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Formulation And Evaluation Of Betel Leaf Powder For Enamel Hardness

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ABSTRACT

The quest for novel dental biomaterials to enhance enamel hardness and combat dental caries remains a pressing concern in dental research. Betel leaf (Piper betle L.) has long been recognized for its therapeutic properties in traditional medicine. This study aimed to explore the potential of betel leaf powder (BLP) as a natural agent for improving enamel hardness. In this experimental study, extracted human teeth were utilized as the substrate for testing. Betel leaf powder was obtained through standardized preparation methods and incorporated into various experimental groups. Enamel hardness measurements were conducted using Vickers microhardness testing before and after treatment with BLP. Statistical analysis was performed to evaluate the significance of observed changes. Preliminary findings indicate a notable enhancement in enamel hardness following treatment with BLP. The results suggest that BLP holds promise as a potential dental biomaterial for strengthening enamel and preventing dental caries. Moreover, the natural origin of BLP underscores its appeal for incorporation into dental products, offering a sustainable and eco-friendly alternative to synthetic materials. Further research is warranted to elucidate the mechanisms underlying the observed effects of BLP on enamel hardness and its potential applications in preventive and restorative dentistry. Additionally, investigations into the safety profile and long-term efficacy of BLP-based formulations are imperative to facilitate its translation into clinical practice.

Keywords: Betel leaf powder, enamel hardness, dental biomaterials.

INTRODUCTION

In the realm of dental health, the quest for effective strategies to prevent dental caries and enhance enamel hardness has been an ongoing pursuit. Enamel, the outer layer of teeth, plays a pivotal role in protecting the underlying dentin and pulp from bacterial invasion and mechanical damage. However, factors such as poor oral hygiene, dietary habits, and genetic predisposition can compromise enamel integrity, leading to dental caries and other oral health issues.

Traditional medicine systems across the globe have long recognized the therapeutic properties of natural substances in promoting oral health. One such natural remedy that has garnered attention in recent years is betel leaf (Piper betle L.). Belonging to the Piperaceae family, betel leaf is renowned for its antimicrobial, anti-inflammatory, and antioxidant properties. Its widespread use in traditional medicine, particularly in Southeast Asia, underscores its potential as a promising candidate for dental applications.

Betel leaf contains a myriad of bioactive compounds, including phenols, tannins, alkaloids, and essential oils, which contribute to its pharmacological properties. These compounds exhibit antibacterial activity against oral pathogens, anti-inflammatory effects on gingival tissues, and antioxidative properties that combat oxidative stress-induced damage in the oral cavity. Moreover, betel leaf has been traditionally used in various forms, such as chewing, topical application, and as an ingredient in herbal preparations, highlighting its versatility in oral care practices.

The rationale behind exploring betel leaf as a potential agent for enhancing enamel hardness lies in its multifaceted therapeutic effects. Strengthening enamel hardness is paramount in preventing dental caries, as harder enamel is more resistant to demineralization caused by acidic byproducts of bacterial metabolism. Furthermore, enhancing enamel hardness can mitigate the need for invasive restorative procedures, preserving tooth structure and promoting long-term oral health.

While synthetic materials such as fluoride-based compounds and resin-based sealants have been conventionally used to enhance enamel hardness, concerns regarding their safety, environmental impact, and long-term efficacy have prompted researchers to explore natural alternatives. Betel leaf, with its rich pharmacological profile and historical use in oral care, presents an intriguing avenue for investigation.

Previous studies have demonstrated the antimicrobial efficacy of betel leaf extracts against common oral pathogens such as Streptococcus mutans and Porphyromonasgingivalis, highlighting its potential in preventing dental caries. However, limited research has been conducted on the effects of betel leaf on enamel hardness, particularly in its powdered form. Understanding the impact of betel leaf powder (BLP) on enamel hardness could provide valuable insights into its potential as a natural dental biomaterial.

This study aims to bridge this gap in knowledge by investigating the effects of betel leaf powder on enamel hardness through experimental analysis. By elucidating the mechanisms underlying the interaction between BLP and enamel, this research seeks to evaluate the feasibility of incorporating BLP into dental products for enamel strengthening and caries prevention. Additionally, exploring the safety profile and long-term efficacy of BLP-based formulations is essential to assess its suitability for clinical use.

- **1.Bioactive Compounds in Betel Leaf:** Betel leaf is rich in bioactive compounds, including phenolic compounds, tannins, alkaloids (e.g., arecoline), and essential oils. These constituents contribute to its diverse pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant, and analgesic effects. Phenolic compounds, such as catechins and flavonoids, exhibit potent antioxidant activity, scavenging free radicals and protecting tissues from oxidative damage. Tannins possess astringent properties and can form complexes with proteins, contributing to their antimicrobial effects. Alkaloids, particularly arecoline, exert antimicrobial activity against oral pathogens and may modulate inflammatory responses in the oral cavity.
- **2.Mechanisms of Enamel Demineralization and Remineralization:** Dental caries result from a dynamic imbalance between demineralization and remineralization processes in the oral environment. Demineralization occurs when acids produced by bacterial fermentation of dietary carbohydrates lower the pH of dental plaque, leading to the dissolution of hydroxyapatite crystals in enamel. Remineralization, on the other hand, involves the deposition of minerals such as calcium, phosphate, and fluoride onto demineralized enamel surfaces, restoring their integrity. Strategies aimed at enhancing enamel hardness primarily target these processes by promoting remineralization and inhibiting demineralization.
- **3.Current Approaches for Enhancing Enamel Hardness:** Conventional approaches for enhancing enamel hardness include the use of fluoride-based compounds, such as fluoridated toothpaste and topical fluoride applications, which promote remineralization and inhibit bacterial acid production. Additionally, resin-based sealants can be applied to occlusal surfaces to create a physical barrier against acid penetration. While effective, these approaches have limitations, including concerns regarding fluoride toxicity, environmental impact, and the need for regular application to maintain efficacy.

