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Evaluation of Toxic Effects of Toothpaste Formation with Moringa Oleifera Purple Bamboo Sea Salt and 9x Bamboo Sea Salt in Animal Model

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ABSTRACT

Introduction: *The demand for natural health products is rising, with Natural Wellness toothpaste being popular for its benefits. Teeth whitening can be done professionally or at home, addressing extrinsic stains caused by substances like coffee and tobacco. Manufacturers innovate to meet the high demand for effective oral care solutions.*

Aim: *To develop a natural toothpaste combining Moringa oleifera, purple bamboo sea salt, and 9x bamboo sea salt, aiming to leverage their antibacterial, anti-inflammatory, and mineral-rich properties for enhanced oral health.*

Materials and Method: *The toothpaste blend includes fluoride for strength, silica for abrasion, glycerin for moisture, sorbitol/xylitol for sweetness, SLS for foaming, xanthan gum for texture, titanium dioxide and hydrogen peroxide for whitening, flavour extracts, preservatives, dicalcium phosphate, sodium saccharin for freshness, and antimicrobial bamboo salts.*

Results: *This study assessed the antibacterial activity of 9x Bamboo Sea Salt and Moringa Oleifera Purple Bamboo Sea Salt against Lactobacillus sp., S. mutans, and E. faecalis, showing significant inhibition zones. The paste showed no cytotoxic effects on mouse fibroblast cells (3T3-L1) across tested concentrations and exhibited no significant histological changes in liver, spleen, kidney, and lung tissues.* ***Conclusion:*** *Toothpaste with Moringa oleifera Purple Bamboo Sea Salt and 9x Bamboo Sea Salt shows strong antimicrobial properties against S. mutans and Lactobacillus sp., is non-toxic to mouse fibroblast cells, and causes no histopathological changes in major organs. This formulation is a safe and effective alternative to conventional toothpaste.*

Keywords: Tooth whitening, Dental discoloration, Oral bacteria, Moringa oleifera, Purple bamboo salt, 9x bamboo sea salt toothpaste formulation

INTRODUCTION

A recent surge in the demand for natural health and wellness goods can be attributed to the growing recognition of the potential benefits of holistic approaches to healthcare. Among them, Natural Wellness toothpaste has garnered attention due to its natural components, which are purported to enhance overall health and vitality.

Tooth whitening has been used in dentistry for many centuries as a conservative and successful way to lighten stained teeth.Teeth can be professionally whitened at a dentist's office by scaling and polishing, bleaching, or utilising prosthetic crowns. Alternatively, the patient can whiten their teeth at home with over-the-counter whitening toothpaste.(1)

The natural colour of permanent teeth is mostly dictated by the dentine, which is influenced by the thickness and translucency of the underlying enamel. Teeth discoloration can result from different pigments being deposited into or onto the tooth(2).A considerable number of toothpastes are available as tooth whitening products. Most appear to contain ingredients that might remove extrinsic stains rather than change natural tooth colour. Extrinsic stain removal could be achieved by physical or chemical means.

Extrinsic discoloration is the term for surface stains on teeth that are often brought on by smoking, drinking coffee or tea, while intrinsic discoloration, such dental fluorosis or teeth stained by tetracycline, is produced by the integration of chromophores into enamel and dentin.(3)

In order to satisfy patients' and consumers' high standards, manufacturers of oral care products are always creating new and improved methods for teeth whitening. As a result, a vast array of product types and methods are currently offered on the market to address the issue of tooth discolouration.(4)

Dental health is a priority for researchers to this day. Health deteriorates as a result of the several illnesses that might affect the teeth(5) The development of different types of bacteria in the oral and dental regions is the cause of dental infections(6). These include anaerobic bacteria including Porphyromonas gingivalis,

