



Formulation And Evaluation Of Toothpaste Of Myristica Fragrans Against Oral Pathogens

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Abstract

This study aimed to develop and assess the efficacy of an anti-bacterial toothpaste formulated with Myristica fragrans against oral pathogens. The toothpaste was prepared using standard procedures, incorporating Myristica fragrans extract known for its antibacterial properties. Various parameters including pH, viscosity, and antibacterial activity against common oral pathogens such as Streptococcus mutans, Lactobacillus acidophilus, and Porphyromonas gingivalis were evaluated. The results demonstrated that the formulated toothpaste exhibited desirable physical characteristics and significant antibacterial activity against tested oral pathogens. Myristica fragrans extract effectively inhibited the growth of these bacteria, suggesting its potential for oral care applications. Therefore, this study suggests that Myristica fragrans could be a promising natural ingredient for the development of effective anti-bacterial toothpaste formulations targeting oral health.

Keyword: Toothpaste, Myristica fragrans

1.Introduction

1.1 Herbal Plants

Herbal Plant contain many active compounds such as alkaloids, steroids, tannins glycosides, volatile oils, resins, phenols, and flavonoids which are deposited in their specific part such as leaves, flowers, bark, seeds, fruits and roots. There are several extraction methods that exist to extract compounds from plants.⁽¹⁾

Herbal medicine is the use of plant, plant part, there water or solvent extracts, essential oil, gums, resins, exudates or other from of advanced products made from plant part used therapeutically to provide proactive supports of various physiological system or, in a more conventional medical sense, to treat, cure, or prevent a disease in animals or humans. ⁽²⁾

CHARACTERISTICS OF MEDICINAL PLANT

Medicinal plants have many characteristics when used as a treatment, as follow

Support of official medicine- The ingredients of plants can be used along with chemical products to achieve the desired outcome.

Preventive medicine- Some components of plants have proved to be effective in preventing or reducing the risk of certain disease (e.g flu), and this can help in reduce the burden and cost of using chemical remedies.

Synergic medicine- Each plant has many compounds that may interact simultaneously leading to either complement or damage the functions of each other, or neutralize their possible negative effects

1.2 Bacteria: - Bacteria are single-celled microorganisms that can be classified into various categories based on their features and characteristics. The classification of bacteria is mainly based on the following:

Shape:

- ❖ Bacillus (Rod-shaped)
- ❖ Spirilla or spirochete (Spiral)
- ❖ Coccus (Sphere)
- ❖ Vibrio (Comma-shaped)

Composition of the cell wall

- ❖ Peptidoglycan cell wall (Gram-positive bacteria)
- ❖ Lipopolysaccharide cell wall (Gram-negative bacteria)

Mode of respiration:

- ❖ Anaerobic Bacteria
- ❖ Aerobic Bacteria

Mode of nutrition:

- ❖ Autotrophic Bacteria
- ❖ Heterotrophic Bacteria

Examples of various types of bacteria include:

- ❖ Escherichia coli (E. coli)
- ❖ Streptococcus pneumoniae
- ❖ Spirillum volutans
- ❖ Vibrio cholerae
- ❖ Staphylococcus aureus
- ❖ Streptococcus pyogenes

Example of anti-bacterial drugs:-

Anti-bacterial drugs are medications that are used to treat bacterial infections. Some examples of anti-bacterial drugs include:

- ❖ Penicillin
- ❖ Amoxicillin
- ❖ Erythromycin
- ❖ Clindamycin
- ❖ Gentamicin
- ❖ Ciprofloxacin
- ❖ Levofloxacin
- ❖ Vancomycin
- ❖ Daptomycin
- ❖ Linezolid

1.3 Oral Pathogens: Oral bacteria exhibit highly specific adherence mechanisms and as a result they colonize and cause disease principally in the oral cavity. Oral pathogens, however, can produce systemic disease and are known causative agents of infective endocarditis. Dental caries and periodontal disease are complex multifactorial diseases with dental plaque as their primary cause. Gram-positive bacteria such as *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacillus* species and some nonmutans streptococci are closely associated with caries formation. Gram-negative bacteria such as *Aggregatibacter actinomycetemcomitans* is associated with aggressive periodontitis, while *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Campylobacter rectus* are associated with chronic periodontitis in adult. Caries and periodontal disease can be prevented by good maintenance of oral hygiene with the use of oral care products such as toothpaste, toothbrush, mouthwash, and oral paste that contain antimicrobial and anticariogenic properties.

