per DPPH assay, the maximum free radical scavenging activity (73.85%) was shown by petroleum ether extract and the minimum activity (34.20%) was shown by methanol extract of basil seeds. Moreover, anticancer activity results clearly indicated that the basil seeds are a potential source of stable bioactive compounds.

7. **Freires et al., 2015** conducted a systematic review to discuss the antibacterial activity of Essential Oils and their isolated constituents in view of a potential applicability in novel dental formulations. Most of the knowledge in the literature is based on in vitro studies assessing the effects of EOs on caries-related streptococci (mainly Streptococcus mutans) and lactobacilli, and on a limited number of clinical trials. The most promising species with antibacterial potential against cariogenic bacteria is: Achillea ligustica, Baccharis dracunculifolia, Croton cajucara, Cryptomeria japonica, Coriandrum sativum, Eugenia caryophyllata, Lippia sidoides, Ocimum americanum, and Rosmarinus officinalis. In some cases, the major phytochemical compounds determine the biological properties of EOs. Menthol and eugenol were considered outstanding compounds demonstrating an antibacterial potential. Only L. sidoides mouthwash (1%) has shown clinical antimicrobial effects against oral pathogens thus far. This review suggests avenues for further non-clinical and clinical studies with the most promising EOs and their isolated constituent's bio prospected worldwide.

8. An invitro study by **Adam et al., 2015** concluded that Ocimum basilicum leaf extract showed strong antimicrobial activity against various strains of bacterial pathogens at all concentrations. 100 bacterial test strains of Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Staphylococcus aureus and Enterococcus faecalis were enrolled in the study. Ethanolic extracts of Ocimum basilicum leaves were prepared at varying concentrations. Against Klebsiella pneumoniae there was significant difference of antimicrobial activity of leaf extract at concentration of 50µg/disc when

compared with the antibiotic's ciprofloxacin, erythromycin and gentamicin (p=0.001, 0.006 and 0.009) respectively.

9. **Vlase et al., 2014** examined the in vitro antioxidant and antimicrobial activities and to characterize the polyphenolic composition of the ethanolic extracts of Hyssopus officinalis, Ocimum basilicum and Teucrium chamaedrys. Qualitative and quantitative analysis of the major phenolic compounds were conducted using high-performance liquid chromatography coupled to mass spectrometry (HPLC-MS). The total polyphenols, caffeic acid derivatives and flavonoids content was spectrophotometrically determined. These extracts contained a large amount of the polyphenolic compounds (77.72, 175.57, and 243.65 mg/g, respectively), and they showed a good antioxidant activity, as witnessed by a number of methods. T. chamaedrys had a high antimicrobial activity. Besides their antioxidant activity, the antimicrobial effect of these extracts confirms the biological activities of these herbal medicinal products.

10. An invitro study by **Kaya et al., 2008** reported that O. basilicum extracts possessed antibacterial activity and caused lysis and eradicated bacteria by degrading bacterial cell walls. The antimicrobial activities of chloroform, acetone and two different concentrations of methanol extracts of Ocimum basilicum L. were studied. These extracts were tested in vitro against 10 bacteria and 4 yeasts strains by the disc diffusion method. While the chloroform and acetone extracts had no effect, the methanol extracts showed inhibition zones against strains of Pseudomonas aeruginosa, Shigella sp., Listeria monocytogenes, Staphylococcus aureus and two different strains of Escherichia coli. The cells of microorganisms, which were treated and untreated with plant extracts, were observed by using the scanning electron microscope. It was

observed that the treated cells were damaged. The results indicated that the methanol extracts of O. basilicum exhibited the antimicrobial activity against tested microorganisms.

11. An invitro study by **Adigüzel et al., 2005** suggested that O. basilicum extracts possessed compounds with antimicrobial properties against C. albicans and some bacterial pathogens. Ethanol, methanol, and hexane extracts from Ocimum basilicum Labiatae (sweet basil) were investigated for their invitro antimicrobial properties. A total of 146 microbial organisms belonging to 55 bacteria, and four fungi, and a yeast species were studied using a disk-diffusion and minimal inhibition concentration (MIC) method. The result showed that none of the three extracts tested have antifungal activities, but anticandidal and antibacterial effects. All three extract of O. basilicum were different in terms of their antibacterial activities. The hexane extract showed a stronger and broader spectrum of antibacterial activity, followed by the methanol and ethanol extracts, which inhibited 10, 9 and 6% of the 146 bacterial strains tested, respectively.

Studies on the chemical composition and properties of Ocimum Basilicum:

12. **Nazir et al., 2021** analyzed Basil (Ocimum basilicum L. var. thyrsiflora) seeds for its physico-chemical, phytochemical, functional, and hydration properties. Proximate composition showed that the seed contain 8.90 % moisture, 9.40 % protein, 33.01 % fats, 5.20 % ash, 43.50 % carbohydrates and 36.30 % total fibre. Gas chromatographic-mass spectrometric analysis showed the presence of omega fatty acids such as alpha-linolenic acid (71.10 %), palmitic-acid (13.72 %), stearic-acid (8.26 %), oleic-acid (1.53 %), arachidioic-acid (1.17 %) and

traces of gamma tocopherol, gamma linolenic-acid, and squalene. The phytochemical analysis of seed showed the presence of 18.24 mg GAE/g phenolic compounds, 0.525 mg QE/g flavonoids, 15.64 mg/g alkaloids, 0.97 mg/g saponins, and 0.134 mg/g triterpenoids. An antioxidant activity of 30.30 % was observed. Furthermore, higher values for water absorption capacity (37.72 g/g) and lower values for oil absorption capacity (6.04 g/g) were observed. Besides, low values for syneresis (7.37 %) and a high emulsification capacity (95.50 %) was obtained. Hydration studies indicated that the presence of ions decreased the water absorption and swelling power of basil seed. Scanning electron microscopy depicted an oblong shape and a smooth surface of basil seed. Findings suggest that basil seed can be used as a promising ingredient in food industry.

13. **Rezzoug et al.,2019** investigated the chemical composition and antioxidant, antimicrobial, and anticancer activities of ethanolic extracts (EEs) and essential oils (EOs) from two species in the Lamiaceae family, Ocimum basilicum L. and Thymus algeriensis Boiss. & Reut., cultivated in the Algerian Saharan Atlas. Phenolic compounds in the EEs from both plants were analyzed by HPLC and demonstrated a rich flavonoid content. Chemical analysis of the essential oil from Ocimum basilicum revealed 26 unique compounds, with linalool (52.1%) and linalyl acetate (19.1%) as the major compounds. The results suggest that the bioactive compounds found in the ethanolic extracts and essential oils from Ocimum basilicum and Thymus algeriensis, with diverse antioxidant, antimicrobial and anticancer activities, may have beneficial applications in nutraceutical and pharmaceutical technologies.

14. **Bucktowar et al in 2016** published a review article on sweet basil seeds (Ocimum basilicum) in world Journal of Pharmacy and Pharmaceutical sciences wherein he described in detail classification, common names, habitat, cultivars of ocimum basilicum, how to obtain sweet basal seeds, beneficial effects of seed on health conditions along with methods and lastly nutritional analysis which concludes that Contains many polyphenolic flavonoids**,** rich in essential oils, high fibre contents, low in calories, contains good amounts of vitamins, minerals such as calcium (244% of RDD) and Potassium (56% of RDD). Hence the author concludes that Sweet Basil Seeds is known in French to be a 'Grains Royales' (English: Royal Seeds) due to a number of hidden health benefits they possess. Sweet Basil seeds were once a rarely known seed to us but slowly these small black seeds have grabbed the attention of the public due to the fact that they come with a myriad of medicinal uses.
15. Parikh et al., 2016 conducted a Preliminary phytochemical analysis using various solvent extract. Aim of this study to determine total phenolic and flavonoid content of methanolic extract of *Ocimum basilicum* seed. Defatted powdered materials of *Ocimum basilicum* seed were extracted with Methanol. The level of phenolic and flavonoid content was determined by Folincoicalteu method and Aluminum chloride colorimetric method with gallic acid and rutin as standard. Determination of phenolic content by 7.15 ± 0.15 (mg GAE/ g extract), while flavonoid content determined by colorimetric method AlCl3 is 3.28 ± 0.27 (mg RE /g extract).
16. **Razavi et al., 2009** conducted a study for optimizing the gum extraction from basil seed. A central composite rotatable design was applied to evaluate the effects of temperature, pH and water⁄seed ratio on the yield, apparent viscosity

and protein content of water-extracted Basil seed gum. All of the variables significantly ($P < 0.05$) affected the extraction yield, whereas the effect of water⁄seed ratio on apparent viscosity and the effects of pH and water⁄seed ratio on protein content were not significant ($P > 0.05$). Numerical optimization determined the optimum extraction conditions based on the highest yield and viscosity and the lowest protein content as being temperature 68.71 ^{0}C, pH 8.09 and water⁄seed ratio 65.98:1.

17. Study by **Bravo et al., 2008** indicated that the mixture of compounds contained in the O. basilicum ethanolic extract was capable of influencing the processes related to foam cell formation through the reduction of cholesterol synthesis and the modulation of the activity of surface scavenger receptors. The study investigated the effects of ethanolic extract of O. basilicum on lipid accumulation in human macrophages. Macrophage treatment with 60 μg/ml, but not 20 μg/ml, of the extract reduced newly synthesized unesterified cholesterol by about 60% and decreased scavenger receptors activity by about 20–30%, evaluated by the internalization of cholesterol carried by [3 H] CE-aggregated-LDL. These findings encourage further studies involving detailed fractionation of the O. basilicum ethanolic extract to identify the components which reduce macrophage lipid accumulation

18. **Hussain et al.,2007** studied the chemical composition, antioxidant and antimicrobial activities of the essential oils from aerial parts of basil (Ocimum basilicum L.) as affected by four seasonal, namely summer, autumn, winter and spring growing variation. The hydro-distilled essential oils content ranged from 0.5% to 0.8%, the maximum amounts were observed in winter while minimum in summer. Samples collected in winter were found to be richer in oxygenated

monoterpenes (68.9%), while those of summer were higher in sesquiterpene hydrocarbons (24.3%). The contents of most of the chemical constituents varied significantly ($p < 0.05$) with different seasons. The essential oils investigated, exhibited good antioxidant activity as measurements by DPPH free radical-scavenging ability, bleaching β-carotene in linoleic acid system and inhibition of linoleic acid oxidation. Evaluation of antimicrobial activity of the essential oils and linalool, the most abundant component, against bacterial strains: Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pasteurella multocida and pathogenic fungi Aspergillus niger, Mucor mucedo, Fusarium solani, Botryodiplodia theobromae, Rhizopus solani was assessed by disc diffusion method and measurement of determination of minimum inhibitory concentration. The results of antimicrobial assays indicated that all the tested microorganisms were affected. Both the antioxidant and antimicrobial activities of the oils varied significantly ($p < 0.05$), as seasons change.

19. **Mathews et al., 1992** studied major food constituents of Ocimum basilicum seeds. The constituents responsible for the swelling properties of these seeds, when dispersed in water were investigated. Ocimum seeds contained a reasonable amount of hemicellulose and cellulose, accounting for their hydrophilic character. They were high in fibre and associated nutritional properties, and considered as a new non-conventional source of fibre.

Studies on pharmacological and health benefits of Ocimum Basilicum:

20. **Borysiuk et al., 2022** studied the anticonvulsant activity of Ocimum basilicum leaf essential oil using in silico method. The paper examines the analysis of the structure of essential oil of Ocimum basilicum using PASS-forecast. With the

help of PASS software, the anticonvulsant activity of individual compounds that are part of O. basilicum was predicted. Essential oils (EO) might have anticonvulsant by interfering with GABAergic neurotransmission.

21. **Mouslli et al., 2022** studied the efficacy and safety of gel products containing Ocimum Basilicum (OB) essential oil for treating acne. The prepared gels were applied alone on twenty volunteers suffering from acne vulgaris. The number of inflammatory lesions and the number of non-inflammatory lesions were significantly reduced at 1- to 4 weeks after treatment. It was found that the prepared gel had effectively treated acne vulgaris without side effects. The dry leaves of OB extract had faster effect compared with the fresh OB leaves extract.

22. **Antonescu et al., 2021** conducted a review to explore the therapeutic potential of Ocimum basilicum and Trifolium pratense in relation with their phytochemical profile and to highlight the pharmacological activity of aqueous or ethanol extracts The antioxidant, antimicrobial, antiviral, antifungal and anti-inflammatory activity of Ocimum and Trifolium species are summarized in this review in order to explore the therapeutic potential of Ocimum basilicum and Trifolium pretense in relation with their phytochemical profile and to highlight the pharmacological activity of aqueous or ethanol extracts. Special attention was devoted to the dermal pathology and wound healing effects, in the context of multiple skin conditions such as acne, eczema boils, psoriasis and rashes. Both extracts (Trifolium sp. and Ocimum sp.) are characterized by high content of antioxidant compounds, which are also responsible for the radiance and resistance of the skin and the slowing down of the aging process by maintaining estrogen levels. Moreover, the potential combined effect of the mixed extract is

pointed out in terms of future applications for wound healing, based on some preliminary results obtained from a “scratch tests” assay performed with respect to human dermal fibroblasts.

23. **Batista et al., 2021** studied the antinociceptive Effect of Volatile Oils from Ocimum basilicum Flowers on Adult Zebrafish. The volatile oil of the O. basilicum flowers was obtained by hydro distillation and analyzed by GC–MS, and the antinociceptive action is evaluated by different stimuli using motor parameters. The analysis of the chemical profile identified fourteen components with linalool (1) as a major chemical constituent (56.37%). The oral administration of volatile oil did not show any acute toxicity or behavior effects in all tested doses. The volatile oil has a pharmacological potential for the treatment of acute pain by modulation of opioid system, N-methyl-d-aspartate receptors (glutamatergic receptor), and the transient receptor potential vanilloid subtype 1 and acid-sensing ion channels. Together, these data provide support for analgesic properties of the volatile oil and contribute to suggest that the adult zebrafish model presents the cheapest, cost-effective pharmacological alternative for the discovery of novel analgesics.

