Original Article

Pro-Argin Technology Based Formulation of Eugenol Containing Toothpaste

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ABSTRACT

Introduction: Formation of cavities and hyper sensitivity are few dental problems, manifested by dental pain. Since long back, volatile oil present in clove buds (Myrtaceae) have been used as dental analgesic. Various toothpastes containing clove oil are marketed. Eugenol is chemically, terpenoids, active principle present in clove oil. It has several pharmacological potentials. Pro-argin technology is novel approach for formulation of toothpaste with arginine (8%) and calcium carbonate. **Aim:** This research was aimed towards formulation of toothpaste containing eugenol by Pro-argin technology and its evaluation. **Materials and Methods:** Toothpaste containing eugenol was formulated with arginine (8%) and calcium carbonate by simple trituration. Formulation was then evaluated for various parameters like pH, spreadability, foaming ability, content determination and also analysed for texture. **Results and Discussion:** On formulation, brownish, aromatic fragrant toothpaste was obtained with acceptable pH, spreadability and foaming ability. About 92.48% of eugenol content of added quantity was determined. **Conclusion:** Eugenol can successfully be formulated in toothpaste by Pro-Argin approach. More than 90% content determination would facilitate maximum release of added eugenol.

Key words: Clove oil, Eugenol, Pro-argin technology, Toothpaste, Texture analysis.

INTRODUCTION

Clove (*Syzygium aromaticum, Eugenia aromatica, Eugenia caryophyllata* or *Eugenia caryophyllus* of family *Myrtaceae*,[1] is a medium size plant with variety of biological activities like antioxidant and antimicrobial activities; thereby, since long ago, it has been advocated in traditional medicine for treatment of various illnesses pertaining to infection and inflammation.[2] As per traditional system of medicine, clove buds and clove oil are used as dental analgesic to relieve dental pain.[3] There are several clove oil based toothpastes available in market, namely Kudos Neem Clove, Colgate sensitive, Colgate Cibaca Vedshakti, Colgate Swarna Vedshakti, Lever Ayush Anti Cavity, Promise clove oil toothpaste, Dabur Herbal Clove toothpaste, Dabur Red toothpaste, Himalaya Sparkling white, Himalaya Active fresh gel, Sensodyne Herbal multi care.

Detail phytochemical analysis of clove buds revealed the presence of flavonoids (kaempferol and quercetin in glycosylated forms), phenolic acids (caffeic, ferulic, elagic, gallic and salicylic acids) and derivatives of hydroxyl-benzoic acids, hydroxy-cinnamic acids and hydroxyl phenyl propanes. Clove buds have also been reported to contain 18% of essential oil (clove oil) which contain eugenol (~75%), eugenyl acetate (~16%), β-caryophyllene (~5%) and α-humulen, β-pinene, limonene, farnesol, benzaldehyde, 2-heptanone, ethyl hexanoate in low concentrations.[2]

Eugenol ($C_{10}H_{12}O_2$; 164.2 g/mol, Figure 1), as a major signature phytocompound of clove/clove oil is a yellowish oily phenolic phenyl propane (2-methoxy-4-(prop-2-en-1-yl) phenol) with spicy aroma. Apart from clove, eugenol has also been reported to be present in several other biological sources like Tolu balsams of *Myroxylon balsamum* (Leguminosae), flowers of *Tilia* species

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(*Tiliacea*), oil of kernels of *Myristica fragrans* (*Myristicaceae*), fruits of *Pimenta dioica* (*Myrtaceae*), oleo-gum resin of *Commiphora molmol* (*Burseracea*), underground organs of *Valeriana officinalis* (*Valerianaceae*).[4]

Among variety of dental problems, dental caries and periodontitis are the major ones. Etiological factor causing dental caries were pathogens *Streptococcus mutans* and *Streptococcus sobrinus*. *S. mutans* were found responsible for formation of cariogenic biofilms which is made up of glucans synthesized from sucrose in presence of glucosyltransferases (*Gtfs*).[5] Also, for their survival, *S. mutans* decrease the pH in their habitat by secreting acids, resulting in demineralization of tooth enamel and thereby dental caries.[6] Periodontitis is a dental inflammatory disease, results from of immune response elicited by colonization of micro-organisms like *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia* and *Treponema denticola* in gingival crevice, reflecting destruction of soft tissues and bone.[7,8] All these clinical manifestations further lead to dental hypersensitivity, characterised by short but sharp pain usually arise when dentin is exposed to thermal, tactile, osmotic, evaporative or chemical stimuli which is not credited to other dental disease.[9] Therefore, it could be noted that prohibiting *S. mutans* to form acids and glucans and inhibiting periodontitis causing pathogens could be an effective therapeutic approach for the fixing of these dental problems. The strategy should also induce analgesia in order to get relief from dental pain associated.