1.4 *Myristica fragrans* Houtt (Family: Myristicaceae) locally known as buah pala in Malay is mostly cultivated for spices in Penang Island, Malaysia. It is composed of the skin, flesh, seed, and mace. Nutmeg is the seed kernel inside the fruit and mace is the fleshy red, net-like skin covering (aril) on the kernel. The main constituents of *Myristica fragrans* have been found to be alkyl benzene derivatives (myristicin, elemicin, safrole, etc.), terpenes, alpha-pinene, beta-pinene, myristic acid, trimyristin, neolignan (myrislignan), and macelignan



Fig. no.1 *Myristica Fragrans*



Fig. no.2 *Myristica fragrans* seeds

Plant Name: Myristica Fragrans

Comen Name: Bombay Mace

Family: Myriticacease

Kingdom: Plantae

Class: Mangnoliopsida

Order: Magnoliales

Species: Fragrance

Chemical Constituents: A neolignane, erythro-surinamensin and a diaryl phenyl propanoid, virolane were isolated from *Myristica fragrans* for the first time

Uses: showed antibacterial effect against *Streptococcus mutans*, *Streptococcus anguish*, *Streptococcus salivarius*, and *Lactobacillus casei*.

Pharmacological effect:

- ❖ Hypolipidemic
- ❖ Hypocholesterolemic effects
- ❖ Antimicrobial
- ❖ Antidepressant
- ❖ Aphrodisiac
- ❖ memory-enhancing
- ❖ antioxidant

Plan of Work:

- Literature Survey
- Selection of Plant
- Material and Method
- ✓ Solvent - ethanol
- ✓ Method – Soxhlet extraction
- Extraction of bio-active compound
- Formulation of toothpaste
- pH
- Viscosity
- Foaming ability and foam stability

5. Methodology used in proposed: -

Materials and Methods

Plant material: Fresh and dry fruits of *Myristica fragrans* were collected from local market of Mandleshwar. (M.P)

Extraction of Plant Material- Plant powder 50g was extracted with 800ml of ethanol in a soxhlet apparatus at 60 °C for 42 hrs.

1. Set up the Soxhlet apparatus: Assemble the round-bottom flask, condenser, and thimble. Place the Myristica fragrance in the thimble.

2. Add ethanol: Pour the 800 ml of ethanol into the round-bottom flask. Make sure the seeds in the thimble are not in direct contact with the ethanol.

3. Start the extraction: Heat the round-bottom flask, causing the acetone to boil and vaporize. The vapor will rise and condense in the condenser, dripping back into the thimble.

4. Continuous extraction: As the condensed acetone drips back into the thimble, it will dissolve more compounds from the Myristica fragrance seeds. This continuous cycle of extraction and condensation will allow for efficient extraction of the desired compounds.

5. Collection: The extracted compounds will gradually accumulate in the round-bottom flask as the extraction process continues.

6. Completion: The extraction process is typically carried out for several hours or until the desired compounds have been sufficiently extracted. The collected solution in the round-bottom flask can then be evaporated to obtain the desired extract.⁽²²⁾

6. Phytochemical screening of *Myristica fragrance* extract

Phytochemical tests were done to find out the presence of bioactive chemical constituents such as alkaloids, flavonoids, carbohydrates, saponins, tannins, steroids and amino acids compounds.

✓ **Test for Carbohydrate-**

Fehling's test: Mix 1 ml Fehling's A and 1 ml Fehling's B solutions, boil for one minute. Add equal volume of test solution. Heat in boiling water bath for 5-10 min. brownish colour is observed.