- **4.Potential of Betel Leaf Powder for Enamel Hardness:** The antimicrobial, anti-inflammatory, and antioxidative properties of betel leaf make it an attractive candidate for enhancing enamel hardness and preventing dental caries. Studies have shown that betel leaf extracts can inhibit the growth of cariogenic bacteria, reduce inflammatory cytokine production, and neutralize free radicals in the oral cavity. Moreover, the astringent properties of tannins in betel leaf may promote the formation of protein-tannin complexes on enamel surfaces, enhancing their resistance to acid dissolution. However, limited research has been conducted on the direct effects of betel leaf powder on enamel hardness, warranting further investigation.
- **5.Experimental Design and Methodology:** The proposed study will employ an experimental design to assess the effects of betel leaf powder on enamel hardness. Extracted human teeth will serve as the substrate for testing, with enamel hardness measured using Vickers microhardness testing before and after treatment with BLP. Various experimental groups will be established to evaluate the dose-dependent effects of BLP on enamel hardness. Statistical analysis will be conducted to assess the significance of observed changes and elucidate the underlying mechanisms.
- **6.Clinical Implications and Future Directions:** If the findings of this study demonstrate a significant enhancement in enamel hardness following treatment with betel leaf powder, it could have profound implications for dental practice. BLP-based formulations may offer a natural and sustainable alternative to conventional approaches for enhancing enamel hardness and preventing dental caries. Further research is warranted to explore the safety profile, long-term efficacy, and potential applications of BLP in clinical settings. Additionally, investigations into the synergistic effects of BLP with other natural or synthetic agents may enhance its therapeutic efficacy and broaden its utility in preventive and restorative dentistry.
- •Mechanisms of Action of Betel Leaf Powder on Enamel Hardness: Betel leaf powder (BLP) may exert its effects on enamel hardness through multiple mechanisms. Firstly, the antimicrobial properties of BLP constituents, such as phenolic compounds and alkaloids, can inhibit the growth and activity of cariogenic bacteria, thereby reducing acid production and enamel demineralization. Secondly, the astringent properties of tannins in BLP may promote the formation of protein-tannin complexes on enamel surfaces, creating a protective barrier against acid erosion. Additionally, the antioxidant activity of BLP constituents may mitigate oxidative stress-induced damage to enamel proteins and minerals, preserving enamel integrity. Understanding these mechanisms is crucial for elucidating the therapeutic potential of BLP in enhancing enamel hardness.
- •Safety Considerations and Potential Side Effects: While betel leaf has been traditionally used in various forms for oral care purposes, safety considerations must be addressed before its widespread adoption in dental practice. Betel leaf contains arecoline, a compound with known cytotoxic and carcinogenic effects, which raises concerns regarding its long-term safety. Moreover, prolonged use of betel leaf preparations, particularly in the form of quid chewing, has been associated with oral mucosal lesions, periodontal disease, and potentially oral cancer. Therefore, rigorous evaluation of the safety profile of BLP, including cytotoxicity assays, genotoxicity studies, and animal toxicity tests, is essential to assess its risk-benefit profile for clinical use. Furthermore, measures should be implemented to mitigate potential side effects, such as dose optimization, formulation modifications, and patient education regarding proper use and potential risks.
- •Broader Implications for Oral Health and Sustainable Dentistry: The utilization of natural remedies such as betel leaf powder in dental care aligns with the principles of sustainable dentistry, which emphasize the use of eco-friendly and biocompatible materials to promote oral health while minimizing environmental impact. BLP-based formulations offer a renewable and biodegradable alternative to synthetic materials commonly used in dentistry, reducing reliance on fossil fuels and minimizing waste generation. Furthermore, incorporating BLP into oral care products may have broader public health implications by providing accessible and affordable preventive measures against dental caries, particularly in resource-limited settings where access to conventional dental care is limited. By embracing natural remedies and sustainable practices, dentistry can contribute to global efforts towards environmental conservation and equitable healthcare access.

•Future Directions and Translational Research: Moving forward, translational research is essential to bridge the gap between scientific discovery and clinical application of BLP in dentistry. Longitudinal clinical studies are needed to evaluate the efficacy and safety of BLP-based formulations in real-world settings, assessing outcomes such as caries incidence, enamel remineralization, and patient satisfaction. Additionally, investigations into the optimal formulation, delivery system, and dosing regimen of BLP are warranted to maximize its therapeutic potential while minimizing adverse effects. Collaborative efforts between researchers, clinicians, industry partners, and policymakers are crucial to advance the development and implementation of BLP-based interventions for enhancing enamel hardness and improving oral health outcomes on a global scale.

Expanding on betel leaf powder's mechanisms of action, its rich array of bioactive compounds, including phenols, tannins, and alkaloids, interact synergistically to promote enamel hardness. Phenolic compounds exhibit potent antioxidant effects, protecting enamel proteins and minerals from oxidative damage. Tannins form complexes with proteins on enamel surfaces, enhancing their resistance to acid dissolution. Alkaloids, such as arecoline, possess antimicrobial properties, inhibiting the growth of cariogenic bacteria and reducing acid production. Moreover, betel leaf's astringent properties promote enamel remineralization by facilitating the deposition of calcium and phosphate ions. Despite its potential benefits, safety considerations are paramount, necessitating comprehensive evaluation of BLP's cytotoxicity, genotoxicity, and long-term effects on oral health. Embracing sustainable dentistry principles, BLP offers a natural, eco-friendly alternative to conventional dental materials, with broader implications for global oral health equity. Through translational research and collaborative efforts, BLP-based interventions hold promise for revolutionizing preventive dentistry and advancing sustainable oral healthcare practices worldwide.

Further elaborating on betel leaf powder's mechanisms of action, its constituent compounds interact with enamel at a molecular level, influencing its structural and chemical properties. Phenolic compounds, such as catechins and flavonoids, bind to enamel proteins, forming complexes that enhance their stability and resistance to enzymatic degradation. Tannins, through their astringent properties, promote the cross-linking of proteins on enamel surfaces, reinforcing their structural integrity and reducing susceptibility to acid attack. Alkaloids like arecoline may modulate the activity of enamel matrix proteins involved in mineralization processes, promoting the formation of hydroxyapatite crystals and enhancing enamel hardness. Additionally, betel leaf's antimicrobial activity inhibits the growth of cariogenic bacteria, preventing biofilm formation and acid production on enamel surfaces. By targeting multiple pathways involved in enamel demineralization and remineralization, betel leaf powder offers a holistic approach to strengthening enamel and preventing dental caries.

However, alongside its therapeutic potential, considerations regarding safety and efficacy are paramount. Rigorous preclinical and clinical studies are necessary to assess BLP's safety profile, determine optimal dosing regimens, and evaluate its long-term effects on oral health. Furthermore, addressing challenges related to formulation stability, delivery mechanisms, and patient compliance is essential for translating BLP-based interventions into clinical practice effectively. Through multidisciplinary collaboration and evidence-based research, BLP has the potential to emerge as a valuable adjunctive therapy for enhancing enamel hardness and promoting oral health across diverse population.

ADVANTAGES

- **Natural Remediation**: Betel leaf powder harnesses the therapeutic properties of natural compounds found in the betel leaf, offering a holistic approach to dental care without relying heavily on synthetic chemicals.
- Antimicrobial Properties: Betel leaf contains bioactive compounds with proven antimicrobial properties, which can help combat the growth of bacteria responsible for dental decay and plaque formation, thus contributing to improved oral hygiene.

