RESEARCH PAPER

Antimicrobial Tooth Powder Formulated from Punica Granatum Fruit Juice: Comparative Analysis of Plain, Spray-Dried, And Freeze-Dried Variants Against Streptococcus Mutans and Candida Albicans.

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ABSTRACT

The goal of the experiment was to prepare an antimicrobial tooth powder using the fruit juice of Punica granatum for the treatment of dental caries. Plain pomegranate fruit juice, spray-dried fruit juice, and freeze-dried fruit juice were compared for their antimicrobial activity against Streptococcus mutans and Candida albicans and further formulated into toothpowder.Punica granatum fruit juice was extracted sprayed and lyophilized. These powders were further transformed into toothpowder. Conversion of liquid juice to solid powder: Pomegranate juice and Neusilin US2 were mixed in a 1:1 weight ratio to obtain a free-flowing juice powder. Spray-dried powder formulation Maltodextrin and β-cyclodextrin were slowly added to deionized water (67%) and heated to 51°C and the resulting solution was obtained. The obtained solution is cooled at room temperature. Pomegranate fruit juice (33%) was added to solution, stirred for 1 hour, and transferred to a spray dryer. Freezing of pomegranate juice Fresh pomegranate juice (20 ml) was filtered and frozen at 2-3 ºC. The cold juice was transferred to a freezer with a 15-minute heating time. After complete drying (20-24 h), Neusilin US2 (3 g) was added as an adsorbent to remove the stickiness of the freeze-dried powder from the powder mass.Pomegranate juice sprayed and freeze-dried fruit juice should have good antimicrobial activity. Based on the obtained data, it can be concluded that 7.5% spray-dried tooth powder and freeze-dried tooth powder have excellent antimicrobial activity in tooth powder...

Keywords: Pomegranate, antioxidant, tooth powder, anti-fungal, anti-microbial.

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INTRODUCTION

Oral health plays a pivotal role in overall well-being, with dental caries, plaque, and gum diseases being betweenutmost prevalent global health concerns[1-2]. The maintenance of oral hygiene primarily depends on the regular use of dentifrices such as toothpaste, tooth powder, and mouthwashes, which aid in the mechanical removal of food debris, plaque, and microorganisms from the oral cavity[3-4]. Dentifrices are designed to be applied to the teeth with the aid of a toothbrush to clean, polish, and protect the accessible tooth surfaces. Over the years, advancements in oral care products have led to the development of formulations with multifunctional claims, including whitening effects, breath freshening, and antibacterial actions[5-7].

Among the common forms of dentifrices, toothpaste is widely used and contains key components such as abrasives, binders, surfactants, humectants, and flavoring agents[8-9]. Tooth powders, though less common today, also serve a similar purpose, primarily employing abrasives like calcium carbonate for cleaning. Mouthwashes, on the other hand, offer an adjunctive approach to oral care, targeting microbial populations and providing temporary relief from halitosis[10-12].

Despite the availability of various oral hygiene products, the incidence of dental diseases such as plaque formation, root caries, and dental caries remains significant[13-14]. The acid produced by bacteria such *Lactobacillus spp., Streptococcus sobrinus,* and *Streptococcus mutans* causes demineralization and loss of dentin, enamel, and cementum in dental caries, an illness caused by bacteria. These bacteria metabolize fermentable carbohydrates, producing acids that compromise the integrity of the tooth structure. If left untreated, dental caries can lead to toothache, infection, and tooth loss[15-17].

Plaque, a soft, sticky biofilm rich in bacteria, forms naturally on the teeth and plays a vital role in pathogenesis of dental caries and periodontal diseases[18-20]. Inadequate plaque removal results in tartar formation, which exacerbates gum inflammation and promotes conditions such as gingivitis and periodontitis. Root caries, another significant concern, typically occurs at the cement-enamel junction, often progressing to wide, flat lesions if not addressed[21-23].

Because of their antibacterial qualities and lower risk of adverse effects, natural products and herbal extracts have recently attracted a lot of attention as potential ingredients in dental care formulations.*Punica granatum* (pomegranate) is a well-documented medicinal plant

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known for its potent antibacterial, antifungal, and antioxidant activities[24-26]. Pomegranate juice, whether in its plain, spray-dried, or freeze-dried form, has demonstrated efficacy against oral pathogens such as Streptococcus mutans and Candida albicans. These properties make it a promising candidate for incorporation into dentifrice formulations aimed at preventing and managing dental caries and other microbial oral infections[27-30].