24. **Dhama et al., 2021** published a comprehensive review which highlighted the phytoconstituent profile, medicinal values, therapeutic applications, and salient beneficial health aspects of O. basilicum, which would help in further exploring this wonderful herb for designing effective pharmaceuticals, drugs and medicines for safeguarding various health issues of humans and animals, while supporting its traditional use in curing various ailments, diseases and health-promoting effects. c. The chemical constituents of a given plant essential oil, per se, Ocimum basilicum, varies widely depending on the geographical area as well

as the applied extraction technique. The therapeutic potential of O. basilicum is broad and ever expanding. Most of the potential activities of O. basilicum are proven either at in vitro or in vivo levels. Hence, further studies have to be conducted in the clinical models of the disease to evaluate and prove the efficacy of the herb in managing different diseases.

25. **Seyed et al., 2021** published a review on the attribution of Ocimum basilicum in the prevention and management of neurodegenerative disorder. The review concluded Ocimum basilicum to be a novel resource for new pharmacotherapeutic discovery and development. Although these efficacious plant genera of prime importance and has potential medical and socioeconomic importance, the pivotal evidence for its neuroprotective potential in novel clinical trials remains lacking. However, with the available wealth of obtainable literature on this medicinal plant, O. basilicum L may function as promising therapeutics for the treatment of NDs.

26. **Bravo et al., 2021** published a systematic review was to study the current state of knowledge and explore the enormous potential of basil seeds as a functional food and source of functional ingredients to be incorporated into foods. Basil (Ocimum basilicum L.) is found worldwide and is used in the food, pharmaceutical, and cosmetic industries; however, the nutritional and functional properties of the seeds are scarcely known. Basil seeds contain high concentrations of proteins (11.4–22.5 g/100 g), with all the essential amino acids except S-containing types and tryptophan; dietary fibre (soluble and insoluble) ranging from 7.11 to 26.2 g/100 g lipids, with linoleic (12–85.6 g/100 g) and linolenic fatty acids (0.3–75 g/100 g) comprising the highest proportions; minerals, such as calcium, potassium, and magnesium, in high amounts; and

phenolic compounds, such as orientine, vicentine, and rosmarinic acid. In addition, their consumption is associated with several health benefits, such as the prevention of type-2 diabetes, cardio-protection, antioxidant and antimicrobial effects, and anti-inflammatory, antiulcer, anticoagulant, and anti-depressant properties, among others. The focus of this systematic review

27. A study by **Khan et al., 2020** concluded that 5% Ocimum basilicum-based (OB) emulgel is an innovative therapeutic approach in wound. The aim of the study was to formulate and evaluate the efficacy of topical application of OB-based emulgel on wound healing in animal model. The prepared formulations (OB emulgel) were assessed for FTIR analysis, stability studies, physical appearance, rheological behavior, spreadability, patch/sensitivity test and in vitro drug release. The in vivo wound healing effect was evaluated and compared with commercially available Silver Sulfadiazine cream Quench in wound-induced rabbits by macroscopic and histopathological evidence. The formulated OB emulgel exhibited good physical properties. The release profile of emulgel was satisfactory and released 81.71 ± 1.7% of the drug in 250 min. In vivo wound healing studies showed that OB emulgel exhibited the highest percent wound contraction similar to the commercial product ($p > 0.05$). Histopathological assessment showed marked improvement in the skin histological architecture after 16 days of OB emulgel treatment.

28. **Shahrajabian et al.,2020** published a review article on the chemical components and pharmacological benefits of Basil (Ocimum Basilicum). Basil is one of the most important crops with essential oils as well as polyphenols, phenolics, flavonoids and phenolic acids and suggested the usage of basil in both food and pharmaceutical industries. Basil has been shown positive effects

against viral, fungal, bacterial and some infections. Basil leaves have been used in treatment of fevers, coughs, flu, asthma, bronchitis, influenza and diarrhea. Basil Seed Mucilage, commonly known as basil seed gum. Basil seed mucilage can be considered as thickening, stabilizing, fat substitute, texturizer, surface-active and emulsifying hydrocolloid. The most important pharmacological uses of basil are anti-cancer activity, radioprotective activity, anti-microbial activity, anti-inflammatory effects, immunomodulatory activity, anti-stress activity, anti-diabetic activity, anti-pyretic activity, anti-arthritic activity, anti-oxidant activity, as a prophylactic agent and in cardiovascular disease.

29. **Mohammadali et al., 2020** investigated the beneficial therapeutic effects of Dill tablet and Ocimum basilicum (Basil) aqueous extract on hypercholesterolemia-induced cognitive deficits and oxidative stress in hippocampus tissues of rats. Hippocampal Aβ (1-42) level was measured. High-cholesterol diet (HCD) significantly increased serum cholesterol, induced deposition of Aβ plaque, altered hippocampus morphology, and impaired memory function, whereas receiving Basil extract or Dill tablet increased antioxidant potency in serum and hippocampus and normalized HCD-induced deleterious effects. Therefore, they can be used as hypocholesterolemic agents.

30. An in vivo study on rats by **Ghazwani et al.,2020** showed that raw or irradiated basil has a therapeutic effect on cardiac damage prompted by arsenic. Albino rats (n=32) were divided into four groups as follows "Control" group received distilled water, "As" group received arsenic(10mg/kg), "As+Basil" group received raw basil(400mg/kg) along with arsenic (10mg/kg) and "As+Irr. basil" group received 400mg/kg of irradiated basil (10kGy) along with arsenic (10mg/kg). To estimate the effect of gamma-irradiation on the antioxidant

properties in basil, Fourier-transform infrared (FTIR) spectroscopy was used. This analysis revealed an increase in the antioxidants compounds in irradiated basil as compared to raw basil. Rats exposed to arsenic showed a significant increase in serum lipid profile, cardiac enzymes as well as increasing the heart oxidative stress with a decline in their antioxidants. The administration of raw or gamma-irradiated basil leaves to arsenic exposed rats significantly reduced the accumulation of lipids and enzymes in serum accompanied by an improvement in antioxidant and oxidative stress of the heart.

31. **Eftekhar et al., 2019** studied the Immunomodulatory and anti-inflammatory effects of hydro-ethanolic extract of Ocimum basilicum leaves and its effect on lung pathological changes in an ovalbumin-induced rat model of asthma. Wistar rats were divided to six groups; non-sensitized, sensitized to ovalbumin, sensitized and treated with dexamethasone (1.25 µg/mL), and O. basilicum extract (0.75, 1.50 and 3.00 mg/mL) in drinking water for 21 days. The improvement effects of O. basilicum on pathological changes, immunological and inflammatory markers in sensitized rats comparable or even more potent than dexamethasone suggests the therapeutic potential of the plant in asthma.
32. **Herrera et al., 2019** studied the effect of Ocimum basilicum, Ocimum selloi, and Rosmarinic Acid on Cerebral Vascular Damage in a Chronic Hypertension Model. administering angiotensin II (AGII; 0.2 µg/kg intraperitoneally (i.p.) for 12 weeks) activates the hypothalamic–pituitary–adrenal (HPA) axis, which caused an increase in corticosterone levels, as well as in proinflammatory cytokines (interleukin 1β (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α)) and macrophage chemotactic protein 1 (MCP-1), and decreased anti-inflammatory cytokines (interleukin 10 (IL-10) and interleukin 4 (IL-4)).

On observing the behavior in the different models, an anxiogenic effect (elevated plus maze (EPM)) and cognitive impairment (water Morris maze (WMM)) was observed in animals with AGII. By administering organic extracts from Ocimum basilicum (Oba-EtOAc) and Ocimum selloi (Ose-EtOAc), and some doses of rosmarinic acid (RA) (6 weeks per os (p.o.)), the damage caused by AGII was stopped by re-establishing corticosterone serum levels and by decreasing the proinflammatory cytokines and MCP-1.

33. **Mili et al., 2019** published a review on Herbs and Herbals Therapy for Dengue wherein it was desribed that Basil fundamental oil had normal insecticidal properties that shield from mosquitoes, making basil a treatment and a safeguard technique. The review explained many home solutions for the control of dengue fever are furthermore free from reactions, for example, utilization of Ipecacuanha, basil, Echinacea, papaya, Astragalus, Neem, grape, kari path, peacock flower, giloy and orange juices etc. As home cures are effectively accessible, reasonable, important and exceedingly viable, it is prescribed to dengue patients to utilize these home cures.

34. **Patel et al., 2019** studied the antihypertensive potential, anti-inflammatory action, and cytotoxicity of Salvia (Chia) and Ocimum (Basil) Seeds In vitro assays were used to determine ACE inhibition and anti-inflammatory activity. The cytotoxicity assay for crude extract of defatted chia and basil seeds was done on human histiocytic lymphoma cell line (U937) by MTS assay. ACE inhibition activity assay showed that ethanolic and hexane extracts of both seeds have distinct ACE inhibitory activity. Both seed extracts had shown higher activity at 150 and 50 µg/mL concentrations respectively. Results of anti-inflammatory assay showed maximum protection of HBRC (Human Red Blood

Cell Membrane Stabilization Method) at 50 and 75 µg/mL concentrations for both seeds in ethanolic and methanolic extracts. MTS assay showed 73.20% and 70.85% cytotoxicity of Chia and Basil seeds at 12.50 and 50µg/mL concentrations respectively. IC50 values of Chia and Basil seeds were found to be 14.09µg/mL and 23.46µg/mL. This study provides direction for future investigation concerning detailed assessment of therapeutic potential of Chia and Basil seeds.

35. **Karima et al., 2019** studied the histological changes that may occur in fundic mucosa of adult male albino rats given aspirin and the protective role of Ocimum basilicum administration. Fundic mucosa of aspirin treated group showed disorganized fundic glands, desquamation of surface epithelial cells, mononuclear cell infiltration and congested blood vessels. Statistically significant increase in the mean area percent of collagen fibers and decrease in the mean thickness of mucous film in PAS stained sections of fundic mucosa of aspirin treated group. These changes were reduced in the coadministration group. It was concluded that Ocimum basilicum aqueous extract has beneficial protective effects against the deleterious effects of aspirin on the fundic mucosa.

36. **Purushothaman et al., 2019** conducted molecular Docking Studies of potential anticancer agents from Ocimum basilicum L. against human colorectal cancer regulating genes. The chemical constituents of Ocimum basilicum were studied against human colorectal biomarkers such as KRAS, NRAS, BRAF (Oncogenes); PIK3CA, P53, DCC (Tumor suppressor genes) through molecular docking. Autodock 4.2 software was used to understand the drug-biomolecular interactions; binding mechanism of drug (ligand) and receptor (target); binding energy and bond length. The mutant type proteins of these markers were

analyzed with specific to their expression codons against these O. basilicum constituents. Results revealed that constituents of O. basilicum have excellent binding energies against colorectal cancer genes.

37. **Sestili et al., 2018** published a review on potential effects of Ocimum basilicum on health. The current status of OB as a nutraceutical, the pharmacology of its bioactive components, the rationale for its indications, and its safety were covered in the review. Due to the polyphenolic and flavonoidic content, OB can be considered as an important ingredient in healthy diets; OB preparations may be effective as chemo preventive agents or adjunctive therapy in the treatment of different clinical conditions. From a toxicological perspective, since the tumorigenic potential of alkenyl benzenes is counteracted by other OB constituents such as nevadensin, it can be concluded that OB consumption in food and preparations is safe. The only concern relates to OB essential oils: in this case, a concentration limit for alkenyl benzenes should be precautionary defined, and the use of plant chemotypes with no or low levels of these alkylbenzenes for the preparation of essential oils should be made compulsory.

38. **Miraj et al., 2016** published a review on the pharmacological effect of Ocimum basilicum. The study overviewed the therapeutic effects of Ocimum basilicum than its nutritive and industrial effects. OBL possesses an inhibitory effect on platelet aggregation induced by ADP and thrombin that is dosedependent and results in an anti-thrombotic effect in vivo which develops progressively over 7 days and disappears over 3-7 days. The possible antihypertensive effects of OBL extract in renovascular hypertensive rats was examined. The effects of OBL on blood pressure, cardiac hypertrophy and ET, are consistent with an effect on ET-converting enzyme, and warrant further exploration. The in vitro

hypoglycemic activity of basil (Ocimum basilicum) aqueous extract was investigated. It concluded that basil aqueous extract via antioxidant and possibly α-glucosidase and α-amylase inhibiting activities, offered positive benefits to control diabetes. The effects of Ocimum basilicum L. tincture in acute inflammation induced with turpentine oil in Wistar male rats was evaluated. Ocimum basilicum tincture significantly reduced the total leukocyte count, monocyte percentage, activation of circulating phagocyte.

39. An in vivo Study was carried out by **Rodrigues et al.,2016** to investigate the chemical composition and systemic anti-inflammatory activity of the Ocimum basilicum essential oil (EOOB) and its major component estragole, as well as its possible mechanisms of action. The Ocimum basilicum essential oil was obtained by hydro distillation and analyzed by GC-MS. The anti-inflammatory action was verified using acute and chronic in vivo tests as paw edema, peritonitis, and vascular permeability and granulomatous inflammation model. The anti-inflammatory mechanism of action was analyzed by the participation of histamine and arachidonic acid pathways. The chemical profile analysis identified fourteen components present in the essential oil, within them: estragole (60.96%). The in vivo test results showed that treatment with EOOB (100 and 50 mg/kg) and estragole (60 and 30 mg/kg) significantly reduced paw edema induced by carrageenan and dextran. The smallest doses of EOOB (50 mg/kg) and estragole (30 mg/kg) showed efficacy in the reduction of paw edema induced by histamine and arachidonic acid, vascular permeability inhibition and leukocyte emigration in the peritoneal fluid. Theses doses were capable of reducing the chronic inflammatory process. This study confirms the therapeutic potential of this plant and reinforces the validity of its use in popular medicine.