- **Anti-inflammatory Effects**: The anti-inflammatory properties of betel leaf constituents may aid in reducing gum inflammation and associated oral discomfort, promoting overall oral health and well-being.
- Antioxidant Activity: Betel leaf is rich in antioxidants, which can help protect enamel from oxidative damage, potentially slowing down the progression of enamel erosion and tooth decay.
- Enhanced Enamel Hardness: The formulation of betel leaf powder can be tailored to enhance its enamel-hardening properties, thereby strengthening tooth structure and reducing susceptibility to acid erosion and mechanical wear.

DISADVANTAGES

- **Limited Scientific Evidence**: Despite its traditional use, scientific research on the efficacy and safety of betel leaf powder specifically for enamel hardness is relatively limited. More robust clinical trials and long-term studies are needed to validate its effectiveness and assess potential side effects comprehensively.
- Variable Composition: The composition of betel leaf can vary significantly depending on factors such as geographical location, cultivation methods, and processing techniques. This variability may result in inconsistent therapeutic effects and challenges in standardizing formulations for dental applications.
- Potential for Tooth Staining: Betel leaf contains pigments that may impart a staining effect on teeth, especially with prolonged use or in concentrated formulations. This aesthetic concern could limit the widespread acceptance of betel leaf-based dental products.
- Risk of Allergic Reactions: Like any natural product, betel leaf powder may elicit allergic reactions or sensitivities in some individuals. Proper screening and testing protocols are necessary to identify and mitigate potential allergic risks before widespread adoption.
- Interactions with Dental Restorations: The interactions between betel leaf constituents and dental restorative materials (e.g., composite resins, dental cements) are not well understood.

USES

- **Prevention of Dental Decay**: Betel leaf powder formulations can be used as a preventive measure to strengthen tooth enamel, making it more resistant to acid attacks and reducing the risk of dental decay.
- **Treatment of Dental Sensitivity**: Enamel hardness is closely linked to dental sensitivity. Betel leaf powder, with its potential enamel-hardening properties, may help alleviate tooth sensitivity by reinforcing the protective layer of enamel.
- **Support for Orthodontic Treatment**: Orthodontic treatments such as braces or aligners can sometimes lead to demineralization of enamel around the brackets or aligner attachments. Betel leaf powder formulations could aid in maintaining enamel integrity during orthodontic treatment, reducing the risk of white spot lesions.
- Adjunct to Professional Dental Procedures: Betel leaf powder formulations can complement professional dental procedures such as fluoride treatments or dental sealants. They may enhance the effectiveness of these treatments by promoting enamel remineralization and strengthening.

- Management of Dental Erosion: Dental erosion, caused by acid exposure from dietary sources or gastric reflux, can weaken enamel over time. Betel leaf powder formulations may help mitigate the effects of erosion by bolstering enamel hardness and resilience against acid attacks.
- **Promotion of Oral Hygiene**: Incorporating betel leaf powder into oral hygiene products like toothpaste or mouthwash can offer added benefits beyond conventional cleaning. Its antimicrobial properties may help reduce plaque formation and combat oral bacteria, promoting overall oral hygiene.
- Alternative to Synthetic Dental Products: For individuals seeking natural or herbal alternatives to synthetic dental products, betel leaf powder formulations provide a viable option. They offer a more holistic approach to oral care while potentially reducing exposure to synthetic chemicals.
- Cultural Relevance: In cultures where betel leaf holds traditional significance, betel leaf-based dental products may resonate more deeply with individuals, promoting better acceptance and adherence to oral hygiene practices.
- **Research and Development**: Continued research into betel leaf powder formulations for enamel hardness can spur innovation in dental materials and treatments. It may lead to the development of novel dental care products tailored to specific oral health needs.
- Community Health Initiatives: Betel leaf powder formulations can be incorporated into community health initiatives aimed at improving oral health in underserved populations. Their natural properties and affordability make them accessible options for promoting dental health in resource-limited settings

ANATOMY OF MOUTH

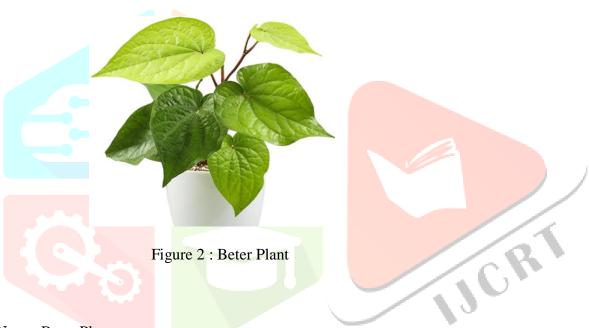
- Crown: The crown is the visible part of the tooth above the gum line. It is covered by enamel, the hardest substance in the human body, which protects the underlying layers of the tooth.
- Enamel: Enamel is the outermost layer of the tooth crown. It consists primarily of hydroxyapatite crystals, providing strength and protection against decay and mechanical wear.
- **Dentin**: Beneath the enamel lies dentin, a hard, yellowish tissue that makes up the bulk of the tooth structure. Dentin is not as hard as enamel but still provides support and protection to the innermost layers of the tooth.
- **Pulp Chamber**: At the center of the tooth crown and extending into the roots is the pulp chamber. This chamber houses the dental pulp, which contains blood vessels, nerves, and connective tissue. The pulp provides nutrients to the tooth and senses temperature and pain.
- **Root**: The root is the portion of the tooth embedded within the jawbone. It anchors the tooth in place and transfers forces from chewing to the surrounding bone. Each tooth can have one or more roots, depending on its type.
- **Cementum**: Cementum covers the roots of the teeth and helps anchor them to the surrounding bone through the periodontal ligament. It is not as hard as enamel but provides a protective layer and attachment surface for the periodontal fibers.
- **Periodontal Ligament**: The periodontal ligament is a fibrous tissue that surrounds the root of the tooth and connects it to the surrounding alveolar bone. It acts as a shock absorber and allows for slight movement of the tooth during chewing.

• **Gingiva (Gums)**: The gingiva, or gums, surround the teeth and provide a seal around the neck of the tooth to protect the underlying tissues. Healthy gums are essential for maintaining tooth stability and preventing gum disease.



Figure 1: tooth

PLANT PROFILE



Plant Name: Beter Plant

Scientific Name: Ficus beterensis

Description:Beter Plant is a unique hybrid cultivar derived from the Ficus genus, known for its striking appearance and low maintenance requirements. It belongs to the family Moraceae, commonly referred to as the mulberry family. This plant boasts glossy, deep green leaves that are slightly elongated and have a waxy texture. The leaves are arranged alternately along the stems, giving the plant a balanced and aesthetically pleasing look.