Enterococcus faecalis, and Candida albicans(7),(8)that cause periradicular periodontitis and odontogenic infections in the root canal system.Up to 30–80%(9) of root canals are reportedly infected with E. faecalis.Furthermore, a significant incidence of root canal infections was revealed by polymerase chain reaction (PCR) research to be caused by the bacteria Tannerella forsythia, Treponema denticola, Dialister pneumosintes, and Prevotella tannerae.These infections are brought on by bacteria forming biofilms on the tooth surface.(10)

A member of the Moringaceae family, Moringa oleifera is a fast-growing, drought-resistant tree native to the Indian subcontinent.(11)The plant Moringa offers an exceptional blend of kaempferol, β-sitosterol, caffeoylquinic acid, zeatin, and quercetin.The therapeutic efficacy of M. oleifera makes it extremely significant. The leaves, roots, seeds, bark, fruit, flowers, and immature pods of this plant have antitumor, antipyretic, antiepileptic, anti inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol-lowering, antioxidant, hepatoprotective, antibacterial, and antifungal activities(12). These parts of the plant are used in traditional medicine, especially in South Asia, to treat a variety of illnesses.(13)

Purple bamboo salt is a uniquely prepared salt made from regular salt and bamboo, following a traditional formula. In Korea, it has been applied to the treatment and prevention of a number of illnesses. Using human mast cell line (HMC-1) as a model, we examined the anti-inflammatory properties of purple bamboo salt.(14).Bamboo salt (BS) has been used to lessen the harmful coolness of salts and has recently demonstrated a number of medicinal benefits.(15)The researchers observed that bamboo salt has antioxidant and anti-inflammatory properties and that it may be utilised as a bamboo salt toothpaste to treat dental conditions and maintain good oral hygiene.(16) The most prevalent type of cancer in the mouth cavity is buccal mucosa carcinoma.It has been demonstrated that the bamboo salt increases the anti-cancer and anti-metastatic effects in mice.(17)(18).When dental patients gurgle with a bamboo solution, their degree of enamel hardness is much higher and their mineral loss is lower than when they gurgle with ordinary water, according to studies. Toothpaste with Bamboo Salt helps to decrease the development of dental plaque and lowers the risk of gingivitis.(19)

The development of HCT-116 cells was suppressed by 53% by a 1% salt concentration of bamboo salt baked nine times (9×). This was more than the effects of salt baked three times (3×) or once (1×; 44% and 41%, respectively), and significantly greater than the effects of solar sea salt (Korean sea salt) and purified salt (22% and 18%, respectively).Bamboo salt (9×) upregulated Bax, caspase-9, and caspase-3, and downregulated Bcl-2, causing cancer cells to undergo apoptosis ($P<.05$.(20)The 9× bamboo salt had the most antioxidant components and properties when compared to the other salts, as determined by mineral element concentration, pH, OH groups, and redox potential amperometric analysis. The in vitro findings demonstrated that the 9× bamboo salt had a greater inhibitory impact than the other salt when it came to oxidative damage caused by hydrogen peroxide (H_2O_2) treatment in HaCaT keratinocytes.(20,21)

This study aims to develops a natural toothpaste combining Moringa oleifera, purple bamboo sea salt, and 9x bamboo sea salt, aiming to leverage their antibacterial, anti-inflammatory, and mineral-rich properties for enhanced oral health.

MATERIALS AND METHODS:

This Toothpaste Blend Features Fluoride for Strength, Silica for Gentle Abrasion, Glycerin for Moisture, Sorbitol/Xylitol for Sweetness, SLS for Foaming, Xanthan Gum for Texture, Titanium Dioxide, Hydrogen peroxide for Whitening, Flavorful Extracts, Preservatives, Dicalcium Phosphate, Sodium Saccharin for Freshness, for Antimicrobial Action, and the Unique Touch of Purple Moringa Oleifera Bamboo Salt and 9x Bamboo Salt.

PREPARATION OF SALT SOLUTION:-

9X Bamboo sea salt (9 times processed sea salt) , 2) SS-M (Moringa.oleifera Purple bamboo Sea salt) 0.5 gm of all the salt samples are weighted in separate sterile containers and mixed with 1 ml of Distilled water.