40. An invitro study by **Kubiça et al., 2013** was done to investigate the antiviral activity of the essential oil of Ocimum basilicum and the monoterpenes camphor, thymol and 1,8-cineole against bovine viral diarrhoea virus (BVDV). The cytotoxicity of the compounds was measured by the MTT (3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide) test, and the antiviral activities were tested by the plaque reduction assay. The oil or compounds were added to the assay in three different time points: a) pre-treatment of the virus (virucidal assay); b) pre-treatment of the cells; or c) post-treatment of the cells (after virus inoculation). The percentage of plaques inhibition for each compound was determined based on the number of plaques in the viral control. The results were expressed by CC_{50} (50% cytotoxic concentration), IC_{50} (inhibitory concentration for 50% of plaques) and SI (selectivity index = CC_{50}/IC_{50}). Camphor (CC_{50} = 4420.12 µg mL-1) and 1,8-cineole (CC_{50} = 2996.10 µg mL-1) showed the lowest cytotoxicity and the best antiviral activities (camphor SI = 13.88 and 1,8-cineol SI = 9.05) in the virucidal assay. The higher activities achieved by the monoterpenes in the virucidal assay suggest that these compounds act directly on the viral particle.

41. **Khair-ul-Bariyah et al., 2012** published a review describing the importance of Ocimum basilicum in the field of herbal medication. Various effects like immunomodulatory, hyperglycemic, hypolipidemic, anti-inflammatory, hepatoprotective, antimutagenic, antimicrobial, antifungal, antioxidant, lipid peroxidation, insect repellency, antiviral, antierythmic, depigmenting, antitoxic and CNS activity analysis reports are exhibited by Ocimum basilicum. The wide range of study on this herbal plant shows that it is very beneficial for the

improvement of current drugs and more work can be done to take advantage of the potential remedial qualities of it.

42. **Bilal et al in 2012** reviewed the literature regarding Ocimum basilicum, specifically for its chemical properties, therapeutic benefits and scientific studies. Studies indicate Ocimum basilicum to possess analgesic, anti-inflammatory, antimicrobial, antioxidant, antiulcerogenic, cardiac stimulant, chemo modulatory, CNS depressant, hepatoprotective, hypoglycemic, hypolipidemic, immunomodulator and larvicidal activities. The drug was also searched for its folkloric claims. It is used in traditional medicine as a tonic and vermifuge, and Basil tea taken hot is good for treating nausea, flatulence, and dysentery. The oil of the plant has been found to be beneficial for the alleviation of mental fatigue, cold, spasm, rhinitis, and as a first aid treatment for wasp stings and snakebites. Preliminary studies have found various constituents of Ocimum basilicum to exhibit a variety of therapeutic effects. These results are very encouraging and indicate that this drug should be studied more extensively to confirm these results and to find other potential therapeutic effects.

Studies on Remineralisation potential:

43. **Renita Soares et al in 2017** conducted a study published in Journal of clinical and diagnostic research with an aim of to evaluate the ability of Casein Phosphopeptide-Amorphous Calcium Phosphate Fluoride (CPP ACPF), Bioactive Glass (BAG), fluoride enhanced Hydroxyapatite (HA) gel and self-assembling peptide P11-4 to remineralize artificial carious lesions in enamel in vitro using a 30 day pH cycling model through surface microhardness analysis and SEM wherein all groups excluding the control group were subjected to

demineralisation following which four of these groups were remineralized using the four demineralizing agents. The treated groups were subjected to pH cycling over a period of 30 days. This was followed by assessment of surface microhardness and SEM for qualitative evaluation of surface changes. Hence it was concluded as Self assembling peptide P11-4 demonstrated promising results by effectively and significantly demineralizing the enamel lesions as compared to other test agents.

44. **Sulistianingsih et al in 2017** conducted study on the remineralization potential of cocoa bean extract (*Theobroma cacao*) to increase the enamel microhardness by comparing with fluorine as synthetic material. Thirty-six maxillary first premolar tooth crowns were cut and planted in the epoxy resin. Teeth were then immersed in demineralization solution at pH 4 for 6 hours. The sample were divided into 2 groups, 18 for the fluorine group and the remaining group of cocoa extract. Vickers microhardness test was used before treatment, both after demineralized and remineralized. The value of enamel microhardness before treatment in the fluorine group was 376.17 VHN as average value and the cocoa extract group was 357.33 VHN. After demineralization in fluorine group was 268.13 VHN and cocoa extract group was 235.93 VHN. After remineralization in fluorine group and cocoa extract group, respectively, 321.08 VHN and 293.86 VHN. The results of the analysis revealed that the level of enamel microhardness after remineralization was not significantly different in both groups ($p>0.05$). The findings indicated the ability of cocoa extract to increase the microhardness of enamel and implies the potential as a fluorine substitution for remineralization.

45. **Gulcin Bilgin Gocmen et al in 2016** published an article in Asian Pacific Journal of Topical biomedicine with an objective to evaluate the effectiveness of herbal medicaments such as ginger, rosemary and honey on re-mineralization of initial enamel lesion. Demineralized human enamel specimens were measured for baseline surface microhardness and fluorescence methods. Ten specimens in each of four groups were used in this in vitro recycling study with the following treatments which applied three times a day: 1) sodium fluoride toothpaste 2) ginger honey 3) ginger-honey-chocolate 4) rosemary oil. Treatment regimens of demineralization and re-mineralization cycle were applied for 21 days. The post-treatment data were obtained by measurements of surface microhardness and fluorescence methods. Data were statistically analysed by ANOVA test with Tukey's honest significant difference test. Finally, he concluded that herbals have enhanced re-mineralization on initial enamel caries.

46. **Abdulraheam et al in 2011** investigated the microscopical changes of different types of tea; Camellia sinensis (black and green), Mentha spicata and Ocimum basilicum on artificially- induced initial enamel caries. Initial enamel caries-like lesions were introduced in 30 sound extracted human 1st premolar teeth using pH cycling procedure. Teeth were divided into 6 groups to test 4 types of tea (black, green, Mentha spicata and Ocimum basilicum) and 2 control solutions (0.05%NaF and de-ionized water as positive and negative control respectively) for enamel re-mineralization, using polarized microscope. The concentration of calcium, inorganic phosphorous and fluoride ions in all tea solutions were also measured. Results revealed that green tea produced the best enamel remineralization, while black tea and Mentha spicata were coming next and result in different mode of re-mineralization. On the other hand, teeth treated

Ocimum basilicum showed mild evidence of re-mineralization. The chemical analysis indicated that both Mentha spicata and Ocimum basilicum had the highest calcium and phosphorous ions levels (higher than their fluoride concentration). Green tea had nearly equal concentration for the 3 tested ions, which were less than its counterpart black tea.

47. **Benjamin et al., 2012** investigated Grape seed extract (GSE) as the potential remineralizing agent. Sound human tooth sections were obtained from the cervical portion of the root and stored in demineralizing solution at 37°C for 96 hours to induce artificial root caries lesions. The sections were divided into four treatment groups including 6.5% grape seed extract, sodium monofluorophosphate (220 ppm) with 0.05% calcium glycerophosphate, 0.5% calcium glycerophosphate and control (no treatment). An *in vitro* pH cycling model was used to cycle the demineralized specimens through treatment solutions, acidic buffer and neutral buffer for 8 days at 6 cycles per day. Subsequently, they were evaluated using confocal laser scanning microscope. Data were analysed using analysis of variance ($p < 0.05$). GSE revealed less demineralization and more remineralization compared with other groups.

48. **Onishi et al., 2007** investigated Gum Arabic which is natural polysaccharide exudate from Acacia Senegal and other related African species of Acacia. It is considered to have an ability to enhance remineralization, because of its high concentration of Ca2+. In this study, investigator evaluated the cariostatic activities of gum Arabic using histopathological methods to determine its effects on remineralization. Following incubation in demineralization solution, human third molars were exposed to 10 mg/ml of gum Arabic, sodium fluoride at 1000 ppm (NaF), or double distilled water (DW, negative control), then subjected to

demineralization–remineralization cycles. Before and after demineralization–remineralization cycles, contact microradiographs of each sample were taken and mineral distribution quantities were calculated. The remineralization ratio of the molars exposed to gum Arabic was similar to that of those exposed to NaF, while the ratios of both were significantly greater than that of those exposed to DW. Gum Arabic enhanced the remineralization of caries-like enamel lesions in vitro, suggesting its inhibitory effects towards dental caries.

MATERIALS AND METHODS

The present study was designed to assess the effect of *Ocimum basilicum* varnish on its remineralization potential. It was an in-vitro study, conducted according to the guidelines of Good Laboratory Practice (GLP)[30] and approved by the Institutional Research and Ethics Committee (IREC) of KLE Vishwanath Katti Institute of Dental Sciences (Reg no:1499, approved on 25/10/2021). *Ocimum basilicum* seeds were authenticated by recognised taxonomist at Indian Council of Medical Research National Institute of Traditional Medicine (ICMR-NITM), Belagavi. The coarse powder of *Ocimum basilicum* seeds was procured from KLE Ayurveda pharmacy of KAHER's Shri B M Kankanwadi Ayurveda Mahavidyalaya, Belagavi, Karnataka.

STUDY DESIGN

Experimental in-vitro study

STUDY SETTINGS

- The Department of Public Health Dentistry, KLE VK Institute of Dental Sciences, KLE Academy of Higher Education and Research, Belagavi, Karnataka
- The Department of Oral and Maxillofacial Surgery, KLE VK Institute of Dental Sciences, KLE Academy of Higher Education and Research, Belagavi, Karnataka
- Indian Council of Medical Research National Institute of Traditional Medicine (ICMR-NITM), Belagavi, Karnataka
- KLE Ayurveda pharmacy of Shri B M Kankanwadi Ayurveda Mahavidyalaya, KLE Academy of Higher Education and Research, Belagavi, Karnataka

- KLE's Prabhakar Kore Basic science research Centre (BSRC), Belagavi, Karnataka
- The Department of Oral and Maxillofacial Pathology and Oral Microbiology, KLE VK Institute of Dental Sciences, KLE Academy of Higher Education and Research, Belagavi, Karnataka
- KLE College of Pharmacy, Belagavi, Karnataka

MATERIALS

Materials used for preparation of extract

1. Coarsely powdered *Ocimum basilicum* seeds
2. Soxhlet apparatus
3. Ethanol 99%
4. Muslin cloth

Materials used for assessing remineralization

1. Teeth samples
   - Extracted premolar teeth
   - 10% formalin
   - Diamond disc
   - Arkansas stone

2. De-mineralizing solution
   - Calcium chloride
   - Sodium di-hydrogen phosphate
   - Acetic acid
   - Potassium hydroxide

Materials for formulation of *Ocimum basilicum* varnish

1. Ethyl acetate
2. Nitrocellulose
3. Fumed silica
4. Iso amyl propionate

Other instruments and equipments

1. Bath sonicator
2. Vortex and CAP 2000+ Viscometer
3. Light microscope (Research microscope Leica™ DM 2500)
4. Image J software
5. Microscopic slides and cover slips
6. Bifluoride 12
7. Electromagnetic stirrer

SAMPLE SIZE ESTIMATION

Sample size for the three groups was assessed using power and effect size by Cohen's statistical power analysis. [31]

Desired Power	Effect size=.2	Effect size=.5	Effect size=.8
0.2	33	7	4
0.3	53	10	5
0.4	74	13	6
0.5	98	17	8
0.6	124	21	9
0.7	156	26	11
0.8	198	33	14
0.9	264	44	18
GPOWER ES:	0.2828	0.7071	1.131

Power and Effect size were estimated based on data procured from the pilot study by fixing *p* value at 0.05 (Alfa=0.05, Power= 80%). The effect size was assumed to be at 0.5. Hence, the sample size was estimated to be 33 in each group.

INCLUSION CRITERIA

Extracted human permanent maxillary and mandibular premolars, macroscopically caries free or orthodontically extracted teeth.

EXCLUSION CRITERIA

Teeth having intrinsic stains, dental caries, gross surface defects like pits, fractures, fluorosis, developmental defects and enamel hypoplasia/hyperplasia

STUDY PROCEDURE

Extract Preparation using Soxhlet method [32]

The preparation of the extract was done by Soxhlet method with 99 percent ethanol as solvent. The powdered seeds (100 gms) were kept in a muslin cloth bag and placed in the body of Soxhlet extractor and ethanol (800ml) was added to it. Soxhlet method with 99 percent ethanol as solvent was employed for preparation of the extract. The Soxhlet extractor apparatus was fastened with clamps and stand to stabilize and support the apparatus, round bottom flask and condenser. The isomantle was used to gently heat ethanol resulting in its evaporation. This evaporated solvent was circulated continuously in multiple cycles from the soxhlet apparatus to the condenser which is connected by a rubber tube to a continuous source of water supply in order to condense the solvent. This cycle is repeated when the condensate reaches the level of siphon, and drips back into the reservoir. This procedure utilized a total of 6 hours to complete 24 cycles at the boiling temperature of ethanol 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 sulfoxide (DMSO) in 20mg/ml ratio to obtain a stock solution.

Phytochemical tests

The phytochemical tests were carried out in KAHER's Shri B M Kankanwadi Ayurveda Mahavidyalaya, Belagavi, Karnataka to investigate the chemical composition and biological activities of ethanolic extract from *Ocimum basilicum.* [33,34]

Sl no.	**Phytochemical constituent**	**Test**	**Procedure and Observations**	**Results**
1	Alkaloids	Mayer's test	2-3 ml filtrate + few drops of Mayer's reagent –Appearance of cream-coloured precipitate	Positive
2	Phenolic compounds	Ferric chloride test	Extract + 2 ml of FeCl solution – Formation of green or blue colour	Positive
3	Detection of flavonoids	Aqueous sodium hydroxide test	Extract + aqueous NaOH – Yellow orange colour	Positive
4	Saponins	Foam test	Extract + water + Sodium Bicarbonate vigorously shaken – Honeycomb like froth	Positive
5	Test for sugars	Benedict's test	Extract + Benedict's solution – heated in boiling water bath – Formation of red precipitate	Positive
6	Tannins	Maxson + Rooney Method	1 ml of extract + 2ml of FeCl – dark green colour	Negative
7	Protein	Ninhydrin Solution test	3ml of extract + 3 drops of 5% Ninhydrin solution heated in boiling water bath for 10 mins – appearance of purple or bluish colour	Positive

Preparation of Ocimum basilicum varnish

The above extract was placed in a bath sonicator along with ethyl acetate for about 30 minutes for the complete dissolution of the extract. After its dissolution, Iso Amyl Propionate and the colloidal solution were added. The contents were mixed in a vortex for about 30 seconds and fumed silica was added to this. The contents were then transferred to an amber-coloured sterile bottle and labelled.