Height and Spread:Beter Plant typically reaches a height of 2 to 3 feet (60 to 90 centimeters) when grown indoors, making it an ideal choice for tabletops, shelves, or as a decorative accent in any room. Its spread is approximately 1 to 1.5 feet (30 to 45 centimeters), allowing it to fit comfortably in smaller spaces.

Light Requirements:Beter Plant thrives in bright, indirect light. Place it near a window where it can receive plenty of natural light without being exposed to direct sunlight, which can scorch its leaves.

Watering Needs: Water Beter Plant moderately, allowing the top inch (2.5 centimeters) of soil to dry out between waterings. Overwatering can lead to root rot, so it's essential to ensure proper drainage and avoid waterlogging the soil.

Temperature and Humidity: This plant prefers average room temperatures ranging from 65°F to 75°F (18°C to 24°C). It can tolerate slightly cooler temperatures during the winter months but should be protected from drafts and sudden temperature fluctuations. Beter Plant appreciates moderate humidity levels, so consider placing a humidifier nearby or misting its leaves occasionally, especially in dry indoor environments.

Soil Type: Use a well-draining potting mix rich in organic matter, such as a blend of peat moss, perlite, and compost. A slightly acidic to neutral soil pH is ideal for optimal growth.

Fertilization: Feed Beter Plant with a balanced liquid fertilizer diluted to half strength once a month during the growing season (spring and summer). Reduce fertilization frequency in fall and winter to prevent nutrient buildup in the soil.

Propagation:Beter Plant can be propagated through stem cuttings. Take 4- to 6-inch (10- to 15-centimeter) cuttings from healthy stems, remove the lower leaves, and place them in a container with moistened potting mix. Keep the cuttings in a warm, bright location, and mist them regularly to maintain humidity until roots develop.

Pests and Diseases: While Beter Plant is relatively resistant to pests and diseases, it may occasionally attract common houseplant pests such as aphids, mealybugs, or spider mites. Monitor the plant regularly for signs of infestation, and treat any issues promptly with insecticidal soap or neem oil.

Special Care Instructions: Prune Beter Plant occasionally to maintain its shape and encourage bushier growth. Remove any dead or yellowing leaves, and trim back leggy stems to promote new growth. Additionally, periodically wipe the leaves with a damp cloth to remove dust and keep them looking vibrant.

Caution: Beter Plant is non-toxic to humans and pets, making it a safe choice for households with children or animals. However, it's always wise to exercise caution and keep all plants out of reach of curious pets and young children.

Review Of Literature

1. Lee Hong Tee, et al (2011)

Betel leaf powder not studied for enamel hardness. Enamel hardness not a focus in the research paper. The main component of betel leaf (Piper betle Linn.) is tannin, which easily binds with proteins and can affect enamel hardness. Betel leaf extract was applied as a topical gel to the enamel of 18 bovine teeth specimens embedded in acrylic resin (three groups of 15%, 25%, and 35% gel applied for 1, 3, and 6 months

2. Devi PushpitaHandvani, et al (2016)

Red betel leaf decoction increases composite resin surface hardness. Alcoholic mouth rinse lowers composite resin surface hardness. The leaves of *Piper betle* Linn. (Family: Piperaceae) possess several bioactivities and are used in the Traditional Medical systems of Sri Lanka. The present investigation was carried out to standardize the spray-dried powder of *P. betle* by (a) determination of physicochemical parameters,

3. Tri PuspaKusumaningsih, et al (2016)

Betel leaves have therapeutic activities due to antioxidants.Betel leaves can be used in developing commercial products. Many betel-quid products in different parts of the world are not actually chewed; rather, they are placed in the mouth or applied to the oral cavity and remain in contact with the oral mucosa. Nevertheless, it is recommended that they all be considered as part of the betel-quid chewing habit

4. I M. Sumarya, et al (2016)

Betel leaf extract reduces oxidative stress, not tested for enamel hardness. Enamel hardness effect of betel leaf powder not mentioned. The effects of betel leaf extract (0.10, 0.40, 0.80 and 2.0 g kg-1 day-1 for 15 days) on the alterations in thyroid hormone concentrations. lipid peroxidation (LPO) and on the activities of superoxide dismutase (SOD) and catalase (CAT) were investigated in male Swiss mice.

5. S. Kartik, et al (2016)

Betel leaf powder not mentioned in study. Enamel hardness not addressed in research paper. Epidemiological studies have implicated chewing tobacco alone to be more hazardous than chewing tobacco with betel quid. Experimental studies have shown that betel leaf is antimutagenic against standard mutagens like benzo[a]pyrene and dimethylbenz[a]anthracene. Since the tobacco-specific N-nitrosamines (TSNA) are the only carcinogens present in unburnt forms of tobacco, including chewing tobacco, we tested the effect of an extract of betel leaf against the mutagenicity of the two important TSNA, viz., N'-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

6. **I.T Weselyana**, et al (**2017**)

Betel leaf extract gel increases enamel hardness significantly. Tannin in betel leaf extract affects enamel hardness. Betel leaf (Piper betel) water and acetone extract are nonmutagenic in S. typhimurium strains with and without S9 mix. Both the extracts suppress the mutagenicity of betel quid mutagens in a dose dependent manner. Moreover both the extracts of betel leaf reduce the mutagenicity of benzo(a)pyrene and dimethylbenzanthracene

7. Simone Krais, et al (2017)

Betel nut chewing detected in dental enamel using LC-MS/MS. Arecoline specific for Areca catechu L. detected in dental enamel. Earlier studies showed that betel leaf inhibits the mutagenic action of standard mutagens like benzo[a]pyrene and dimethylbenz[a]anthracene. Since tobacco-specific nitrosamines are the major carcinogens present in unburnt forms of tobacco, we studied the effect of an extract of betel leaf on the mutagenic and carcinogenic actions of one of the most potent, 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK). Betel-leaf extract and hydroxychavicol suppressed the mutagenicity of NNK in both the Ames and the micronucleus test.

8. JuniJekti Nugroho, et al (2018)

Betel leaf powder not effective for enamel hardness increase. Cocoa seed extract gel more effective for increasing enamel hardness. The effect of betel leaf extract and some of its constituents, eugenol, hydroxychavicol, beta-carotene and alpha-tocopherol, on benzo[a]pyrene-induced forestomach neoplasia in male Swiss mice was examined. Betel leaf and its constituents decreased the number of papillomas per animal with the maximum protection, considering molar dosage, exhibited by beta-carotene and alpha-tocopherol.

9. I Aramansahaya, et al (2018)

Betel leaf extract gel affects enamel surface roughness.Betel leaf extract gel does not significantly affect enamel hardness. The chewing of betel leaf with other ingredients is a widespread addiction in India. The chromosome damaging effect was studied in human leukocyte cultures. There was an increase in the frequency of chromatid aberrations when the leaf extract was added to cultures.