Eugenol possess inhibitory effects against *S. mutans* at different levels like inhibiting acid production; synthesis of water-insoluble glucans and repressed the adherence of *S. mutans* to hydroxyapatite beads.[10,11] Eugenol also exhibit anti-microbial potential against the gram-negative oral pathogens, particularly *Prevotella intermedia, Porphyromonas gingivalis* and *Actinomyces viscosus*.[10] It has also been reported that eugenol has antinociceptive ability alleviating neuropathic pain, proven in mice.[12] In recent years, several attempts were to prepare nanoformulations containing eugenol and their characterization. Ahmad *et al.* 2018a formulated eugenol-encapsulated poly ε-caprolactone–nanoparticles and coated them with chitosan polymer. Their intranasal administration enhanced the eugenol bioavailability in rat brain, claimed after studying eugenol brain and plasma pharmacokinetic parameters. Toxicity studies revealed that eugenol-encapsulated poly ε-caprolactone –nanoparticles were safe.[13] Ahmad *et al.* 2018b screened the wound healing effects of eugenol nanoemulsion, formulated using excipients such as Tween-80, Labrasol and water. Further, it was applied topically in rats to study *in vivo* anti-inflammatory activity and *in vitro* skin permeation.[14] Ahmad *et al.* 2019 made eugenol nanoemulsion and characterized by particle size, PDI and transmittance; then formulate eugenol nanoemulsion–gel. Further, they characterized eugenol nanoemulsion–gel for pH, gelling capacity, syringeability, mucoadhesion, irritancy studies and *in vitro* drug release.[15] Hence, it can be concluded that treatment with eugenol can be effective therapeutic approach which can overcome these dental problems. As per our literature survey, surprisingly, so far no research attempt has been made to formulate toothpaste with isolated active principle of clove, eugenol. With the hypothesis that Pro-Argin based formulation of eugenol into toothpaste could be better option to treat dental problems, the present research includes formulation of toothpaste with eugenol by Pro-Argin technology and studies different pharmaceutical evaluation parameters.

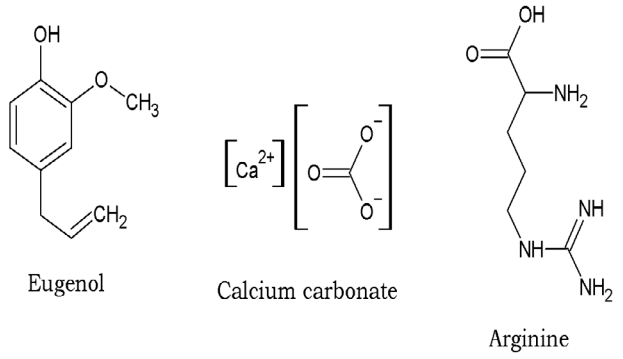

Figure 1: Structures of eugenol, calcium carbonate and arginine.

MATERIALS AND METHODS

Isolation of eugenol from clove oil and preparation of its UV-Visible spectroscopic calibration curve

Clove oil was purchase from Analab Fine Chemical Ltd. Mumbai and eugenol was isolated from it, following procedure described by Kokate, 2014.[16] Calibration curve was obtained by measuring absorbance of ethanolic solutions of eugenol (1, 2, 4 µg/ml).

Pro-Argin technology based formulation of eugenol containing toothpaste

Based on Pro-Argin technology, toothpaste was formulated by addition of Arginine (8%) and calcium carbonate to drug eugenol and other excipients in the quantity mentioned in Table 1. Possible chemical reaction of eugenol with mixture of arginine (8%) and calcium carbonate was first determined by FT-IR spectral analysis. All ingredients were added in increasing order of their

quantities and toothpaste was prepared by simple trituration. Purified water was added until the desired consistency attained.

Evaluation of toothpaste

Toothpaste was then evaluated for the various pharmaceutical parameters, right from preliminary organoleptic criteria to the drug content and texture analysis.

Colour, odour and taste

Colour, odour and taste were tested by sensory and visual inspection.

Foaming capacity

About 1 gm of toothpaste was added to 10 ml of water taken in measuring cylinder of 50 ml. It was then shaken for 10 min, after which volume of foam was measured directly by subtracting initial volume of 10 ml from the observed volume. The process was repeated thrice.

Determination of pH

About 0.5 g of toothpaste was diluted with 50 mL of distilled water in a 100 mL beaker and stirred well to. Its pH checked using previously calibrated pH meter within next 5 min. The process was repeated thrice.