Sl No.	Ingredients	Per 5ml of varnish
1	*Ocimum basilicum* extract	80mg
2	Ethyl acetate	2ml
3	Colloidal solution	2ml
4	Iso Amyl Propionate	1 mg
5	Fumed Silica	100 mg

Evaluation for Physical Parameters of varnish[35]:

1. **Colour Matching:** The test area required adequate artificial daylight illumination. The specimen was held at eye level, 25 cms away and viewed perpendicularly, in order to match and note the colour of the indigenously prepared varnish with the shade guide. The date of varnish preparation was also noted to estimate its shelf life. The intra examiner kappa value was estimated by considering the mean value of six specimens of the same shade after observing for a period of 1 week.
2. **Rate of evaporation:** At the outset, the weight of a sterile glass slide was recorded and 100 microlitre of varnish was evenly distributed on it and weighed again. A stop watch was used to estimate the time required for the

complete evaporation of varnish and the return of the slide to its original weight.

3. **Viscosity:** It was assessed using CAP 2000+ Viscometer, Brookfield. Two ml of varnish was placed on the viscometer plate and the values were noted after conducting the test as per the manufacturer's instructions.
4. **pH of the varnish:** pH strips were used to establish the pH values of the varnish.
5. **Film forming ability:** Sectioning of the tooth samples were obtained by slow speed diamond disc. Fifty micro litres of varnish was applied using the applicator tip. The samples were dried and observed under light microscope to assess the morphology of the film formed by the application of varnish.
6. **Cytotoxicity assessment:** Healthy, adult, human Primary Gingival Fibroblasts (HGF) ATCC PCS-201-018 were assessed for cell toxicity. Dulbecco's modified Eagle's medium (DMEM; Gibco laboratories, Grant Is; NY, USA) was employed to cell culture. This media was supplemented with 10%FBS (Fetal Bovien Serum, Gibco, Invitrogen) Cat No-10270106, Antibiotics-Antimycotic 100X solution (Thermofisher Scientific)-Cat no-15240062. Approximately $5x10^3$ cells/wells were seeded in a micro plate with 96-wells. Plate was stored overnight in controlled conditions with 37^oc temperature, 95% humidity and 5% CO_2. Each sample concentration of 300 microlitres was processed and the incubation period of the cells was extended by another 24 hours. The cells in the well were washed with Phosphate buffer solution and 80 microlitre of methyl-thiazol-tetrazolium (MTT) staining solution was added. Further incubation of plate was carried out at 37^oc. After 4h, 200 microlitre of di-methyl sulfoxide (DMSO) was injected in each well for the

dissolution of formazan crystals, and rate of absorbance was recorded using 570mm micro plate reader.

Formula: Surviving cells (%) = Mean OD of test compound/ Mean OD of negative control x 100 graph Pad Prism Version5.1

Preparation of demineralizing solution

2.2 mM calcium chloride, 2.2 mM NaH2PO4, 0.5 M acetic acid, were mixed with 1 litre of distilled water by an electromagnetic stirrer at 800 rpm and pH was adjusted at 4.4 with 1M KOH by pH meter in KLE's Prabhakar Kore Basic science research centre, Belagavi, Karnataka.

- Calibration of digital pH meter was done by following the below mentioned steps:

a) The pH mode was selected, temperature control knob was set to 25 ^{o}C.

b) The electrode was cleaned with de-ionized water and blotted dry with a tissue.

c) Further the electrode was immersed in the solution of pH 7 buffer, by adjusting and setting the display on the calibrate button to '7' (cal).

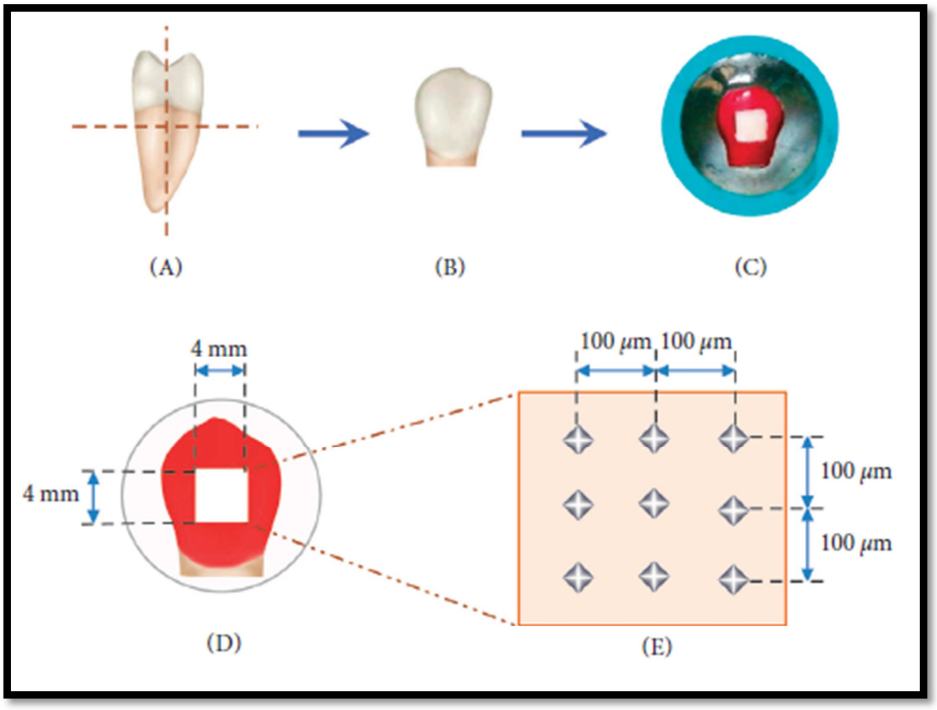
d) The electrode was taken out of the buffer and replaced again in the solution of pH 2 buffer, allowing the display to stabilize at '2' by adjusting calibrate button (cal).

e) The electrode was cleaned with de-ionized water and blotted dry with a tissue and the bottles were capped to be reused later.

- After preparation of the solution, the pH electrode was rinsed and placed back in the storage solution.

Preparation of 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. Each enamel surface was marked with a square area of 4x4mm to mimic an enamel window buccally. The remainder of the enamel specimen was coated with acid-resistant nail varnish so as to generate lesions only in the enamel window. This helped to standardize, determine and compare the lesion depth between the enamel window and the unaffected region covered with nail varnish. The enamel specimens were divided randomly into three groups mainly fluoride varnish group (group 1), *Ocimum basilicum* varnish test group (group 2) and Placebo-distilled water group (group 3). The samples were immersed in demineralising solution for 72-hour cycle to simulate the daily acid challenges in oral cavity. After immersing each enamel specimen in a demineralizing solution, artificial subsurface carious lesions were created. Each group was subjected to re-mineralization 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 laboratory produced saliva which contained 2.00 g/L methyl p-hydroxybenzoate, 10.0 g/L sodium carboxymethyl cellulose, 8.38 mmol/ L KCl, 0.29 mmol/L MgCl2$6H2O, 1.13 mmol/L CaCl2$2H2O, 4.62 mmol/L KH2PO4, 2.40 mmol/L K2HPO4; and pH was adjusted to 7.0 using KOH to simulate normal pH of oral environment. There was no precipitation detected during the experiment. The saliva was changed each day and the treatment materials were freshly prepared in every application. The samples were continuously immersed in artificial saliva that was blended by a magnetic stirring machine except during the time of application.



Photograph 1: Representation of "Enamel window"

***Enamel specimens and* evaluation of re-mineralization potential**

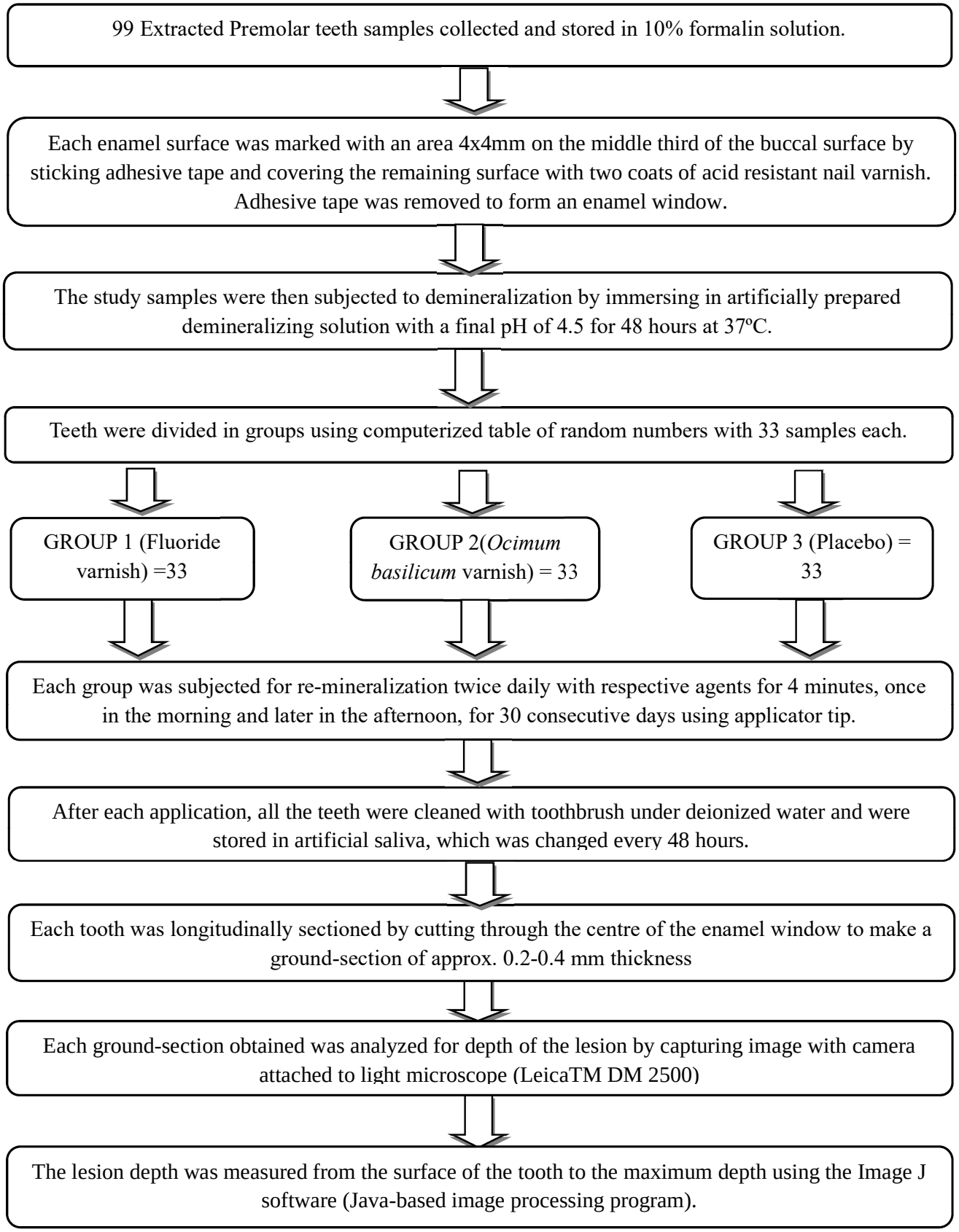
Each sample was sectioned longitudinally in the labio-lingual direction through the window using diamond disk to produce approximately 700 to 1000 μm thick sections. These sections were further grinded on Arkansas stone to produce 150 to 250 μm paper thin ground sections. The light microscope (Leica™ DM 2500) was used to analyze demineralisation and re-mineralization. The light microscope was standardized for visualization of ground sections by an expert. A trained examiner calibrated the image J 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 CCD shading camcorder (Leica DFC320) attached to the microscope. These images were scaled using Image-J 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 meters from the examiner.

Statistical Analysis

Data obtained was entered in Microsoft Excel 2020 and analyzed using the IBM Corp. Released 2012. IBM SPSS® Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp. The data was subjected to normality (Shapiro-Wilk) test and it was found to fall under normal distribution. The mean and standard deviation (SD) of the lesion depth from each group were obtained. The values were tabulated and statistically analyzed using one-way ANOVA. Comparison of each group with the control group was analyzed 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.