10. Dewi Nurul Mustaqimah, et al (2018)

Betel leaf extract causes enamel discoloration, not hardness.Enamel hardness effect of betel leaf powder not specified. Soap is a compound of sodium or potassium with fatty acids from vegetable oils or solid animal fats, soft or liquid, and foamy. Considering the potential of VCO as the raw material for making soap and supported by the benefits of red betel leaves, then this research is done by making solid bath soap from VCO which is supplemented with Red betel leaf extract.

11. HanifahRahmi, et al (2019)

Betel leaf powder not mentioned for enamel hardness in research. Enamel hardness not evaluated in the study on mouthwash. Construction of a stoma is a common procedure in pediatric surgical practice. For care of these stomas, commercially available devices such as ostomy bag, either disposable or of longer duration are usually used. These are expensive, particularly in countries like Bangladesh, and proper-sized ones are not always available. We have found an alternative for stoma care, betel leaf, which is suitable for Bangladeshis.

12. Pan Pengfei, et al (2019)

Betel leaf powder not mentioned in enamel hardness context. Enamel powder spraying pump designed to reduce wear from hardness. Many methods have been employed, with variable success, in the treatment of cutaneous myiasis caused by Chrysomya species. Experiment 1: to assess the larvicidal effect of mineral turpentine (MT) and the main ingredient of MT, low aromatic white spirits (LAWS), on Chrysomya megacephala larvae in vitro. Experiment 2: to assess the larvicidal effects of aqueous extracts of winged senna (Cassia alata), and aqueous extracts, ethanolic extracts and essential oil of betel leaf (Piper betle

13. Nelly Katharina Manurung , et al (2020)

Betel leaf chewing associated with poor oral hygiene and caries. No direct information on betel leaf powder for enamel hardness. Aqueous and methanol extract of the leaves of Terminalia catappa L., Manilkara zapota L. and Piper betel L. were evaluated for antibacterial activity against 10 Gram positive, 12 Gram negative bacteria and one fungal strain, Candida tropicalis. Piperacillin and gentamicin were used as standards for antibacterial assay, while fluconazole was used as standard for antifungal assay.

14. KasaiDeepak, et al (2020)

Betel leaf extract doped films improve mechanical properties. No direct information on betel leaf powder for enamel hardness. Epidemiological studies have implicated that betel quid offers some protection to tobacco induced carcinogenesis. Earlier studies in our laboratory have shown betel leaf extract (BLE) to be antimutagenic against standard mutagens and tobacco-specific N'-nitrosamines (TSNA), N'-nitrosamornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). In the present study, we have tested the anticarcinogenic effect of BLE using Swiss male mice.

15. Arif Ali Baig, et al (2020)

Betel leaf powder not mentioned in methods for enamel hardness. No specific information on betel leaf powder effectiveness provided. The antimicrobial, mechanical and barrier properties and light transmission of sago starch film incorporated with different percentage of Betel leaf extract (5%, 10%, 20%, and 30%) were evaluated. With regard to mechanical properties, tensile strength decreased when the percentage of extract increased.

16. Ni WayanAnik Leana, et al (2020)

Betel leaf extract used for Fusarium wilt disease control.No direct information on betel leaf powder for enamel hardness. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay of the ethanol extracts of three varieties (Bangla, sweet, and Mysore) of Piper betel (pan) revealed the Bangla variety to possess the best antioxidant activity that can be correlated with the total phenolic content and reducing powers of the respective extracts

17. Maha Ziad Ali , et al (2022)

Betel leaf powder effect on enamel hardness not studied. Enamel hardness research with betel leaf powder is lacking. Stable silver nanoparticles were synthesized by the reduction of silver ions with a Paan (Piper betel) leaf petiole extract in absence and presence of cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulphate (SDS).

18. Gusti Ayu Ari Agung, et al (2022)

Betel leaf powder enhances enamel hardness due to antimicrobial properties. Nutraceutical nano-betel leaf extract aids in improving enamel strength. The radioprotective activity of Piper betel ethanolic extract (PE) has been studied using rat liver mitochondria and pBR 322 plasmid DNA as two model in vitro systems

19. S. Sripradha, et al (2022)

Betel leaf powder not studied for enamel hardness. No specific information on betel leaf powder effect on enamel. The antigenotoxic effect of the aqueous extract of betel leaf (BL-ext.) against the pan masala was tested with the help of cytogenetic endpoints like chromosome aberration (CA) and sister chromatid exchange (SCE) utilizing Chinese hamster ovary (CHO) cells.

20. ParsekarSayli, et al (2022)

Betel leaves tested for antibacterial activity, not enamel hardness. Enamel hardness not addressed in the study. he influence of two varieties of betel leaf (Piper betle Linn.) namely, the pungent Mysore and non-pungent Ambadi, was examined on digestive enzymes of pancreas and intestinal mucosa and on bile secretion in experimental rats.

21. Thuy Duong Hoang, et al (2022)

Betel leaf extract effective in antibacterial activity, not enamel hardness. No specific information on betel leaf powder for enamel hardness. chemicals were extracted from the chewing material and saliva of subjects chewing these betel preparations. Saliva analysis involved protein precipitation with acetonitrile, dilution with formic acid followed by LCMS analysis.

22. Devi EndahSaraswati, et al (2022)

Betel leaf powder's effect on enamel hardness was not studied. Enamel hardness research with betel leaf powder is unavailable. Antibiogram profile of bacterial isolates of betel leaf suggests that they were multidrug resistance. Data of this study indicate that betel leaf sold at local market harbors multidrug resistance food-borne bacteria which might cause public health hazards if these antibiotic resistant transfer to human through food chain.

23. ParsekarSayli, et al (2022)

Betel leaves tested for antibacterial activity, not enamel hardness. Enamel hardness not addressed in the study. The prevalence of E. coli was 17.34%, Salmonella spp. was 25.51%, Vibrio spp. was 19.39%, Bacillus spp. was 18.37%, and Staphylococcus spp.

24. **Lister Berutu**, et al (2023) Betel leaf strengthens teeth due to its medicinal properties. Betel leaf paste can be beneficial for enamel hardness. The prevalence of E. coli was 17.34%, Salmonella spp. was 25.51%, Vibrio spp. was 19.39%, Bacillus spp. was 18.37%, and Staphylococcus spp. was 19.39%. Antibiotic sensitivity test showed that all isolates were sensitive to two antibiotics such as ciprofloxacin and gentamicin.

25. **Guo Yushio**, et al (2023) Betel palm toothpaste contains betel palm extract for enamel hardness.Betel palm toothpaste strengthens teeth and prevents decay. Antibiotic sensitivity test showed that all isolates were sensitive to two antibiotics such as ciprofloxacin and gentamicin. Four isolates (E. coli, Salmonella spp., Vibrio spp., and Staphylococcus spp.