ANTIMICROBIAL ACTIVITY:-

Antimicrobial Activity of respective salt samples against the bacteria's, E. faecalis, S. Mutans, and Lactobacillus sp. . MHA agar was utilised for this activity to determine the zone of Inhibition .Muller Hinton agar was prepared and sterilised for 45 minutes at 120 lbs . Media poured into the sterilised plates

and let it stable for solidification. The wells were cut using the well cutter and the test organisms were swabbed. The salt samples were loaded and the plates were incubated for 24 hours at 37 deg c . After the incubation time zone of inhibition was measured.

INGREDIENTS:-

S.No	List of Ingredients	By Weight
1	Sodium Fluoride	0.1-1 Gm
2	Silica	4-7gm
3	Sorbitol /Xylitol	15-25gm
4	Glycerin	15-25gm
5	Sodium Lauryl Sulphate	1-2gm
6	Xanthan Gum	1-3gm
7	Titanium Dioxide	0.5-1.5gm
8	Peppermint Oil	1-2gm
9	L-Menthol	1-2gm
10	Sodium Benzoate	0.1-0.5gm
11	Di Calcium Phosphate	4-7gm
12	Sodium Saccharine	1-3gm
13	Hydrogen Peroxide	1-3gm
14	Strontium Chloride	3-7gm
15	Sodium Pyrophosphate (Sapp)	1-3gm
16	Citric Acid	1-2gm
17	9x Bamboo Sea Salt	5-10gm
18	9x Moringa Olie Purple Bamboo Sea Salt	3-5gm
19	Aqua	Qs

1. **Sodium Fluoride**: This ingredient helps prevent tooth decay by strengthening tooth enamel and making it more resistant to acids from plaque bacteria and sugars.
2. **Aqua (Water)**: Serves as a solvent to dissolve other ingredients and give the toothpaste its desired consistency.
3. **Silica**: Acts as a mild abrasive to help remove plaque and surface stains from teeth.
4. **Glycerin**: Functions as a humectant to prevent the toothpaste from drying out and helps maintain moisture in the product.
5. **Sorbitol**: Another humectant that adds sweetness to the toothpaste and helps keep it moist.
6. **Sodium Lauryl Sulphate (SLS)**: Creates foam to aid in distributing the toothpaste throughout the mouth and between teeth. It also acts as a detergent to assist in cleaning teeth.
7. **Xanthan Gum**: Acts as a binder and thickener to stabilize the toothpaste and give it the desired texture.
8. **Titanium Dioxide**: Provides whitening and opacity to the toothpaste formula.
9. **Flavouring Agent**: Adds taste to the toothpaste, typically to make it more pleasant for users.

10.**L-Menthol**: Provides a cooling sensation and contributes to the flavor and breath-freshening properties of the toothpaste.
11.**Sodium Benzoate**: Acts as a preservative to prevent microbial growth and maintain the stability of the toothpaste.
12.**Di Calcium Phosphate**: Another mild abrasive that helps in cleaning teeth and polishing them.
13.**Sodium Saccharine:** Adds sweetness to the toothpaste.
14.**Hydrogen Peroxide**: Can be included for its whitening properties, helping to lighten teeth stains.
15.**Strontium Chloride:** Sometimes used for its desensitising properties to reduce tooth sensitivity.
16.**Sodium Acid Pyrophosphate**: Often included to help prevent tartar buildup and maintain whiteness of teeth.
9X Bamboo Sea Salt, 9X Moringa Oleifera Purple Bamboo Sea Salt: These specialised salts likely provide minerals and potentially additional oral health benefits, though specific details would depend on the formulation and manufacturer's intentions.
Each of these ingredients plays a specific role in cleaning, protecting, and enhancing the toothpaste, ensuring it effectively cleans teeth, freshens breath, and maintains oral hygiene.