SCHEMATIC REPRESENTATION OF THE METHODOLOGY



Photograph 2: *Ocimum basilicum* plant (Lamiaceae)

Photograph 3: *Ocimum basilicum* seeds (Sweet basil seeds, Falooda seeds, Tukmaria, Sabja seeds, Thai holy basil, Sabja vethai, Hazbo,Tuk malanga, Basica cultive, Basilien Kraut)

a)

b)

Photograph 4: a) *Ocimum basilicum* seeds coarsely powdered
b) *Ocimum basilicum* seeds finely powdered

a)

Soxhlet Apparatus for preparation of *Ocimum basilicum* crude extract

b)

IKA Rotary evaporator to extract ethanol from crude extract

c)

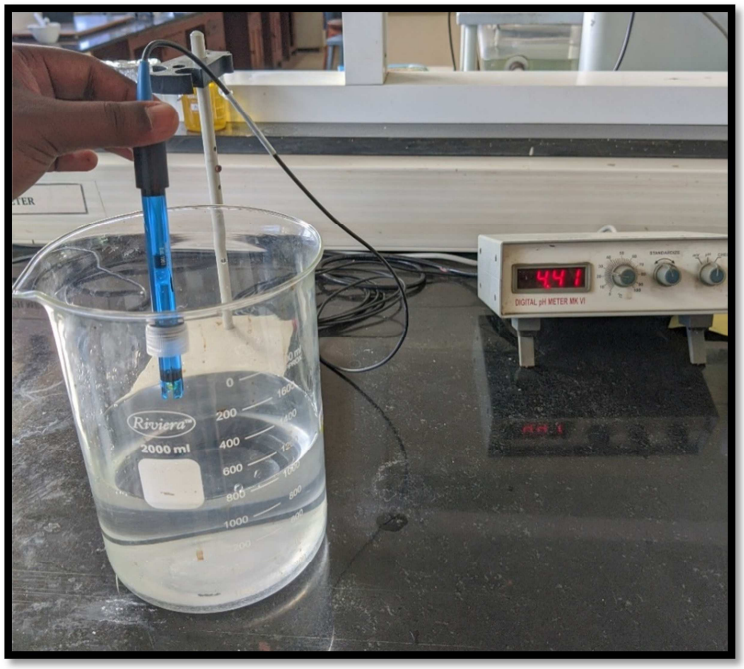
Crude extract collected in crucible after each cycle

d)

Total extract prepared from 100gm of *Ocimum basilicum* powder and 800 ml of ethanol

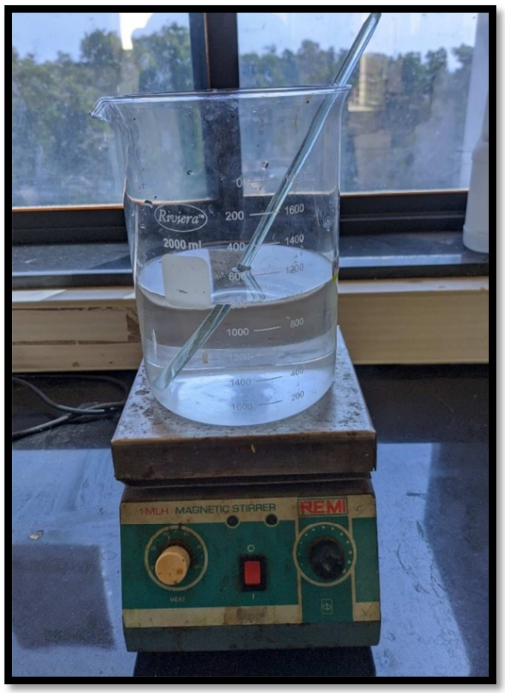
Photograph 5: Extract preparation

Photograph 6: Representative teeth samples

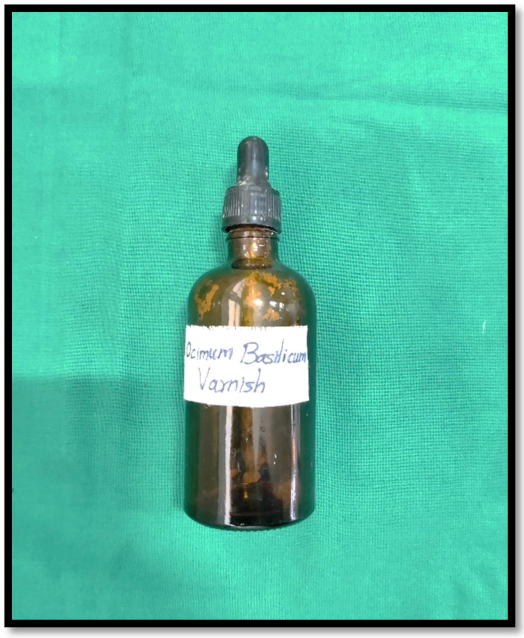


Photograph 7: Demineralization solution pH set at 4.4

Photograph 8: Ingredients for preparation of varnish



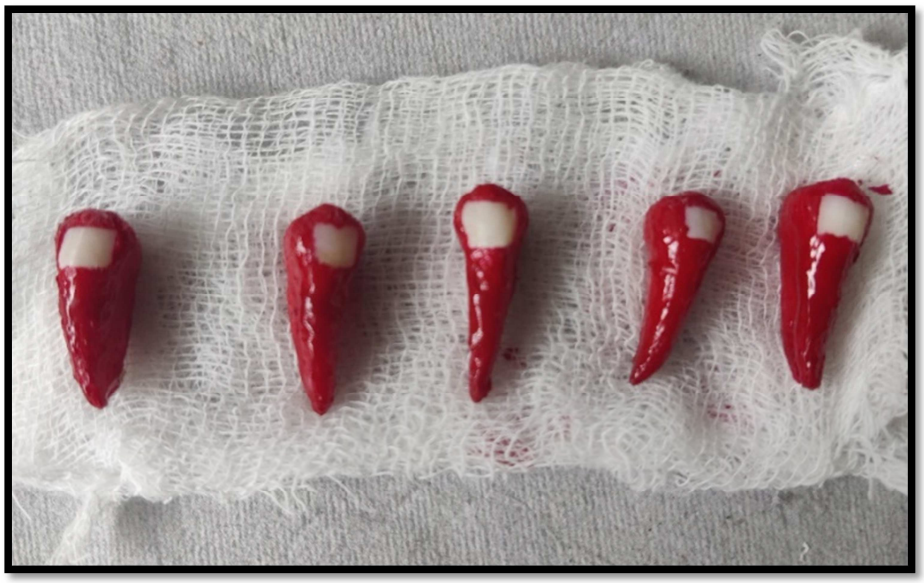
Photograph 9: The mixture of extract and all the chemicals in the water bath and magnetic stirrer



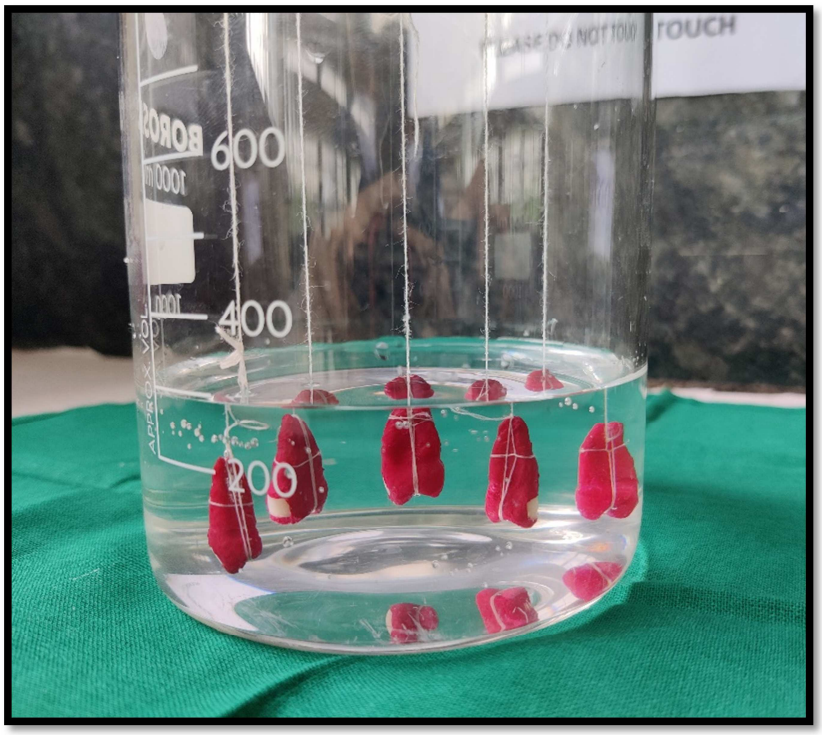
Photograph 10: *Ocimum basilicum* varnish in amber coloured bottle (Study agent)

Photograph 11: Bifluoride varnish (Control agent)

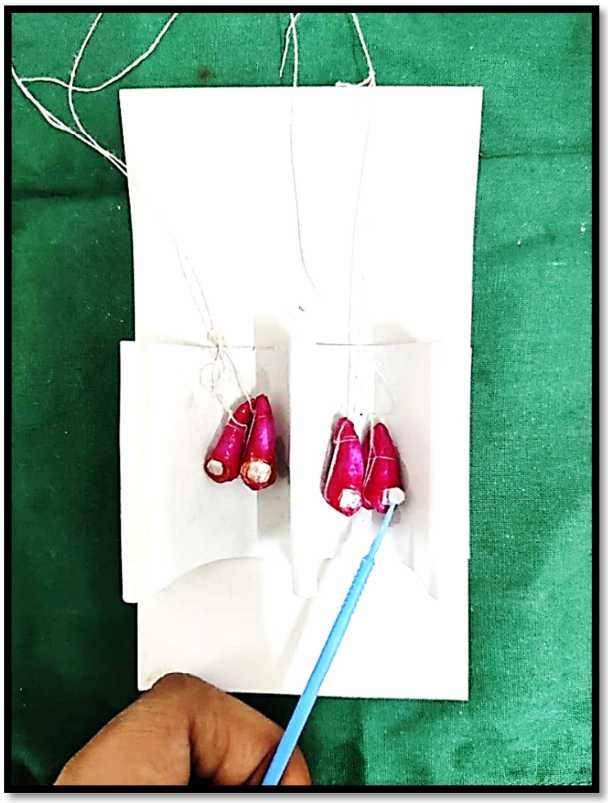
Photograph 12: Nail varnish application for the entire teeth surface leaving a window



Photograph 13: Creating a window on the buccal surface of the teeth

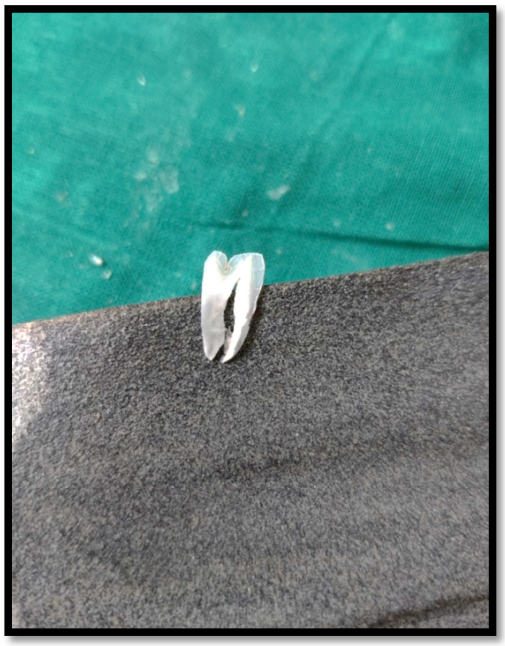


Photograph 14: Teeth immersed in demineralizing solution

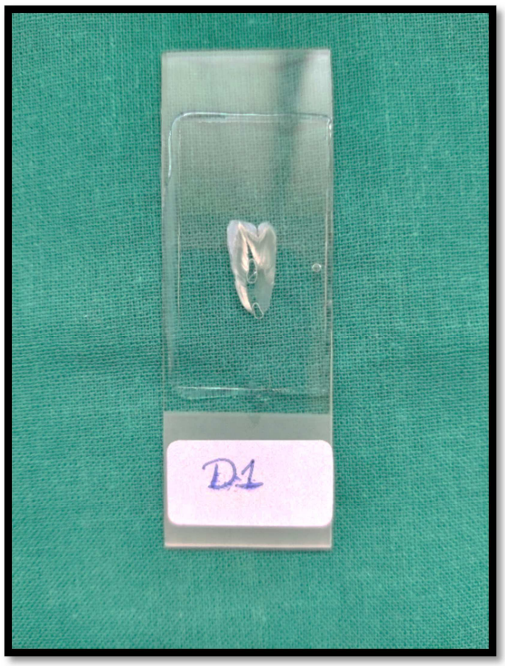


Photograph 15: Applying control and test agent everyday

a)

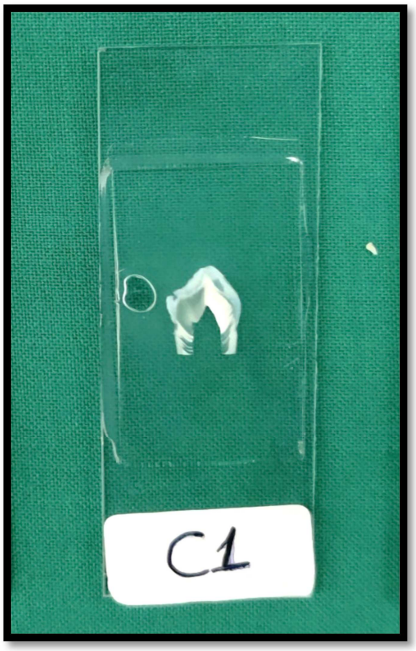


b)



c)

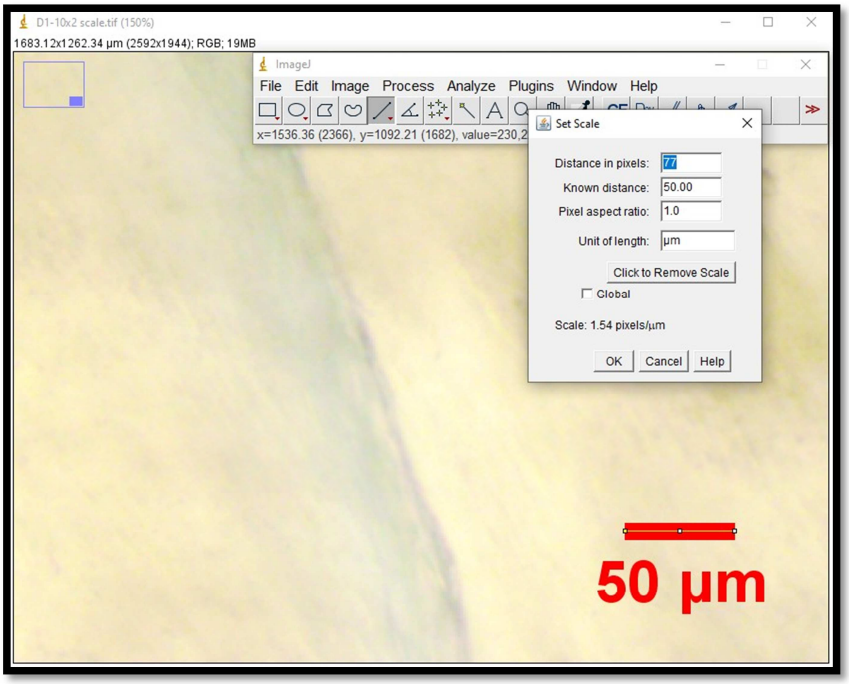
d)