✓ **Test for Glycosides-**

Liebermann reaction: Mix 2 ml extract with chloroform. Add 1-2 ml acetic anhydride and 2 drops conc. H₂SO₄, from the side of test tube. Green colour appears.

✓ **Test for Saponin-**

Foam test: Shake the drug extract or dry powder vigorously with water. Persistent foam observed.

✓ **Test for Anthraquinone Glycosides-**

Borntrager's test : To 3 ml extract, add dil. H₂SO₄, Boil and filter. To cold filtrate, add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammoniacal layer turns pink or red.

✓ **Test for Alkaloid-**

Mayer's test: 2-3 ml filtrate with few drops Mayer's reagent gives ppt.

✓ **Test for steroid-**

Liebermann reaction: Mix 2 ml extract with chloroform. Add 1-2 ml acetic anhydride and 2 drops conc. H₂SO₄, from the side of test tube. Green colour appears.

(23)

| S.no | Product | Test | Result |
|------|---------------|----------------------------|----------|
| 1. | Carbohydrate | Fehling's test | Positive |
| 2. | Glycosides | Liebermann's test | Positive |
| 3. | Anthraquinone | Borntrager's test | Negative |
| 4. | Alkaloids | Dragendorff's test | Positive |
| 5 | Steroid | Liebermann Burchard's test | Positive |
| 6 | Saponin | Foam test | Negative |

Table no. 1 Phytochemical screening of seeds extract of *Myristica fragrance*.

Preparation of herbal toothpaste

These preparations are preferably made in stainless steel mixer container, for large scale manufacture filled with slowly rotating blades. It can be done in a planetary mixer or similar mixer used for semisolid preparations. Small scale batch can be made in a glass container

The gum is mixed with a suitable quantity of humectant, without any water, the proper dispersion. Chloroform or alcohol can also be used dispersion of binding agents. Other colloids may be dispersed in water. Preservatives can be dissolved in glycerine or water. Methyl cellulose should be mixed with cold water, but ethyl cellulose should be dispersed in warm water. Other powder ingredients are sifted together and added gradually to mucilaginous mixture with continuous gentle stirring. Then aqueous media is mixed and set further to get the product. Flavour and detergent should be added at the last

In an alternative method the binder is premixed with solid abrasives and other powders and then poured in a suitable mixer (dough type mixer) along with aqueous solution of the humectant, preservative, sweetening agent, and mixing is done. After obtaining a homogeneous paste, flavor, and detergent. ⁽²⁴⁾

| s.no | Ingridients | Concentration (% w/w) | Role of ingredients |
|------|------------------------|--------------------------|----------------------------------|
| 1. | Nutmeg powder | 5 gm | Antibacterial and abrasive agent |
| 2. | Clove powder | 0.2 gm | Thickning agent |
| 3. | Methyl paraben | 0.2 gm | Preservative |
| 4. | Menthol | 0.1 gm | Cooling agent |
| 5. | Titanium dioxide | 0.4 gm | Whitening agent |
| 6. | Sodium lauryl sulphate | 2.5 gm | Detergent |
| 7. | Water | 7 ml | Vehicle |

Table no. 2 Ingridients used in toothpast

7. Evaluation Parameter

Organoleptic parameters

Organoleptic parameters including the colour, texture, appearance, taste and odour were evaluated by sensory and visual inspection of both the toothpastes.

pH levels

10 g of each toothpaste was dissolved in 10 ml of deionised water and stirred well to make a suspension in a 100 ml beaker. The pH was then measured using an EI pH meter.

Foaming ability

5 g of each toothpaste was weighed into a 100 ml glass beaker. 10 ml of distilled water was added to it and allowed to stand for 30 minutes (allowing the toothpaste to disperse in the water). The contents were stirred, and the slurry was transferred to a 250 ml graduated measuring cylinder. The residue in the beaker was rinsed and transferred with 5-6 ml portion of the water to the cylinder. The contents of this cylinder were then stirred to get a uniform suspension. A stopper was placed on the cylinder and subjected to 12 shakes.