RESULT

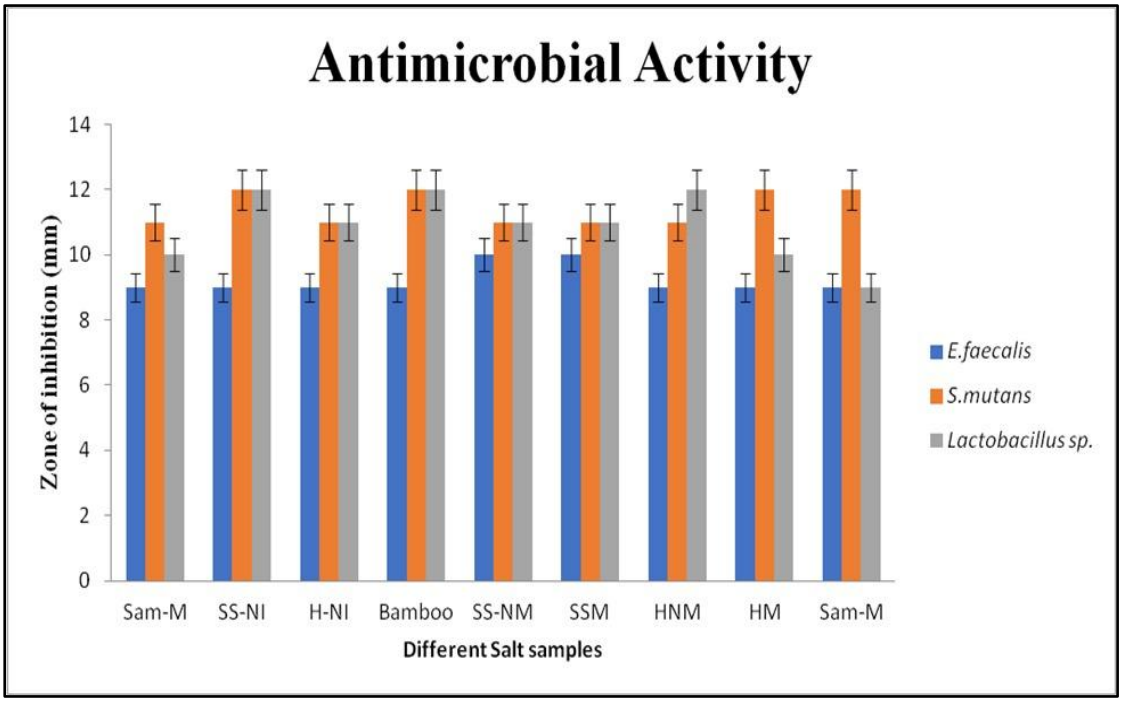


Fig 1. Antibacterial effects of different salt samples.