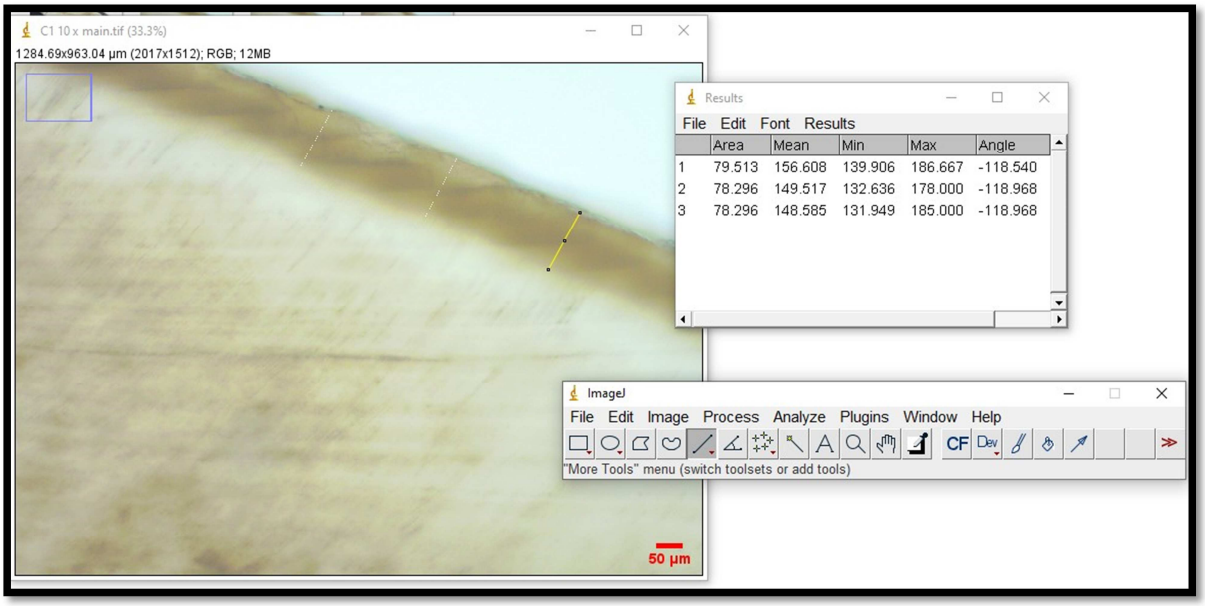
Photograph 16: a) Ground sectioning of the tooth samples
b) Representative mounted ground section in Demineralizing group
c) Representative mounted ground section in Remineralizing group
d) Representative mounted ground section in Control group

Photograph 17: Light microscope (LeicaTM DM 2500)

Photograph 18: Direct observation under light microscope with attached screen display by an investigator



Photograph 19: Standardization of scale for lesion depth measurement in image J software



Photograph 20: Light microscopy 4X magnification image of samples in Image J software to analyze for lesion depth

RESULTS

The present in vitro study was designed to evaluate and compare *Ocimum basilicum* varnish on the remineralizing potential of artificially created initial enamel caries. Lesion depths were recorded in all group samples using light microscope and analyzed with Image J software. Upon application of remineralizing 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 remineralization was observed in the samples of *Ocimum basilicum* varnish as compared to test groups of fluoride varnish application [Figure 1].

The data obtained from the study was subjected to statistical analysis. The mean (± SD) pre-treatment lesion depth across the groups ranged from 242.11 ± 26.144μm to 352.66 ± 34.531μm. Comparisons between pre-test lesion depths in all groups were statistically insignificant (p = 0.380). The mean (± SD) lesion depths between the pre-treatment groups are shown in Table 1.

Pre and post treatment lesion depths were calculated for all three groups. In Group 1 positive control group (Fluoride varnish), the average lesion depth was 148.62μm with a range of 124.59μm to 166.61μm. In the group 2 test group (*Ocimum Basilicum* varnish), the average lesion depth was 180.52μm with a range of 158.12μm to 218.48μm and in the Group 3 negative control group (Placebo), the average lesion depth was 282.45μm with a range of 216.32μm to 348.30μm. ANOVA analysis 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 2.

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 remineralization post pH cycling. However, there was a considerable increase in mean lesion depth in group 3 with $p=0.028$ [Table 3 and Figure 3]. The Intra group lesion depth was compared for the mean difference among the groups. 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 analysis ($p<0.001$) [Table 4 and Figure 4]. Inter group analysis within the groups for lesion depths revealed a statistically significant difference by Tukey's post hoc analysis ($p<0.001$) [Table 5].

The percentage of mean lesion depth recovery was calculated for all the experimental groups. The highest recovery rate of 45.938% 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%. The relative percentage of reduction of lesion depths was observed in Figure 5 and Table 6. Inter group analysis for percentage of reduction within the groups for lesion depths revealed statistically significant difference by Tukey's post hoc analysis ($p<0.001$) [Table 7].

The MTT assay results obtained for the *Ocimum basilicum* varnish and the fluoride varnish control revealed that it to be non-toxic to gingival fibroblast cells as the viability of cells was maintained. It was observed that the viability of the cells was more towards *Ocimum basilicum* varnish as compared to the control group by 20 %. The IC50 (concentration at which the drug induces up to 50% cell death) obtained for

Ocimum basilicum varnish was 47 lg/ml, and that obtained for fluoride varnish was below 36 lg/ml. The statistically significant difference observed between *Ocimum basilicum* varnish and fluoride varnish indicated that *Ocimum basilicum* varnish provided better cytocompatibility than fluoride varnish and showed no toxic effects on fibroblasts [Table 8].

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

Groups	**Mean**	**SD**	**SE**	**95% Confidence Interval for Mean**		**Minimum**	**Maximum**	**F**	***p-Value***
				Lower Bound	Upper Bound				
Group 1	278.112	29.625	5.157	267.607	288.617	250.94	361.22	0.978	0.380
Group 2	284.280	26.144	4.551	275.009	293.550	242.11	346.89		
Group 3	273.911	34.531	6.011	225.01	352.66	273.911	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: *$p \leq 0.05$ is considered statistically significant, **$p \leq 0.001$ Highly significant.

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

Groups	**Mean**	**SD**	**SE**	**95% Confidence Interval for Mean**		**Minimum**	**Maximum**	**F**	***p*-Value**
				Lower Bound	Upper Bound				
Group 1	148.62	9.626	1.675	145.204	152.031	124.59	166.61	381.297	**<0.001***
Group 2	180.52	11.876	2.067	176.307	184.729	158.12	218.48		
Group 3	282.45	32.172	5.60	271.043	293.858	216.32	348.30		

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: *$p \leq 0.05$ is considered statistically significant, **$p \leq 0.001$ Highly significant.

Table 3: Mean comparison of pre and post treatment lesion depth in tests groups

Groups	**Mean**	**SD**	**Mean difference**	**Std. Error Mean**	**95% Confidence Interval of the Difference**		***t***	***p-Value***
					Lower Bound	Upper Bound		
Group 1								
Pre	278.112	29.625	129.495	5.931	117.414	141.575	21.834	<0.001
Post	148.62	9.626						
Group 2								
Pre	284.280	26.144	103.762	4.874	93.835	113.689	21.29	<0.001
Post	180.52	11.876						
Group 3								
Pre	273.911	34.531	-8.539	3.718	-16.113	-0.966	-2.297	0.028
Post	282.45	32.172						

All values are expressed as mean ± standard deviation (SD) in parentheses; Statistical test used: Paired T test; 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, **$p \leq 0.001$ Highly significant.

Table 4: Comparison of intra lesion mean difference between the groups

Groups	**Mean**	**SD**	**SE**	**95% Confidence Interval for Mean**		**Minimum**	**Maximum**	**F**	***p*-Value**
				Lower Bound	Upper Bound				
Group 1	129.495	34.069	5.931	145.20	152.03	95.36	217.74	222.176	**0.001***
Group 2	103.762	27.996	4.873	176.30	184.73	40.53	168.45		
Group 3	-8.539	21.359	3.718	271.04	293.86	-64.12	46.08		

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: *$p \leq 0.05$ is considered statistically significant, **$p \leq 0.001$ Highly significant.

Table 5: 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, **$p \leq 0.001$ Highly significant.

Table 6: Mean percentage change in lesion depth

Groups	Mean	SD	SE	95% Confidence Interval for Mean		Minimum	Maximum	F	*p*-Value
				Lower Bound	Upper Bound				
Group 1	45.938	6.873	1.196	43.501	48.376	36.75	61.68	408.256	<0.001*
Group 2	36.015	6.938	1.207	33.555	38.475	16.74	48.56		
Group 3	-3.552	8.418	1.465	-6.537	-0.567	-27.87	17.56		

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, **$p \leq 0.001$ Highly significant.

Table 7: Inter-group comparison of mean percentage change in lesion depth

(I) Factor		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group 1	2	9.923	1.833	<0.001*	5.561	14.286
	3	49.491	1.833	<0.001*	45.128	53.853
Group 2	1	-9.923	1.833	<0.001*	-14.287	-5.561
	3	39.567	1.833	<0.001*	35.204	43.929
Group 3	1	-49.491	1.833	<0.001*	-53.853	-45.128
	2	-39.568	1.833	<0.001*	-43.929	-35.205

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, **$p \leq 0.001$ highly significant.

Table 8: Mean optical densities (OD) of surviving cells of study groups at a wavelength of 570 nm

Conc	OD	Mean	% viability	Results as observed	IC 50 conc.	IC50 (µg)
NC	0.164	0.287	100	No lysis	Nil	No cell death at higher concentration
	0.439			No lysis		
	0.259			No lysis		
OBV	0.269	0.275	95.592	No lysis	47 lg/ml	
	0.245			No lysis		
	0.31			No lysis		
FV	0.154	0.217	75.522	No lysis	36 lg/ml	
	0.264			No lysis		
	0.233			No lysis		

NC=Negative control; OBE=*Ocimum Basilicum* Varnish; FV=Fluoride Varnish; OD=Optical Density; IC50=Half maximal inhibitory concentration; nm=nanometer; µg=microgram

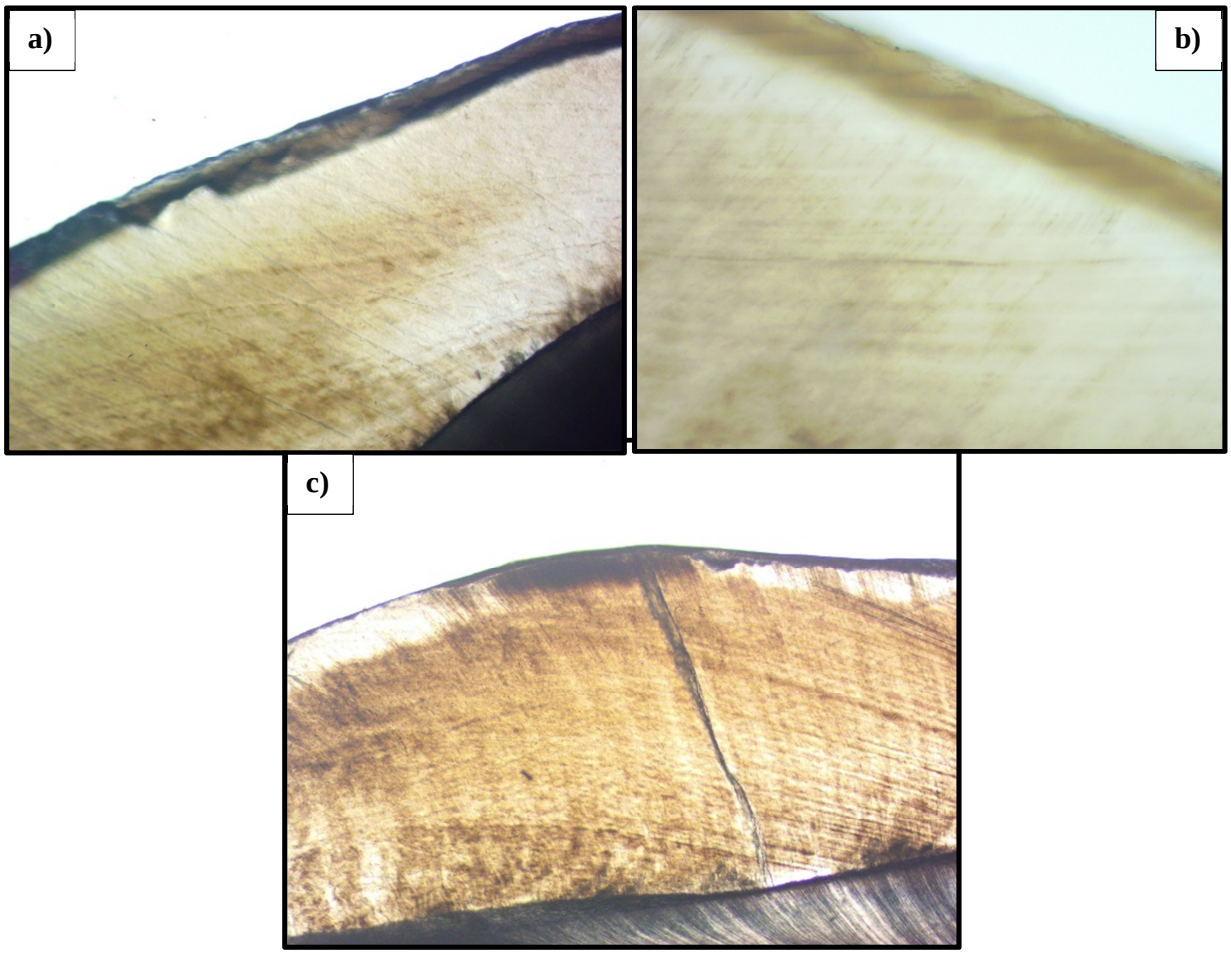
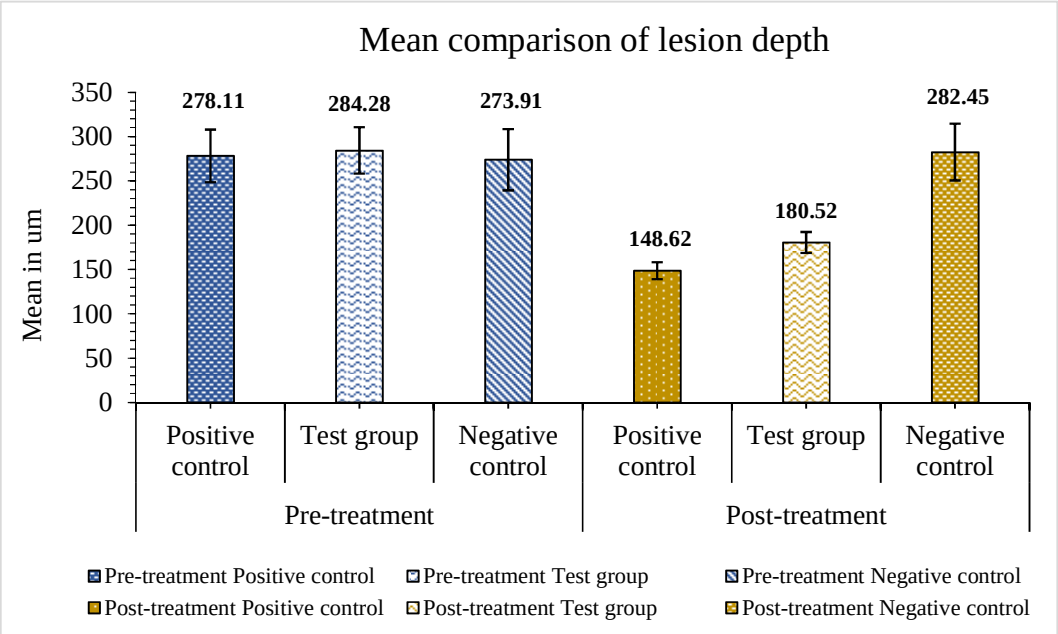