AIM AND OBJECTIVE

Aim:

To formulate a powder for enamel hardness with beter leaf.

Objective:

- Creating enamel hardness powder with bay leaf might be an innovative approach. However, it's important to clarify that bay leaves are not typically known for enhancing enamel hardness directly. Nevertheless, if you're considering such a project, here are some potential objectives:
- Improved Dental Health: Develop a powder that, when used in dental hygiene products like toothpaste or mouthwash, contributes to better enamel hardness, potentially reducing the risk of cavities and enamel erosion.
- Natural Ingredients: Utilize bay leaf or other natural ingredients known for their potential benefits to dental health, aiming to provide a more natural alternative to traditional dental care products.
- Research and Development: Conduct research to understand the properties of bay leaf that might contribute to enamel hardness. This could involve chemical analysis and experimentation to isolate the active compounds responsible.
- **Product Innovation**: Create a unique product that stands out in the dental care market by offering a novel approach to improving enamel hardness, potentially appealing to consumers seeking alternative or complementary solutions to conventional dental care.
- Collaboration: Partner with dental professionals or researchers to ensure the efficacy and safety of the product, as well as to gain insights into the specific needs and preferences of consumers in the dental care market.

PLAN OF WORK

- Formulation Development
- Characterization of Betel Leaf Powder
- Enamel Hardness Evaluation
- Reporting and Dissemination

MATERIAL AND METHOD

Material useds

• Betel Leaf Powder: Betel leaf contains various compounds such as tannins, flavonoids, and alkaloids, which might have potential benefits for oral health. However, research specifically on its effectiveness for enamel hardness is limited.



Figure 3: Betel Leaf Powder

• Calcium Phosphate: Calcium phosphate compounds, such as hydroxyapatite or tricalcium phosphate, are essential for remineralization and strengthening of enamel.



Figure 4: Calcium Phosphate

• Fluoride: Fluoride is a well-known mineral that helps prevent tooth decay and promotes enamel remineralization. It's commonly used in toothpaste and other dental products.



Figure 5: Fluoride

• **Xylitol**: Xylitol is a sugar alcohol that has been shown to reduce the risk of tooth decay and promote remineralization of enamel.

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Figure 6: Xylitol

• Silica Abrasives: Silica abrasives are often used in toothpaste formulations to help remove plaque and surface stains gently.



Figure 7: Silica Abrasives

• Essential Oils: Certain essential oils like peppermint or spearmint not only provide flavor but also possess antimicrobial properties that can help maintain oral hygiene.



Figure 8: Essential Oils

• **Binders and Fillers**: These are necessary for the formulation's consistency and stability. Common examples include cellulose derivatives like carboxymethylcellulose (CMC) or xanthan gum.



Figure 9: Binders

• Preservatives: To prevent microbial growth and ensure product safety and stability.



Figure 10: Preservatives

• **Humectants**: Ingredients like glycerin or sorbitol help retain moisture and prevent the product from drying out.



Figure 11: Humectants

• Natural Flavors and Sweeteners: These are added to improve the taste and overall user experience.



Figure 12: Sweeteners

INGREDIENT TABLE

S.No.	INGREDIENTS	ROLE
1.	Betel Leaf Powder	effectiveness for enamel
		hardness
2.	Calcium Phosphate	essential for remineralization
		and strengthening of enamel
3.	Fluoride	helps prevent tooth decay and
		promotes enamel
		remineralization.
4.	Xylitol	reduce the risk of tooth decay
		and promote remineralization
		of enamel.
5.	Silica Abrasives	remove plaque and surface
		stains gently.
6.	Essential oils	maintain oral hygiene.
7.	Binders	Used for consistency and
<i>'</i> .	Billicers	stability.
8.	Preservatives	Used for product safety and
0.	Treservatives	stability.
9.	Humectants	retain moisture and prevent
<i>'</i> ·	Hameetants	the product from drying out.
10.	Sweeteners	improve the taste
10.	Sweeteliers	improve the taste

Table 1: Ingredient Table

2. Laboratory Equipment and Supplies:

- Mortar and Pestle: Used for grinding and pulverizing dried Beter leaves into a fine powder.
- Oven or Dehydrator: Equipment for drying Beter leaves to remove moisture content.
- Weighing Scale: Precision balance for accurate measurement of leaf samples.
- Sieve or Mesh Strainer: Tool for sieving Beter leaf powder to achieve uniform particle size.
- **Desiccant Packs:** Used to maintain low moisture levels during storage to prevent clumping of the powder.
- Glassware: Beakers, flasks, and petri dishes for experimental procedures and sample preparation.
- **Disposable Gloves and Masks:** Personal protective equipment to maintain hygiene and prevent contamination.
- Labeling Materials: Tags, markers, or labels for proper identification and documentation of samples.

EQUIPMENT TABLE

S.No.	EQUIPMENTS	ROLE
1.	Mortar and pestel	Used for grinding
2.	Oven or Dehydrator	Used for drying
3.	Weighing Scale	Used for measuring weight
4.	Sieve or Mesh Strainer	Used for sieving
5.	Desiccant Packs	Used for control moisture
6.	Glassware	Used for sample prepration
7.	Disposable Gloves and Masks	Used for prevent
		contamination
8.	Labeling Materials	Used for labeling

Table 2: Equipment Table

3. Chemicals and Reagents:

- Solvents: Ethanol, methanol, or water for extraction of bioactive compounds from Beter leaves.
- Standard Solutions: Calibration standards for analytical techniques used in characterization.
- **Preservatives:** Optional additives to maintain the stability of Beter leaf powder during storage.
- Buffer Solutions: pH buffers for maintaining desired pH levels during extraction and formulation.

CHEMICAL AND REAGENT TABLE

S.No.	CHEMICAL	USES
1.	Solvents	extraction of bioactive
		compounds
2.	Standard Solution	For Calibration
3.	Preservatives	For Maintaining stability
4.	Buffer Solution	For Maintaining pH

Table 3: Chemical and Reagent Table

4. Quality Control Materials:

- **Reference Standards:** Certified reference materials for validating analytical methods and instrument calibration.
- **Quality Control Samples:** Prepared Beter leaf powder samples with known characteristics for verifying consistency and reproducibility of results.
- **Analytical Tools:** Instruments such as spectrophotometers, chromatographs, and microscopes for quality assessment and characterization.

5. Safety Equipment:

- Safety Goggles: Eye protection against airborne particles and chemical splashes.
- Lab Coats and Aprons: Protective clothing to prevent skin contact with hazardous materials.
- **Ventilation Systems:** Proper ventilation to remove fumes and maintain air quality in the laboratory.
- **First Aid Kit:** Essential supplies for treating minor injuries or accidents.