Determination of spreadability

About 1 gm of toothpaste was placed at the centre of a clear glass plate of size 10 and same another plate was carefully placed on it so that sliding was avoided and pressed with weight of 2 kg. After 3 mins, diameter of toothpaste spread on bottom glass plate was measured. The process was repeated thrice.

Texture analysis

Further, toothpaste was evaluated for different texture parameters representing human sensory perceptions namely, firmness and adhesiveness or cohesiveness by using Texture Pro CT V1.3 Build 15 manufactured by Brookfield Engineering Labs, Inc. The results, in the form of graphs and other data were generated using software, Texture Pro CT V1.3 and formulation was evaluated in triplicate.

Content determination

In the present experiment, eugenol was added as drug. Its content in this newly formulated toothpaste was determined using calibration curve method. About 0.1 gm of toothpaste was shaken with 10 ml of ethanol and filtered. The volume of filtrate was again made upto 10 ml using ethanol. Its absorbance was measured at 280 nm based on calibration curve previously prepared for eugenol, concentration was determined.

RESULTS AND DISCUSSION

Isolated eugenol was found to occur as dark yellow liquid. It had absorbance maximum at 280 nm. Calibration curve of absorbance measured at 280 nm, measured for ethanolic solutions of different concentrations of eugenol (1, 2, 4 µg/ml) was not straight line; so Microsoft Excel based best fit equation was applied for further calculations of content determination from newly formulated toothpaste.

FT-IR spectrum (Figure 2) of blend made by mixing eugenol, arginine and calcium carbonate showed several peaks. Broad peak at 3334 cm^{-1} could be assigned to OH stretching vibrations of hydroxyl group (intramolecular H-bonding of carboxylic acid group of arginine and free hydroxyl group of eugenol). It might had overlapped NH stretching vibrations of amino and imine group of arginine. Peak at 1025 cm^{-1} could indicate C-O stretching vibrations of carboxylic group. Aromatic ring could be predicted by peaks at 1600, 1447 and 911-738 cm^{-1}. Peaks at 1265 and 1228 cm^{-1} were typical for ester linkage. Existence of these peaks proved that addition of eugenol to main excipients like arginine and cal-

Table 1: Formulation of toothpaste.

Ingredient	Content (%)
Eugenol	3
Calcium carbonate	40
Arginine	8
Carboxy methyl cellulose	2.5
Sodium lauryl sulphate	2.5
Methyl paraben	0.001
Saccharine sodium	0.001
Glycerine	10
Pomegranate extract	10
Distilled water	Quantity sufficient

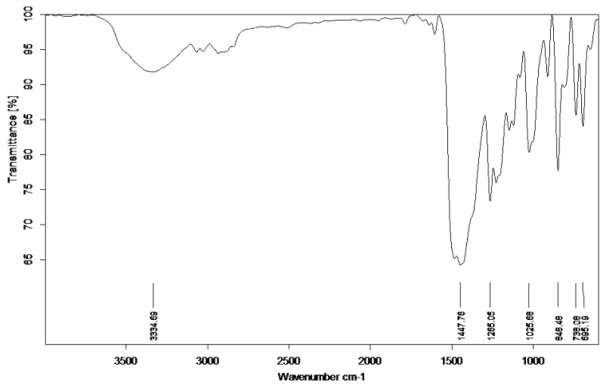

Figure 2: FT-IR spectrum of eugenol, arginine and calcium carbonate blend.

cium carbonate was compatible; no chemical reaction occurred in these ingredients, so that mixture was just physical blend.

So far, very few attempts have been made to formulate toothpaste containing clove oil or extract. Ellepola *et al.* 2011 compared antifungal activity of various marketed toothpaste brands against clinical isolates of *Candida albicans* and *Candida dubliniensis*; and found that brand Dabur Herbal, United Arab Emirates containing clove oil was most active against *Candida* species and thereby made higher zone of inhibition (tested by Sabouraud agar plate method).[17] Abhay *et al.* 2014 added clove oil to polyherbal toothpaste for taste masking of bitter extracts used.[18] Haque *et al.* 2014 formulated polyherbal tooth gel containing hydroalcoholic extract of clove and found that formulation containing 10 % extract has pH 6.9 and maximum zone of inhibition against *E. coli, S. typhi*, *S. aureus* and *P. aeruginosa*.[19] Hence, we can claim, our formulation with eugenol, as an active ingredient of clove oil was definitely a unique attempt.