Figure 1: Light microscope 4X magnification representative sample lesion depth a) Fluoride varnish b) *Ocimum basilicum* varnish c) Placebo

Figure 2: Mean comparison of pre and post treatment lesion depth in tests groups



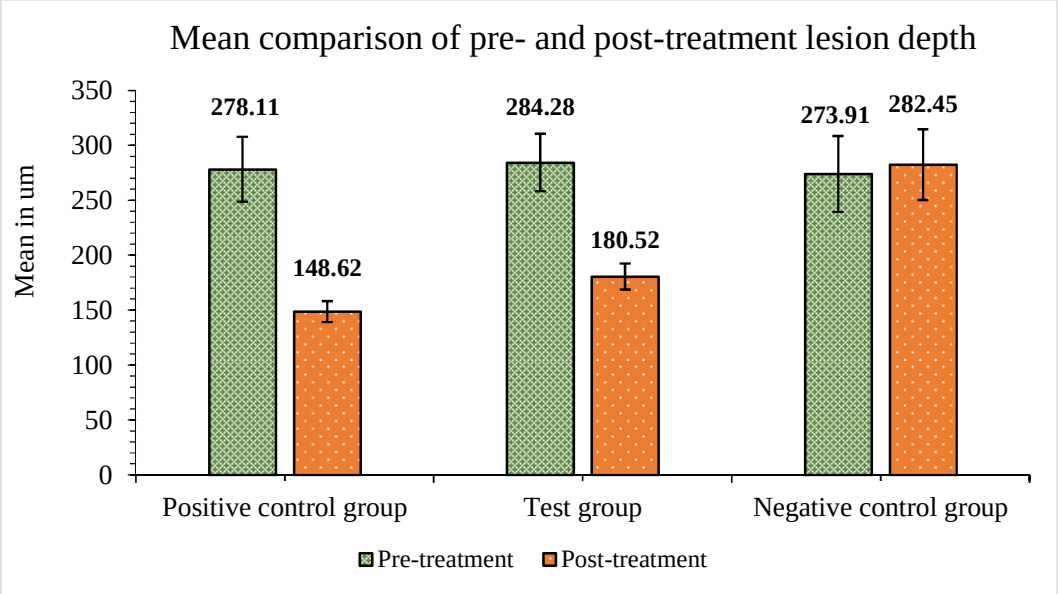


Figure 3: Mean comparison in between groups with respect to lesion depth

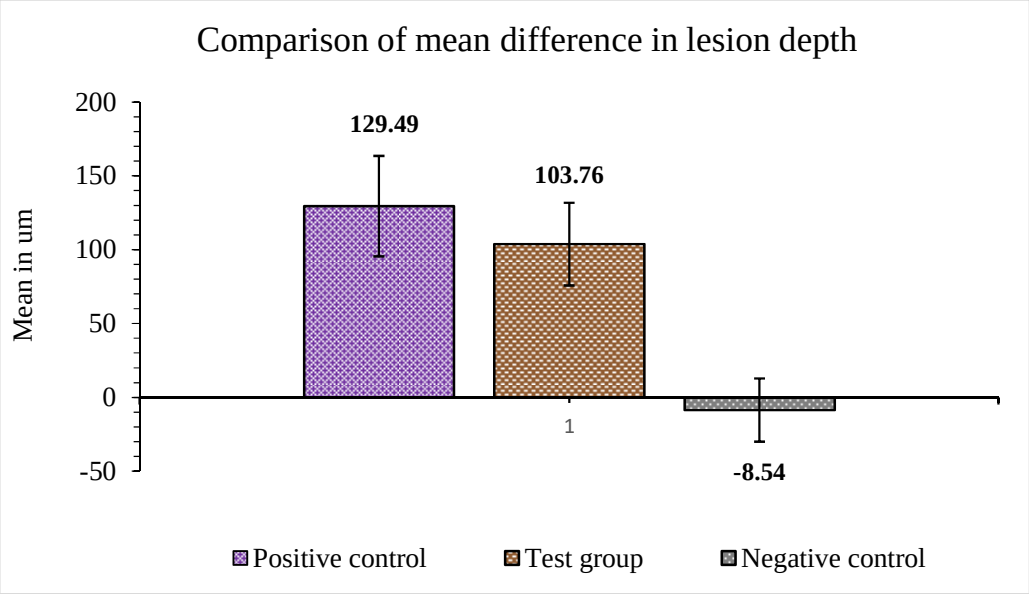


Figure 4: Comparison of intra lesion mean difference between pre- and post-treatment groups

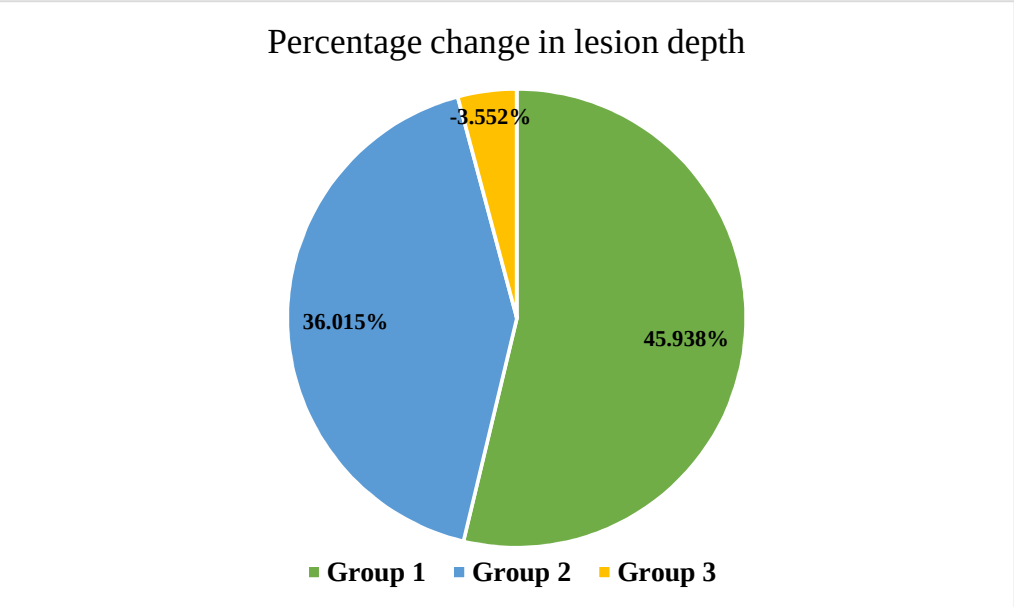


Figure 5: Percentage change in lesion depth in between the groups

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.[38] The current approach in medicine is shifting towards utilizing the ingredients derived from nature.[57] Recognizing medicinal plants becomes vital and requires development since medications generated from natural materials are thought to be generally safe and economical.[58] 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.[59]

Ocimum basilicum Linn. is a medicated herb that has traditionally been used to treat a variety of health conditions.[39] The present study investigation pertains to the aspects of oral health care. The requisites of suitable physiochemical conditions for mineral dissolution determines the onset of dental caries.[40] The diagnosis of early stages of carious lesions, as well as the use of non-invasive remineralization treatment for these lesions, has the potential to represent a substantial development in the clinical management of the disease.[41] *O. basilicum* seeds are derived from Ocimum popularly known as Tulsi which is an aromatic plant belonging to the family Lamiaceae indigenous to the Indian subcontinent and is a widely cultivated plant across the Southeast Asian tropics.[24] The plants are predominantly herbs or shrubs annuals or perennials dwell.[25] According to research, *O. basilicum* seeds exhibit antibacterial, antioxidant, and anticancer properties.[42] 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.[42-44] In the

current study, *O. basilicum* varnish demonstrated remineralizing properties. It has been established that the preservation of the supersaturated state of calcium around the tooth surface decreases demineralization and promotes remineralization.[45] 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.[29]

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 utilised.[46] These in-vitro systems are the spearhead of caries research, because they are less costly and take less time than conventional testing methods.[47] This study used an in-vitro pH-cycling model to assess the remineralization 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.[46,47] 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.[48]

The current study used standardised demineralizing solutions to construct artificial carious lesions. It is considered to be more reproducible than actual carious lesions, thus making the experimental model deemed more reliable.[49] They facilitate the examination of several lesion locations at different time periods to analyze the remineralizing phenomenon.[50] The artificial carious lesions used in this investigation

had lesion depths of 242–361 μm; replicating a carious lesion that has been active for 12 months in the oral cavity.

A single section technique was used for preparing the samples in this study, to enable the possibility of measuring the mineral changes after multiple periods of exposure to the test agents at the same site according to Exterkate *et al.,*[51]. It eliminates the issues associated with other models that involve reintroducing sections into the medium for additional examination. Although the use of the single section technique is tedious, it can provide the exact measure of de/remineralization.[52]

In the interest of standardization, all of the specimens in the current study were subjected to 30 days of pH cycling. During the pH cycle, the specimens were treated twice daily with respective agents for 4 minutes, once in the morning and later in the afternoon. This helps to mimic the real-life situations to a greater extent that occur in-vivo according to evidence obtained by Buzalaf *et al.,*[48] and Itthagarun *et al.,*[53]. "pH cycling" refers to in-vitro experimental techniques that include exposing substrates, enamel, or dentin, to alternating solutions of remineralization and demineralization.[54] These combination tests are intended to simulate the dynamic changes in mineral saturation and pH that occur throughout the caries process.[55] As stated by Ten Cate *et al.*,[11] contemporary pH cycling models have been the preferred option for many caries researchers using in-vitro techniques because give a more accurate simulation of the caries process for both mechanistic studies and for profile evaluations of varnishes.

In the current study, light microscope was used for qualitative assessment of lesions and microradiography as a quantitative measure of lesion mineralization as it shows highly defined radiolucent areas as demineralization of the lesions and radiopaque surface layer as remineralized section of the lesions. In the current

investigation, the light microscope was devised to be the appropriate approach for measuring lesion depth supported by Sadoon *et al*.,[56] reported a significant degree of discrimination between demineralized and normal areas of tooth samples using light microscopy.

It is widely known that applied fluoride combines into enamel crystals, resulting in the formation of fluorapatite crystals, which increases the enamel's capacity to resist the acid challenge.[60] A source of ions containing fluoride may be viable and aid in the maintenance of supersaturated enamel minerals. 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.,*[61] stating fluoride varnish increases the surface hardness and reduces the dissolution of enamel in acid.[61] The explanation for this might be the short contact period of fluoride varnish with enamel in our investigation. Nevertheless, fluoride varnish was administered twice to simulate a professional clinical situation. 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.[62]

In the current study, non-defective, non-carious premolar teeth were used. All the teeth were subjected to a freshly prepared demineralizing solution with a standardized pH of 4.4. All teeth were subsequently subjected to a freshly produced re-mineralizing treatment regimen with a consistent pH, with all surface treatments conducted by a single operator. Samples were subjected to 30 days of pH cycling twice daily with topical application of all the agents. Fluoride varnish and *O.*

basilicum varnish group samples revealed a significant reduction in lesion depth. This could be because of the subsurface remineralization of the white spot lesion thereby proving *O. basilicum* varnish to have a preventive role in lesion formation and lesion progression. However, samples in Placebo during the test period showed an increase in lesion depth compared to the pre-test readings. This could be because of the progression of the carious lesion as there was no protective or inhibitory effect. This study has verified the effect of *O. basilicum* varnish on tooth mineralization by reduction of lesion depth significantly. The present study demonstrated the convenience and efficacy of light microscope in lesion depth measurement.

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. The mineralisation inhibition effects of salivary proteins, pellicles and plaque were not considered. The variations in the characteristics and quantities of these factors, which vary between individuals, need standardization in all in-vitro studies. Other confounding factors involve the possibility of experimental errors and dissimilarities in the micro-structure of the enamel between specimens. Furthermore, the specimens in the pH cycle were subjected to repeated cycles of remineralization and demineralization which is more aggressive than the acid attacks that a tooth is exposed to, on a daily basis in the oral cavity. Hence, it is apparent that no in-vitro model can be a realistic substitute for the conditions that prevail in the oral cavity. However, these models provide a helpful technique of evaluating and incorporating

novel products designed to cause remineralization before they are tried in human individuals.