Abrasiveness

A pea size amount of both the toothpastes was placed on separate clean plastic microscope slides and one drop of distilled water added to it. A clean cotton swab was rubbed on the toothpaste sample in a back- and-forth motion 30 times using short strokes. This was followed by carefully rinsing the slide and drying it with a soft tissue. The slide was examined under a dissecting microscope illuminated from above to determine the number of scratches on the surface of the slide. It was rated on a scale of 0 (no scratch) to 5 (high degree of scratches).⁽²⁶⁾

Gritty matter

A small amount of each toothpaste was rubbed into a piece of butter paper. The number and intensity of scratches that appeared on the butter paper was recorded as being absent or present. ⁽²⁷⁾

Homogeneity

A normal amount of force was applied on both the toothpastes which were contained in separate tubes at room temperature. It was observed whether the toothpaste extruded homogeneously from the tube or not. ⁽²⁸⁾

Stability

Some amounts of the toothpastes were transferred into 3 glass test tubes and a stopper was placed on them. These test tubes were heated at 45 degrees Celsius for 72 hours, allowed to cool and the content was examined visually for homogeneity, signs of fermentation and other deterioration results. It was reported as pass or fail. ⁽²⁹⁾

Spread ability.

The Brookfield CT3 texture analyser was used to measure the spread ability of the two toothpastes. The test was performed using the fixture base table and spread ability accessor. A conical shaped sample holder was filled evenly with the sample while the cone analytical probe was forced down into each sample at a defined test speed (2 mm/s) and to a defined depth (15 mm). The hardness of both toothpastes was recorded from the graph obtained, which is inversely proportional to their spread ability. ⁽³⁰⁾

Isolation of Oral Pathogens: The dental plaque sample was inoculated on blood agar, MRS and MacConkey plates and incubated for 18-24 hours at 37°C streak plate technique and the pathogens were isolated and identified by Bergey's manual. ⁽³¹⁾

8. Discussion

In recent years, there has been a paradigm shift from the usage of chemical to herbal products amongst the public. This could be due to the individual benefits offered by the herbal ingredients and the harmful effects of the chemical ones. A variety of ingredients have been used to formulate herbal toothpastes, with the exception of *Myristica fragrans* (Houtt.) or nutmeg. According to studies, nutmeg contains 25–30% fixed oils and 5–15% volatile oils, including substances like elimicin, myristic acid, dihydroguaiaretic acid, myristicin, lignan compounds, and various volatile oils. Recent research indicates that the mace, or aril of *Myristica fragrans* (Houtt.), has antibacterial effectiveness against the cariogenic *Streptococcus mutans*. However, nutmeg (the seed kernels of *Myristica fragrans* (Houtt.)) has not been used widely in studies against oral microorganisms.

9. Conclusion and result:

In conclusion the present study revealed that the ethanolic extract *myristica fragrans* can give antibacterial activity against oral pathogens. The results of the present study reveal that in comparison to the standard Dabur Red toothpaste, the formulated novel nutmeg toothpaste exhibits significant foaming ability, abrasivity, spreadability; and comparable texture, consistency, colour, gritty matter, homogeneity and stability properties.

However, it exhibits less favourable odour and taste. Thus, within the limitations of this study, it can be concluded that there is convincing evidence for the satisfactory physicochemical properties of the nutmeg toothpaste. However, further research is warranted to test other significant properties of the toothpaste.

| Sr.No. | Property | Nutmeg toothpaste |
|--------|--|---|
| 1 | Organoleptic parameters: | |
| | Colour | Brownish |
| | Texture | Smooth |
| | Appearance | Paste-like |
| | Taste | d) Slightly bitter |
| | Odour | Pungent |
| 2 | pH | 6.6 |
| 3 | Foaming ability (foam in cm) | 33 (acceptable) |
| 4 | Abrasiveness (on a scale of 0-5) | 2 |
| 5 | Gritty matter | Absent |
| 6 | Homogeneity (pass/fail) | Pass |
| 7 | Stability (pass/fail) | Pass |
| 8 | Spreadability (inversely proportional to hardness) | Hardness: 39.9g (more spreadability) |

Table no.3 Results of the physicochemical evaluation of the nutmeg

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