As per simple sensory and visual inspection, toothpaste had acceptable light brownish colour, refreshing aromatic odour and characteristic taste giving feel of eugenol. As such eugenol is very spicy and pungent in taste but saccharine sodium was a sweetener added to improve the extreme spicy taste of eugenol in toothpastes. Foaming capacity of toothpaste was found to be 10 ml. This foaming action is very important for better patient compliance as it provides the feel of being clean and healthy at oral cavity. It was the due function of anionic surfactants, sodium lauryl sulphate added. The pH was observed in range of 6.5 to 8.0. Spreadability in terms of diameter was found to be 5 cm.

Texture is a combination of mechanical, geometrical and surface attributes of a product as perceived by means of mechanical, tactile, visual and auditory receptors in humans. To determine these attributes, Szczesniak, 1963 launched the concept of 'two-bite test' or texture profile analysis (TPA) which uses various protocols, one of which is one-deformation.[20] One-deformation tests, here determined various parameters such as firmness (the maximum force in the first compressive deformation of an object), 1700 G; adhesiveness or cohesiveness (the maximum negative force in the first deformation), -700 G. Using instrument Texture analysers, all texture attributes could be determined by applying controlled forces to the semi solid formulation and recording its response in the form of force of deformation and time Figure 3. As such, these parameters do not have any fixed selection criteria or any pharmacopoeial or regulatory standards, but based on the requirements specific for particular product; these parameters

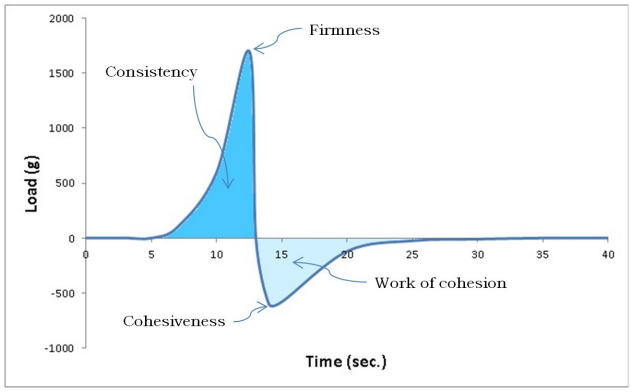

Figure 3: Texture analysis report.

can be characterized and standardized.[21] Formation of gel phase containing a consistent distribution of all ingredients was due to viscosity and rheology modifier, carboxy methyl cellulose, added. Homogeneity of formulation was because of glycerine, humectant used to avoid 'capping-off', i.e. drying out of paste at the dispensing point due to loss of moisture. It also favoured a smooth and glossy appearance. The curve of load as a function of time showed decrease in firmness in next deformation. This could be related to a destruction of the original microstructure of the system established on standing at ambient conditions.

Theoretically, about 0.3 gm (1.498 %) of eugenol was added to the formulation. Now, from the toothpaste, eugenol contents was found to be 0.13 mg/gm of toothpaste, indicating presence of 2.74 mg (0.13 × 20) of eugenol contents, thereby representing determination of 92.48% of theoretical eugenol content.

CONCLUSION

Eugenol is an active ingredient present in clove oil, responsible for antimicrobial effect in oral cavity and analgesic for dental pain. IR spectrum revealed that eugenol, calcium carbonate and arginine, on mixing, do not get interacted with each other and retain their properties. Toothpaste so formulated was brown with acceptable odour and skin compatible pH. Addition of sodium lauryl sulphate exhibited sufficient foaming of toothpaste. Maximum eugenol content was determined from toothpaste, indicating sufficient release of eugenol. Using Pro-argin technology, it could be concluded that eugenol can be formulated in toothpastes and combination can be used even for dental sensitivity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

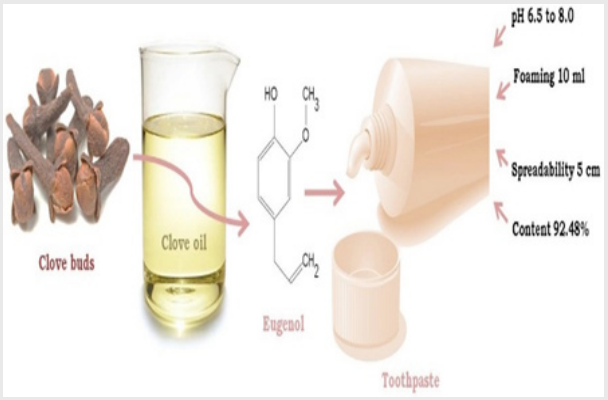
ABBREVIATIONS

FT-IR: Fourier-transform infrared spectroscopy; **PDI:** Polydispersity Index; **TPA:** Texture profile analysis.