Recommendations

It is recommended that further controlled in-vivo studies and extensive clinical trials be conducted to ascertain the true clinical efficacy of *O. basilicum* varnish. It is critical to assess the effectiveness in lesions on various surfaces (e.g., occlusal, where most lesions develop) and with validated outcomes for remineralization. Reduction of outcome reporting and publication bias is crucial in the validation of novel agents for remineralization. Further studies are required to elaborate the mechanism of action and the clinical protocol of application *of O. basilicum* varnish for maximum efficacy. Overall, the experimentation with clinical trials of *O. basilicum* constituent in various local delivery systems is to be evaluated for its efficacy and effectiveness. Studies focusing on wide age group populations and independent groups should be a research priority.

CONCLUSION

Several remineralizing techniques have advanced greatly in recent years. Most of these treatments work by establishing steady systems capable of delivering bioavailable calcium and phosphate unswervingly to the lesion or the surrounding biofilm to encourage remineralization. One significant challenge for remineralizing therapies is to provide optimal concentration of minerals without surface precipitation which limits the efficacy of the therapy. Slow and prolonged delivery of minerals might favour subsurface mineral gain. Both groups showed increased remineralization capacity than the negative control group, as evidenced by higher mean remineralized value. Although, the intra group lesion mean depth in the fluoride varnish group was higher as compared to the *O. basilicum* varnish group, the latter has shown homogenous remineralization of enamel subsurface lesions in situ significantly slowing the progression of the lesion. Hence the new *O. basilicum* based remineralization agent appears to have potential as a non-invasive alternative to topical fluorides in the therapy of early carious lesions. Light microscopy was potent in determining demineralized and remineralized zones, and it may therefore be utilised as a diagnostic tool in diagnosing, qualitatively analysing enamel lesions, and the remineralization potential of agents. It is imperative to note that remineralisation in-vitro may not exhibit variability when compared to changes that ensue in the oral cavity in vivo. Therefore, direct extrapolations to clinical situations must be executed judiciously.

SUMMARY

Despite the fact that the frequency of illness has reduced since the introduction of fluorides, dental caries remains a serious public health concern in the majority of communities. However excess use of fluoride causes fluorosis. The focus of caries research has lately switched to the development of approaches for early identification and non-invasive treatment of carious lesions. The non-invasive treatment of early carious lesions by re-mineralization has the potential to be a major advancement in the clinical management of the disease. The remineralization process needs suitable quantities of calcium and phosphate ions. Thus, *O. basilicum* seed extract containing a relatively high amount of calcium (244% of recommended daily dose RDD) and potassium (56% RDD) is being evaluated for re-mineralizing because of its availability, feasibility and safety. A thorough literature search revealed a gap regarding the re-mineralizing potential of *O. basilicum* seeds. The present study was conducted with the aim to evaluate and compare the re-mineralizing potential of *O. basilicum* varnish and fluoride varnish.

O. basilicum seeds extract was prepared using the Soxhlet method which was vortexed with IP graded chemicals to obtain varnish. Extracted human permanent premolars were collected and distributed 33 samples in each of the three groups of fluoride varnish, *O. Basilicum* varnish and placebo (as determined by statistical sampling method). Specimens were surface polished and carved to make a 4*4 mm in diameter ‘enamel window’. Each of the samples were exposed to demineralizing solution to produce an initial enamel lesion of 242–361μm in depth in this enamel window. Then, pH cycling model was adopted to simulate the changes occurring in the oral cavity where in specimens were treated twice daily with respective

remineralizing agents for 4 minutes, once in the morning and later in the afternoon. The specimens were immersed in laboratory saliva. The pH cycling step was repeated twice daily for 30 days. The laboratory saliva was changed each day and these treatment regimens were freshly prepared for every application. Each sample was sectioned longitudinally and grinded to 0.2-0.4 mm thickness. The specimens were observed under light microscope and recorded. These images were examined using image J software for any significant changes suggesting re-mineralization. The mean value of each sample was tabulated to evaluate it for statistical analysis. The data were evaluated using ANOVA and post hoc analysis.

The mean (± SD) pre-treatment lesion depth across the groups ranged from 242.11 ± 26.144μm to 352.66 ± 34.531μm. Comparisons between pre-test lesion depths in all groups were statistically insignificant ($p = 0.380$). The reduction in mean lesion depth after pH cycling was maximum for group 1, followed by group 2 ($p<0.001$) indicative of effective remineralization post pH cycling. However, there was a considerable increase in mean lesion depth in group 3 with $p=0.028$. The percentage of recovery was calculated for all the experimental groups. The highest recovery rate of 45.938% 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%. Inter group analysis for lesion depths revealed a statistically significant difference by Tukey's post hoc analysis ($p<0.001$). The MTT assay results obtained for the *O. basilicum* varnish and the fluoride varnish control revealed it to be non-toxic to gingival fibroblast cells as the viability of cells was maintained. It was observed that the viability of the cells was more towards *O. basilicum* varnish as compared to the control group by 20%.

The *O. basilicum* varnish demonstrated homogenous remineralization of enamel subsurface lesions in situ and significantly slowed the progression of lesions. Furthermore, remineralizing efficacy has been demonstrated to be slightly lower than the fluoride varnish. Hence the novel *O. basilicum* based remineralization agent appears to have potential as a non-invasive alternative to topical fluorides in the therapy of early carious lesions.

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ANNEXURE – I - ETHICAL CLEARANCE CERTIFICATE

Research and Ethics Committee
KLE V K INSTITUTE OF DENTAL SCIENCES
KLE University
Accredited 'A' Grade by NAAC Placed in Category 'A' by MHRD (GoI)
Nehru Nagar, Belagavi - 590 010, Karnataka State
☎: 0831-2470362 Web: http://www.kledental-bgm.edu.in
FAX: 0831-2470640 E-mail:principal@kledental-bgm.edu.in

Sl. No. : 1453

CERTIFICATE

This is to Certify that the synopsis titled

Effective evaluation of remineralicing potential of Ocimum basilicum varnish and flouride varnish on initial enamel caries An invitro study

Submitted by

Dr. _____ *P. G. Student /*

Staff, Guided by _____ *from Department of*

Public Health Dentistry *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 05/05/2021

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

ANNEXURE – II – WAIVER INFORMED CONSENT FORM

Department of Public Health Dentistry

KAHER VK institute of dental sciences, Nehrunagar Belagavi

Comparative evaluation of re-mineralizing potential of *Ocimum basilicum* varnish and fluoride varnish on initial enamel caries: An In-vitro study

Waiver of informed consent form

It is not feasible to obtain individual informed consent of donors of specimens used in this study. However, I assure that confidentiality of the participant information will be ensured and no identifying information related to study participants will be disclosed in any report/ publication arising from the study.

Postgraduate
Department of Public
Health Dentistry
KLE VKIDS

Professor and head
Department of Public
Health Dentistry
KLE VKIDS

ANNEXURE – III – AUTHENTICATION

राष्ट्रीय पारम्परिक चिकित्साविज्ञान संस्थान
ICMR-NATIONAL INSTITUTE OF TRADITIONAL MEDICINE
(भूतपूर्वक्षेत्रीयआयुर्विज्ञानअनुसंधानकेन्द्र**Formerly Regional Medical Research Centre)**
Nehru Nagar, Belagavi-590 090

Dr. Harsha Hegde
Scientist-E
harshah@icmr.gov.in

भारतीयआयुर्विज्ञानअनुसंधानपरिषद
INDIAN COUNCIL OF MEDICAL RESEARCH
स्वास्थ्यअनुसंधानविभाग, स्वास्थ्यऔरपरिवारकल्याणमंत्रालय, भारतसरकार
Department of Health Research,
Ministry of Health & Family Welfare, Govt. of India

Date: 10-03-2021

AUTHENTICATION

This is to authenticate that the plant material brought by , I year MD, Department of Public Health Dentistry, KLE VK Institute of Dental Sciences, Belagavi, is identified as ***Ocimum basilicum* L.** belonging to family Lamiceae. The herbarium specimen of the same has been deposited in our herbaria with accession number RMRC-1630.

Harsha Hegde
Scientist-E

ANNEXURE – IV – PHYTOCHEMICAL SCREENING

SHRI B M KANKANAWADI AYURVED MAHAVIDYALAYA
A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)
(Re-Accredited 'A' Grade by NAAC (2nd Cycle) || Placed under Category 'A' by MHRD GoI)
CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Outward No:-BMK/CRF/ /2021-22

Reference No:CRF/RM/12/2021-22
Submitted by:Dr.Atreya J. Pai Khot
Sample : *Ocimum basilicum*
Product : Plant
(* N/A - Not Available)

Batch No. : NA
Sample Qty : 10gm

Registration Dt:-02/01/2021
Requisition no :----
Part/Form: Seed
Report Date :16/01/2021

TEST REPORT

Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Preliminary Phytochemical Screening:

TESTS	WATER
Test for Carbohydrates	Positive
Test for Flavonoids	Positive
Test for Alkaloids	Positive
Test for Tannins	Negative

ANALYST

Shri B. M. Kankanwadi Ayurved Mahavidyalaya • K.L.E. University
CRF
SHAHAPUR, BELGAUM

AUTHORISED SIGNATORY

ANNEXURE – V – DATA COLLECTION PROFORMA

Department of Public Health Dentistry
KAHER VK institute of dental sciences, Nehrunagar Belagavi
Effective evaluation of re-mineralizing potential of *Ocimum basilicum* varnish and fluoride varnish on initial enamel caries: An In-vitro study

Data Collection Proforma

Sr No	Sample No	Date of sample demineralization	Date of sample re-mineralization	Depth of the lesion obtained on image J software in units
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
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26				
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28				
29				
30				
31				
32				
33				

ANNEXURE – VI – PERMISSION LETTER FOR VARNISH PREPARATION

From,

27/3/2021

Postgraduate,
Department of Public Health Dentistry,
KLE VK Institute of Dental Sciences,
Belagavi

To,

The Principal
KLE College of Pharmacy
Belagavi

Respected Sir/Madam,

(Through proper channel)

Subject: Permission for preparation of extract and varnish in the Department of Pharmaceutics

, I[st] year postgraduate student of the Department of Public Health Dentistry in KLE VKIDS Belagavi, am conducting a research for my thesis on; "**Comparative evaluation of re-mineralizing potential of *Ocimum basilicum* varnish and fluoride varnish on initial enamel caries: An in-vitro study**" under the guidance of Professor and Head, department of Public Health Dentistry, KLE VKIDS, Belagavi. I would like to request you to permit me to prepare an extract and varnish under the supervision and able guidance of in the department of Pharmaceutics. This is an integral part of research study, wherein I will take responsibility of procuring all the necessary chemicals required for the same.

Kindly grant me permission for conducting the aforementioned procedures.

Thanking You

Postgraduate
Department of Public Health Dentistry
KLE VKIDS, Belagavi

Dr. Anil V. Ankola MDS
Professor & Head
Department of Public Health Dentistry
KLE VKIDS, Belagavi

Professor and Head
Dept. of Public Health Dentistry
KLE VK Institute of Dental Sciences
Nehru Nagar, Belagavi-58

ANNEXURE – VII – DEPARTMENT PERMISSION LETTER FOR OUTCOME ASSESSMENT

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH

(KLE UNIVERSITY'S) KLE VK INSTITUTE OF DENTAL SCIENCES, BELAGAVI

Date: 16/12/2020

To,
Head of Department
Department of Oral and Maxillofacial Pathology and Microbiology
KLE VK Institute of Dental Sciences
Belagavi, Karnataka

From

I[st] year MDS
Department of Public Health Dentistry
KLE VK Institute of Dental Sciences
Belagavi, Karnataka

(Through proper channel)

Subject: Permission to conduct ground sectioning of teeth and microscopic analysis in your Department

Respected Ma'am

(I[st] MDS, Dept. of Public Health Dentistry) would like to request you to allow me to conduct groundsectioning of teeth and microscopic analysis in your departmentfor my dissertation study-**"Comparative evaluation of re-mineralizing potential of *Ocimum basilicum* varnish and fluoride varnish on initial enamel caries: An in-vitro study"** under the guidance of in your department.

Kindly grant me the permission for the same.

Thanking you in anticipation

Yours Sincerely

Dr.Anil V. AnkolaMDS
Professor & HOD
Department of Public
Health Dentistry ,
KLE VKIDS

Reader
Department of Public
Health Dentistry
KLE VKIDS

Post graduate
Department of Public
Health Dentistry
KLE VKIDS

ANNEXURE – VIII – SPECIFICATION DATA SHEET OF ARTIFICIAL SALIVA

NANOCHEMAZONE INC.

An ISO 9001:2015 Certified Company
Manufacturer of Nanomaterials, Micropowders & Specialty Chemicals
www.nanochemazone.com

SPECIFICATIONS DATA SHEET

Product Name	**Artificial Saliva**	Batch Number	NCZBS-0920B
Pack Size	100 mL to 1000 ml	Mfg. Date	Sept-2020
Solubility	Miscible in Water as Aqueous solution	Product Number	NCZ-APS-0012
pH	6.8 ± 0.1 @25 ± 0.4°C (customizable)		
CAS No.	7732-18-5		
Boiling Point	100 deg C		
Density	1.05		
Color	Colorless		
Odor	Odorless		
Stabilizer	Customizable		

Primary Application: Research and product testing.

Components(customizable)

pH = 6.8 ± 0.05 @ 25 ± 0.4 °C (Y/N) **Y**

CAS: 7447-40-7 Potassium Chloride **<0.5%**

CAS: 7778-77-0 Potassium Phosphate **<0.1%**

CAS: 333-20-0 Potassium Thiocyanate **<0.05%**

CAS: 57-13-6 Urea **<0.05%**

CAS: 7647-14-5 Sodium Chloride **<0.05%**

CAS: 7732-18-5 Water, distilled water, deionized water **99-100%**

Amylase Enzyme: Present(customizable)

Storage Conditions: store at 8 ° to 25° C, For Prolong stoatge keep at -20° C

Shelf-life	**Six Months (without Stablizer), Two years (with Stabilizer)**
Condition	ready-to-use solution

Remarks: *Sample complies as per the above specification.*

NANOCHEMAZONE INC.
38 Truesdale Cres, Guelph, Ontario, Canada N1G 5H3
Contact: +1-365-888-7013 (Canada); +49-15215183436 (Germany)
Email: contact@nanochemazone.com
www.nanochemazone.com