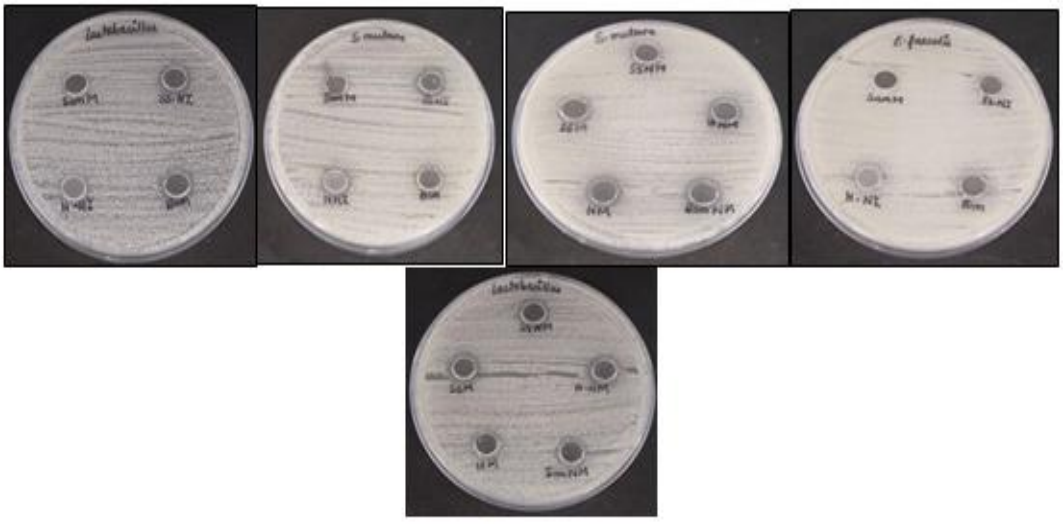


Fig 2. Agar plates showing zone of inhibition of different salts.

S.No	Sample Salt Name	E.Faecalis	S.Mutans	Lactobacillus Sp.