REFERENCES

1. Bownik A. Clove essential oil from *Eugenia caryophyllus* Induces Anesthesia, Alters Swimming Performance, Heart functioning and Decreases Survival Rate during Recovery of *Daphnia magna*. Turk J Fish Aquat Sci. 2015;15(1):157-66.
2. Cortés-Rojas DF, DeSouza CRF, Oliveira WP. Clove (*Syzygium aromaticum*): A precious spice. Asian Pac J Trop Biomed. 2014;4(2):90-6.
3. Curtis EK. In pursuit of palliation: Oil of cloves in the art of dentistry. Bull Hist Dent. 1990;38(2):9-14.
4. Evans WC. Trease and Evans Pharmacognosy. Elsevier 15th Ed. New Delhi. 2005.
5. Hamada S, Slade HD. Biology, immunology and carioge¬nicity of *Streptococcus mutans*. Microbiol Rev. 1980;44(2):331-84.
6. Belli WA, Marquis RE. Adaptation of *Streptococcus mutans* and *Enterococcus hirae* to acid stress in continuous culture. Appl Environ Microbiol. 1991;57(4):1134-8.
7. Apatzidou DA, Riggio MP, Kinane DF. Quadrant root planning versus same day full mouth root planing II. Microbiological findings. J Clin Periodontol. 2004;31(2):141-8.
8. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. Periodontol. 1994;5:78-111.
9. Jena A, Shashrekha G. Comarison of efficy of three different desensitizing agents for in-office relief of dentin hypersensitivity: A4 week clinical study. J Conserv Dent. 2015;18(5):389-93.
10. Cai L, Wu CD. Compounds from *Syzygium aromaticum*[1] possessing growth inhibitory activity against oral pathogens. J Nat Prod. 1996;59(10):987-90.
11. Xu JS, Li Y, Cao X, Cui Y. The effect of eugenol on the cariogenic properties of *Streptococcus mutans* and dental caries development in rats. Experimental and Therapeutic Medicine. 2013;5(6):1667-70.
12. Kurian R, Arulmozhi DK, Veeranjaneyulu A, Bodhankar SL. Effect of eugenol on animal models of nociception. Indian J Pharmacol. 2006;38(5):341-5.
13. Ahmad N, Ahmad FA, Bedi S, Sharma S, Umar S, Ansari MA. A novel Nanoformulation Development of Eugenol and their treatment in inflammation and periodontitis. Saudi Pharm J. 2019;27(6):778-90.
14. Ahmad N, Ahmad R, Alam MA, Ahmad FA. Quantification and Brain Targeting of Eugenol-Loaded Surface Modified Nanoparticles through Intranasal Route in the Treatment of Cerebral Ischemia. Drug Res. 2018a:68(10):584-95. https://doi.org/10.1055/a-0596-7288
15. Ahmad N, Alam MA, Ahmad FJ, Sarafroz M, Ansari K, *et al*. Ultrasonication techniques used for the preparation of novel Eugenol-Nanoemulsion in the treatment of wounds healings and anti-inflammatory. J Drug Del Sci Tech. 2018b;S1773-2247. https://doi.org/10.1016/j.jddst.2018.06.003
16. Kokate CK. Practical pharmacognosy. Vallabh Prakashan. 5th Ed. New Delhi. 2014.
17. Ellepola ANB, Khan ZU, Chandy R, Philip L. A Comparison of the Antifungal Activity of Herbal Toothpastes against Other Brands of Toothpastes on Clinical Isolates of *Candida albicans* and *Candida dubliniensis*. Med Princ Pract. 2011;20(2):112-7. http://10.1159/000321199
18. Abhay S, Dinnimath BM, Hullatti KK. Formulation and Spectral Analysis of New Poly Herbal Toothpaste. J Drug Del Ther. 2014;4(6):68-74.
19. Haque M, Singh AK, Maurya SK, Seth A. Formulation development, physico-chemical characterization and evaluation of antimicrobial activity of herbal tooth gel. J Chem Pharm Res. 2014;6(3):1279-85.
20. Szczesniak AS. Classification of textural characteristics. J Food Sci. 1963;28(4):285-9.
21. Meher JG, Yadav NP, Sahu JJ, Sihna P. Determination of required hydrophilic-lipophilic balance of citronella oil and development of stable cream formulation. Drug Dev Ind Pharm. 2013;39(10):1540-6.

PICTORIAL ABSTRACT



SUMMARY

Eugenol was extracted from clove oil and formulated in toothpaste using Pro-Argin technology approach by adding excipients mainly arginine and calcium carbonate. Toothpaste obtained was having acceptable organoleptic properties and analysed for its texture and also quantified for eugenol. IR interpretation shows no interaction between eugenol, arginine and calcium carbonate.

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