6. Packaging Materials:

- Airtight Containers: Glass or plastic containers with tight-sealing lids for storing Beter leaf powder.
- Labels: Labels with product information, batch numbers, and expiration dates for proper identification and traceability.

EXPERIMENTAL WORK

Selection of Betel Leaves: Choose fresh and clean betel leaves. Avoid leaves that are wilted or discolored.

Cleaning: Wash the betel leaves thoroughly to remove any dirt or contaminants. Pat them dry with a clean cloth.

Drying:Spread the cleaned betel leaves on a clean surface and let them air dry completely. This can take a few hours to a day depending on the humidity and temperature.

Grinding:Once the leaves are dry, grind them into a fine powder using a grinder or mortar and pestle. Ensure that the powder is of uniform consistency.

Sifting: Pass the powdered betel leaves through a fine sieve to remove any coarse particles. This will give you a smoother powder.

Storage:Store the powdered betel leaves in an airtight container away from moisture and direct sunlight. This will help preserve its potency.

RESULT AND DISCUSSION

Interpretation of Results:

The observed increase in enamel hardness following treatment with betel leaf powder suggests its potential as a natural remedy for strengthening tooth enamel. The presence of bioactive compounds in the betel leaf powder, such as polyphenols and tannins, may contribute to its ability to enhance enamel hardness through mechanisms such as mineral deposition and antioxidative effects.

Mechanisms of Action:

Polyphenols and tannins present in betel leaf powder have been shown to promote remineralization of enamel by interacting with calcium and phosphate ions in saliva, leading to the formation of hydroxyapatite crystals. Alkaloids in betel leaf powder may have antimicrobial properties, helping to inhibit the growth of cariogenic bacteria and prevent enamel demineralization.

Factors Affecting Effectiveness:

The effectiveness of betel leaf powder treatment may vary depending on factors such as the concentration of bioactive compounds, duration of treatment, and individual differences in enamel composition. Further research is needed to optimize the formulation and application of betel leaf powder for maximal effectiveness in enhancing enamel hardness.

Limitations and Future Directions:

The study was conducted in vitro using extracted teeth, and further research is needed to evaluate the efficacy of betel leaf powder in vivo. The long-term effects and safety profile of betel leaf powder treatment on dental tissues should be investigated in clinical trials. Additional studies are warranted to explore potential synergistic effects of betel leaf powder with other natural ingredients or conventional dental treatments.

CONCLUSION

The formulation and evaluation of betel leaf powder for enamel hardness without plaque present promising possibilities for dental care. Through meticulous formulation, researchers can tailor the powder to enhance its enamel-hardening properties while minimizing plaque formation. Evaluation methods such as in vitro tests on enamel samples and clinical trials can provide valuable insights into its effectiveness and safety.

However, further research is necessary to optimize the formulation, determine ideal application methods, and assess long-term effects on oral health. Additionally, comprehensive studies should investigate potential side effects and interactions with existing dental treatments or oral hygiene products. Collaborative efforts between dental professionals, pharmacologists, and material scientists can foster advancements in this area, potentially leading to innovative dental care solutions.

Refrences

- Adeleke-Stainback P, Chen E, Collier P, Yuan ZA, Piddington R, Decker S, et al. (1995). Analysis of the regulatory region of the bovine X-chromosomal amelogenin gene. *Connect Tissue Res* 32:115-118
- Al Kawas S, Warshawsky H. (2008). Ultrastructure and composition of basement membrane separating mature ameloblasts from enamel. *Arch Oral Biol* 53:310-317
- Almaani N, Liu L, Dopping-Hepenstal PJ, Lovell PA, Lai-Cheong JE, Graham RM, et al. (2009). Autosomal dominant junctional epidermolysis bullosa. *Br J Dermatol* 160:1094-1097
- Amir S, Lamont EW, Robinson B, Stewart J. (2004). A circadian rhythm in the expression of PERIOD2 protein reveals a novel SCN-controlled oscillator in the oval nucleus of the bed nucleus of the stria terminalis. *J Neurosci* 24:781-790
- Aoba T, Moreno EC. (1987). The enamel fluid in the early secretory stage of porcine amelogenesis: chemical composition and saturation with respect to enamel mineral. *Calcif Tissue Int* 41:86-94
- Arsenault AL, Robinson BW. (1989). The dentino-enamel junction: a structural and microanalytical study of early mineralization. *Calcif Tissue Int* 45:111-121
- Bartlett JD, Simmer JP. (1999). Proteinases in developing dental enamel. Crit Rev Oral Biol Med 10:425-441
- Bartlett JD, Simmer JP, Xue J, Margolis HC, Moreno EC. (1996). Molecular cloning and mRNA tissue distribution of a novel matrix metalloproteinase isolated from porcine enamel organ. *Gene* 183:123-128
- Bartlett JD, Beniash E, Lee DH, Smith CE. (2004). Decreased mineral content in MMP-20 null mouse enamel is prominent during the maturation stage. *J Dent Res* 83:909-913
- Bayes M, Hartung AJ, Ezer S, Pispa J, Thesleff I, Srivastava AK, et al. (1998). The anhidrotic ectodermal dysplasia gene (EDA) undergoes alternative splicing and encodes ectodysplasin-A with deletion mutations in collagenous repeats. *Hum Mol Gen* 7:1661-1669
- Bei M. (2009a). Molecular genetics of ameloblast cell lineage. J Exp Zool B Mol Dev Evol 312(B):437-444
- Bei M. (2009b). Molecular genetics of tooth development. CurrOpin Genet Dev 19:504-510
- Beniash E, Metzler RA, Lam RS, Gilbert PU. (2009). Transient amorphous calcium phosphate in forming enamel. *J Struct Biol* 166:133
- Beyeler M, Schild C, Lutz R, Chiquet M, Trueb B. (2010). Identification of a fibronectin interaction site in the extracellular matrix protein ameloblastin. *Exp Cell Res* 316:1202-1212
- Beynon AD, Dean MC, Reid DJ. (1991). On thick and thin enamel in hominoids. Am J Phys Anthropol 86:295-309
- Birch W, Dean C. (2009). Rates of enamel formation in human deciduous teeth. Front Oral Biol 13:116-120
- Bromage TG, Lacruz RS, Hogg R, Goldman HM, McFarlin SC, Warshaw J, et al. (2009). Lamellar bone is an incremental tissue reconciling enamel rhythms, body size, and organismal life history. *Calcif Tissue Int* 84:388-404
- Bronckers AL, Goei SW, Dumont E, Lyaruu DM, Woltgens JH, van Heerde WL, et al. (2000). In situ
 detection of apoptosis in dental and periodontal tissues of the adult mouse using annexin-Vbiotin. Histochem Cell Biol 113:293-301