1.	9x Bamboo sea salt (9 times processed sea salt)	9	12	12
2.	SS-M (M.oleifera Purple bamboo Sea salt)	10	11	11

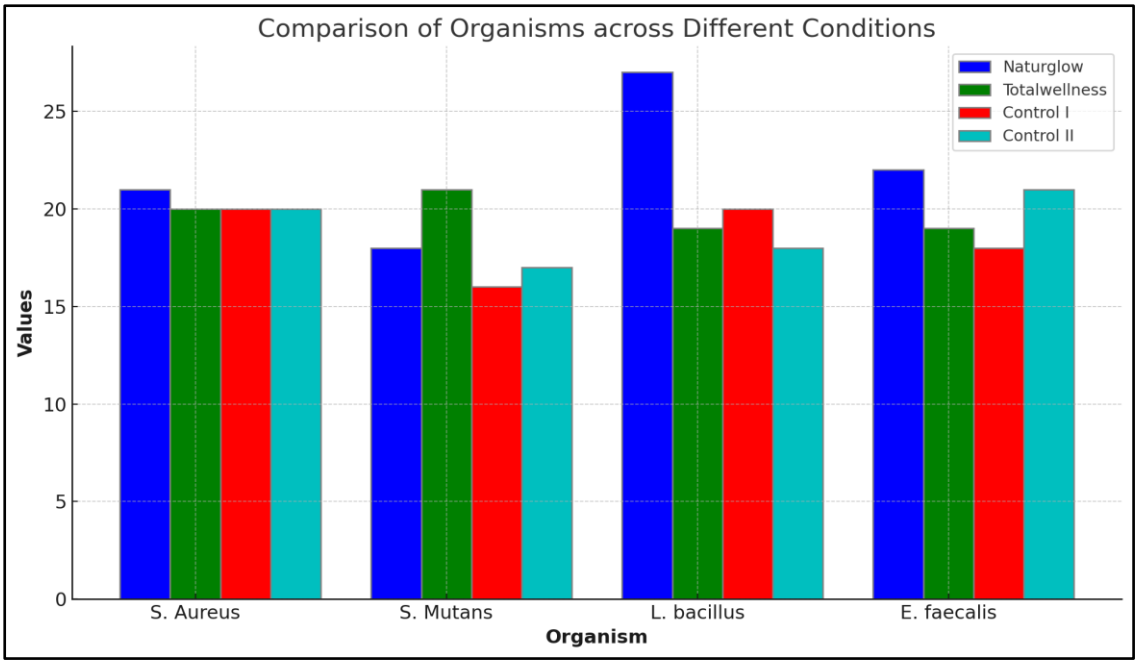
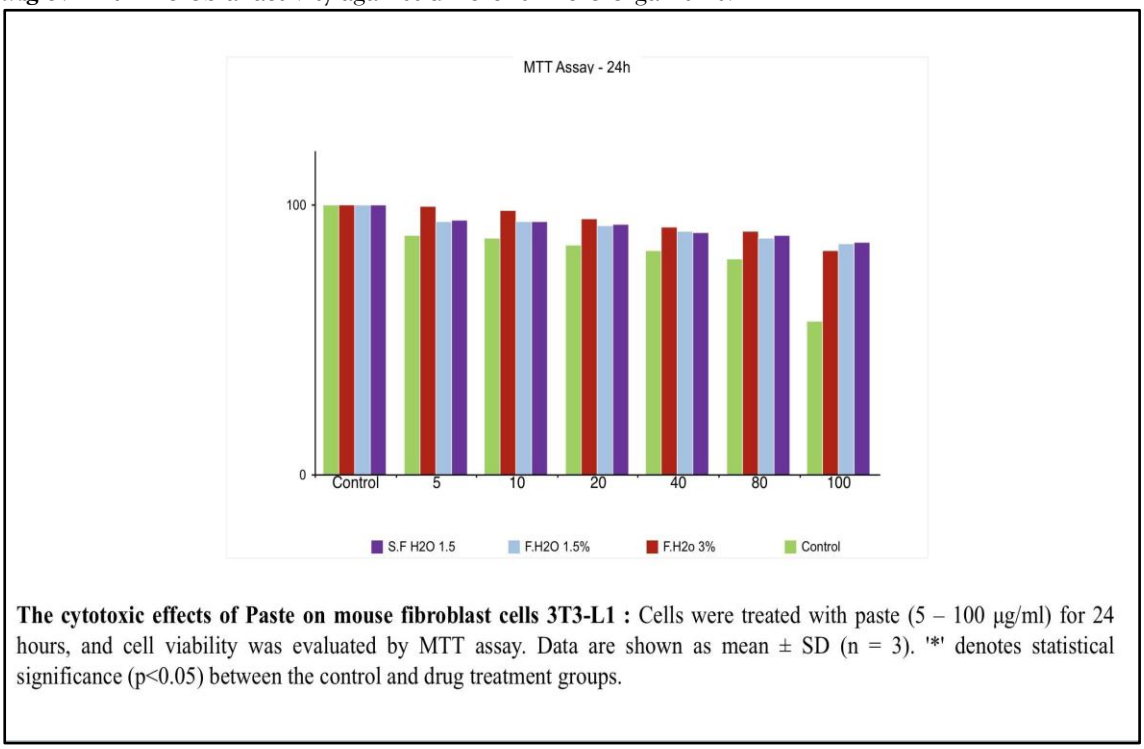