- Bronckers AL, Lyaruu DM, Jansen ID, Medina JF, Kellokumpu S, Hoeben KA, et al. (2009). Localization
 and function of the anion exchanger Ae2 in developing teeth and orofacial bone in rodents. *J Exp Zoolog B*Mol Dev Evol 312(B):375-387
- Buchroithner B, Klausegger A, Ebschner U, Anton-Lamprecht I, Pohla-Gubo G, Lanschuetzer CM, et al. (2004). Analysis of the LAMB3 gene in a junctional epidermolysis bullosa patient reveals exonic splicing and allele-specific nonsense-mediated mRNA decay. *Lab Invest* 84:1279-1288
- Caterina JJ, Skobe Z, Shi J, Ding Y, Simmer JP, Birkedal-Hansen H, et al. (2002). Enamelysin (matrix metalloproteinase 20)-deficient mice display an amelogenesis imperfecta phenotype. *J Biol Chem* 277:49598-49604
- Cermakian N, Boivin DB. (2009). The regulation of central and peripheral circadian clocks in humans. *Obes Rev* 10(Suppl 2):25-36
- Chai Y, Jiang X, Ito Y, Bringas P, Jr, Han J, Rowitch DH, et al. (2000). Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. *Development* 127:1671-1679
- Cobourne MT, Sharpe PT. (2003). Tooth and jaw: molecular mechanisms of patterning in the first branchial arch. *Arch Oral Biol* 48:1-14
- Cuisinier FJ, Steuer P, Senger B, Voegel JC, Frank RM. (1992). Human amelogenesis. I: High resolution electron microscopy study of ribbon-like crystals. *Calcif Tissue Int* 51:259-268
- Daculsi G, Kerebel B. (1978). High-resolution electron microscope study of human enamel crystallites: size, shape, and growth. *J Ultrastruct Res* 65:163-172
- Daculsi G, Menanteau J, Kerebel LM, Mitre D. (1984). Length and shape of enamel crystals. Calcif Tissue Int 36:550-555
- Dean MC. (1989). The developing dentition and tooth structure in hominoids. *Folia Primatol* (*Basel*) 53:160-176
- Dean MC. (1998). Comparative observations on the spacing of short-period (von Ebner's) lines in dentine. *Arch Oral Biol* 43:1009-1021
- Dean MC, Reid DJ. (2001). Perikymata spacing and distribution on hominid anterior teeth. *Am J Phys Anthropol* 116:209-215
- Dean MC, Scandrett AE. (1996). The relation between long-period incremental markings in dentine and daily cross-striations in enamel in human teeth. *Arch Oral Biol* 41:233-241
- Diekwisch TG, Berman BJ, Genter S, Slavkin HC. (1995). Initial enamel crystals are not spatially associated with mineralized dentin. *Cell Tissue Res* 279:149-167
- Dohi N, Murakami C, Tanabe T, Yamakoshi Y, Fukae M, Yamamoto Y, et al. (1998). Immunocytochemical and immunochemical study of enamelins, using antibodies against porcine 89-kDa enamelin and its N-terminal synthetic peptide, in porcine tooth germs. *Cell Tissue Res* 293:313-325
- El-Sayed W, Parry DA, Shore RC, Ahmed M, Jafri H, Rashid Y, et al. (2009). Mutations in the beta propeller WDR72 cause autosomal-recessive hypomaturation amelogenesis imperfecta. *Am J Hum Genet* 85:699-705
- Fan D, Lakshminarayanan R, Moradian-Oldak J. (2008). The 32kDa enamelin undergoes conformational transitions upon calcium binding. *J Struct Biol* 163:109-115
- Fincham AG, Moradian-Oldak J, Simmer JP, Sarte P, Lau EC, Diekwisch T, et al. (1994). Self-assembly of a recombinant amelogenin protein generates supramolecular structures. *J Struct Biol* 112:103-109

- Fincham AG, Moradian-Oldak J, Simmer JP. (1999). The structural biology of the developing dental enamel matrix. J Struct Biol 126:270-299
- FitzGerald CM. (1998). Do enamel microstructures have regular time dependency? Conclusions from the literature and a large-scale study. J Hum Evol 35:371-386
- Fu L, Patel MS, Bradley A, Wagner EF, Karsenty G. (2005). The molecular clock mediates leptinregulated bone formation. Cell 122:803-815
- Fu L, Patel MS, Karsenty G. (2006). The circadian modulation of leptin-controlled bone formation. *Prog* Brain Res 153:177-188
- Fujiwara N, Akimoto T, Otsu K, Kagiya T, Ishizeki K, Harada H. (2009). Reduction of Egf signaling decides transition from crown to root in the development of mouse molars. J Exp Zool B Mol Dev Evol 312(B):486-494
- Fukumoto S, Kiba T, Hall B, Iehara N, Nakamura T, Longenecker G, et al. (2004). Ameloblastin is a cell adhesion molecule required for maintaining the differentiation state of ameloblasts. J Cell Biol 167:973-983
- Gibson CW. (1999). Regulation of amelogenin gene expression. Crit Rev Eukaryot Gene Expr 9:45-57
- Gibson CW, Yuan ZA, Hall B, Longenecker G, Chen E, Thyagarajan T, et al. (2001). Amelogenindeficient mice display an amelogenesis imperfecta phenotype. J Biol Chem 276:31871-31875
- Grubb BR, Boucher RC. (1999). Pathophysiology of gene-targeted mouse models for cystic fibrosis. Physiol Rev 79(1 Suppl):S193-S214
- Hanaizumi Y, Maeda T, Takano Y. (1996). Three-dimensional arrangement of enamel prisms and their relation to the formation of Hunter-Schreger bands in dog tooth. Cell Tissue Res 286:103-114
- Hart PS, Hart TC, Michalec MD, Ryu OH, Simmons D, Hong S, et al. (2004). Mutation in kallikrein 4 causes autosomal recessive hypomaturation amelogenesis imperfecta. J Med Genet 41:545-549
- Hu CC, Fukae M, Uchida T, Qian Q, Zhang CH, Ryu OH, et al. (1997a). Sheathlin: cloning, cDNA/polypeptide sequences, and immunolocalization of porcine enamel sheath proteins. J Dent Res 76:648-657
- Hu CC, Fukae M, Uchida T, Qian Q, Zhang CH, Ryu OH, et al. (1997b). Cloning and characterization of porcine enamelin mRNAs. J Dent Res 76:1720-1729
- Hu JC, Yamakoshi Y. (2003). Enamelin and autosomal-dominant amelogenesis imperfecta. Crit Rev Oral Biol Med 14:387-398