Fig 3. Anti microbial activity against different micro organisms.



The cytotoxic effects of Paste on mouse fibroblast cells 3T3-L1 : Cells were treated with paste (5 – 100 µg/ml) for 24 hours, and cell viability was evaluated by MTT assay. Data are shown as mean ± SD (n = 3). '*' denotes statistical significance ($p<0.05$) between the control and drug treatment groups.

Fig 4. MTT assay for control and experimental toothpaste.

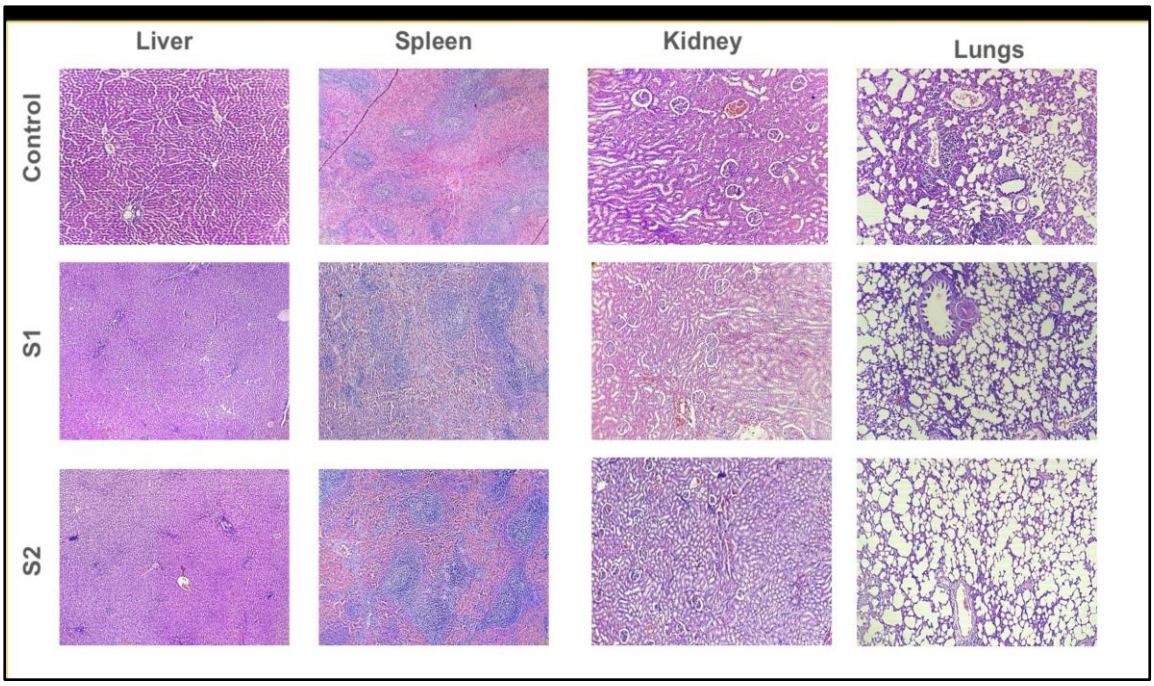


Fig 5. Histological analysis of Liver, spleen, kidney and lungs.

DISCUSSION

The antibacterial activity of several salt samples, such as 9x Bamboo Sea Salt and Moringa Oleifera Purple Bamboo Sea Salt, was assessed in this study against Lactobacillus sp., S. mutans, and E. faecalis. According to our results, both salt samples showed appreciable zones of inhibition against every bacterial strain that was examined. Particularly, the 9x Bamboo Sea Salt displayed inhibitory zones measuring 9 mm for E. faecalis and 12 mm for Lactobacillus sp. and S. mutans. Likewise, the Moringa Oleifera Purple Bamboo Sea Salt demonstrated inhibitory zones measuring 10 mm for E. faecalis and 11 mm for S. mutans and Lactobacillus sp. The Cytotoxic Effects of Paste on Mouse Fibroblast Cells (3T3-L1),the MTT assay results indicated that the paste, at concentrations ranging from 5 to 100 μg/ml, does not significantly affect the viability of 3T3-L1 cells after 24 hours of treatment. This suggests that the paste, regardless of its composition (S.F H2O 1.5%, F.H2O 1.5%, F.H2O 3%), exhibits no cytotoxic effects on the mouse fibroblast cells within the tested concentration range. The consistent cell viability close to the control group across all concentrations implies the potential safety of the paste for applications involving similar cell types. The absence of significant differences ($p<0.05$) between the treated groups and the control underscores the paste's biocompatibility under the conditions tested.The histological examination of liver, spleen, kidney, and lung tissues from the control and treated groups (S1 and S2) revealed no significant differences in tissue architecture and cellular morphology. The treatment groups included exposure to hydrogen peroxide, 9x moringa oleifera purple bamboo salt (sea salt), and 9x bamboo salt (sea salt). The lack of observable histological changes suggests that these treatments do not induce notable tissue damage or alterations at the microscopic level.

Over the years, several innovative toothpaste formulations have been released, and toothpaste has been used extensively for mechanical plaque management in conjunction with toothbrushes for many years. Dentifrice compositions have been changed to suit demands for better dental health care and to accommodate additional features such flavour modifications, reduced dentin hypersensitivity, less plaque buildup, increased fluoride, and tooth brightness(22).Toothpaste using purple bamboo sea salt, 9x bamboo sea salt, and Moringa oleifera is explored in this book. Moringa coagulant protein (MCP), one of the components of MO, flocculates microorganisms by adsorption and charge neutralisation (23). These components were chosen because they may have positive effects on dental health, such as being anti-inflammatory, antimicrobial, and remineralizing. The chance for delayed defensive mechanisms, such as oxidative radical scavenging and mucosal layer repair, to be implicated was created by the five days of treatments with evaluated extracts. Polyphenolic chemicals may be found in large quantities in the leaves, flowering tissues, and seeds of Moringa plants(24) .Despite the study's promising results, there are several limits to take into account. The complex environment of the human oral cavity is not perfectly replicated by the in vitro nature of antimicrobial tests. Clinical trials to assess the effectiveness and safety

of these salt-infused toothpaste formulations should be a part of future research.These results align with the antimicrobial properties of extracts from Moringa Oleifera and bamboo salt that have been studied (25). Higher inhibition zones for S. mutans and Lactobacillus.sp indicate that these salts would be especially useful against probiotic and cariogenic bacteria, which are essential for maintaining dental health (26).Our findings, which show significant antimicrobial activity against common oral infections, further complement these findings. Our study's comparison of several salt samples offers a broader view of how it could be utilised in oral health products.

The "miracle tree," Moringa oleifera, has drawn interest for its therapeutic and nutritional qualities (27).Moringa extract, which is abundant in vitamins, minerals, and antioxidants, has been investigated for its potential to reduce inflammation and fight oral infections. Nowadays, not just in Korea but in many other Asian nations as well, bamboo salt is among the most well-known traditional medicinal therapies. In traditional medicine, brushing with bamboo salt has been used to treat periodontal disease, toothaches, and foul breath. Compared to solar and refined salts, bamboo salts have greater concentrations of potassium, calcium, magnesium, and iron(28).Furthermore, longer-baked bamboo salt has a higher mineral content than solar and filtered salts. Increasing the amounts of these minerals in the salt is crucial to boosting its anti-inflammatory and antioxidant properties(29). Prior research has shown that the combined application of bamboo salt and sodium fluoride solution boosted tooth surface strength and may have partially remineralized teeth (30)(30,31). These toothpaste formulas represent a major breakthrough in oral care products because of their dual action, which provides both antibacterial and tooth-whitening properties (32,33). These formulas improve dental health and oral hygiene by drastically lowering dangerous microorganisms and stimulating tooth whitening (34). Also appealing to customers looking for safer, more environmentally friendly toothpaste substitutes is their natural composition(35)

CONCLUSION

This study demonstrates that incorporating Moringa oleifera Purple Bamboo Sea Salt and 9x Bamboo Sea Salt into toothpaste formulations offers significant antimicrobial properties, particularly against S. mutans and Lactobacillus sp. The MTT assay results indicate that these formulations are non-toxic to mouse fibroblast cells, maintaining high cell viability across a range of concentrations. Furthermore, histopathological evaluations reveal no significant differences in tissue architecture or cellular morphology in liver, spleen, kidney, and lung tissues, confirming the safety and efficacy of these toothpaste formulations. By effectively reducing bacterial growth and enhancing overall oral hygiene and dental health, these natural salt-based toothpaste formulations represent a valuable advancement in oral care products. They offer a promising, safe, and effective alternative to conventional toothpaste, contributing to improved dental health and hygiene practices.

FUTURE SCOPE

Future research should include extended toxicological studies to ensure long-term safety of Moringa oleifera Purple Bamboo Sea Salt and 9x Bamboo Sea Salt. Mechanistic studies can elucidate their antimicrobial properties. Clinical trials are needed to validate efficacy in humans, while comparative studies with commercial toothpaste will highlight their benefits. Exploring antimicrobial effects on diverse pathogens and optimising formulations can enhance efficacy. In vivo studies will assess their impact on oral health. Developing additional products like mouthwashes can expand their use. Regulatory approval and consumer education initiatives will facilitate market introduction and public awareness of these natural alternatives, ultimately leading to safer and more effective oral hygiene solutions.

CONFLICT OF INTEREST

There is no conflict of interest

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