Considering the growing resistance to synthetic antimicrobial agents and the increasing demand for herbal-based oral care products, current study is focused on formulation and evaluation of an antibacterial toothpaste incorporating *Punica granatum* juice[31-33]. By comparing the antimicrobial efficacy of different forms of pomegranate juice—plain, spray-dried, and freeze-dried—against *Streptococcus mutans* and *Candida albicans*, the study aims to develop a safe, effective, and natural dentifrice formulation for the management of dental caries[34-35].The prime objective of this study is to formulate and evaluate an antibacterial toothpaste containing *Punica granatum* juice and to assess comparative antimicrobial efficacy of its different processed forms against key oral pathogens. The objective of the research is to determine if pomegranate can replace conventional dental care products in warding off cavities, plaque, and the diseases that may develop from them[36-37].

MATERIAL & METHODS

MATERIAL

The pomegranate fruits used in the formulation of the toothpaste were locally sourced from a fruit vendor in Pune during January 2018. Neusilin US2 was graciously provided by Gangwal Chemicals Pvt. Ltd., Mumbai, India. Mumbai-based Hi-Media Laboratories supplied maltodextrin and nutritional agar. The β-Cyclodextrin was received from Research-Lab Fine Chem Industries in Mumbai, India. Streptococcus mutans was purchased from Monera Labs, Pimpri, Pune.Sabouraud agar and other required microbiological media were also obtained from Hi-Media Laboratories, Mumbai, India[11-12].

METHODS

Pomegranate juice was extracted by cold pressing fruit grains through a screw juicer and stored refrigerated. To make powder (PJP), juice was mixed with Neusilin US2 (1:1 w/w). For spray drying, maltodextrin (17%) and β-cyclodextrin (3%) were dissolved in heated water, mixed with juice, then spray dried at 180°C inlet temperature and 3 ml/min feed rate to produce spray dried powder (SDJ). For freeze drying, filtered juice was frozen at 2–3°C, lyophilized for 20–24 hours, then mixed with Neusilin US2 (3 g) to reduce stickiness, yielding freeze dried powder (FDJ). Both powders were stored in closed containers.

Preformulation study

Organoleptic characteristics

Pomegranate juice, spray-dried and freeze-dried powder samples were analyzed for color, odor, and appearance.

Determination of solubility

To determine solubility, pomegranate powder was placed in different test tubes containing different solvents (distilled water, DMSO, acetone, methanol, ethanol). Afteraddition of each portion of solvent, the test tube was shaken vigorously and visually inspected.

Flow properties

Flow properties of pomegranate juice, spray dried and freeze-dried powders were determined as follows:

The device was placed on the surface. Powder (1 g) was placed on the flat surface of the apparatus. The tilt was manually increased by 1.5 degrees/sec. The downward angle of the powder was recorded as the slip angle.Gliding angle specification Angle from 0 to 80°.

Determination of antibacterial activity

Cup plate method

Preparation of inoculum

The caries-causing fungal pathogen selected in this study is*Streptococcus mutans* ATCC 25175 (obtained from Monera Lab Pimpri, Pune). To make testing inoculum, bacteria were cultivated on nutrient agar at 37° C for 24 hours.

Preparation of Nutrient agar media

The media was prepared by Beef extract (10 g), peptone (10g), sodium chloride (5.0g), agar (20 g), water (1000 ml) and adjusted to pH to 7.0. Then the media was sterilised at 121ºC, 15 lbs pressure for 15 min in autoclave.

Determination of zone of inhibition

The antibacterial activity of pure pomegranate juice powder, spray dried powder and freeze dried powder sample was measured by cup plate method. Using a laminar flow unit, sterile media is poured into Petri dish and the sterile condition are maintain at depth of 3-4 mm a uniform medium was spread on a Petri dish after solidification. The subculture was placed on surface of agar media and spread on medium through the help of loop. After the culture had stabilized, a 6 mm diameter hole was punched with a sterile cork borer and scraped from the Petri dish. Various concentrations of pure pomegranate juice powder, spray-dried powder, and freeze-dried powder 100, 500, 1000, 1500 mg/ml were dissolved in dimethyl sulfoxide and added to beakers. The next step was to incubate the Petri dishes at 37°C for a full day. Using a zone reader, the inhibition zones were measured after incubation[17].

Broth dilution method

A nutritional broth tube with a twofold concentration was made and labeled. For the sake of the negative control, the first tube, UT, was not infected. A control tube (CT) without pomegranate juice and inoculum was used. Tubes c, d, e, f, and g were each given a different concentration of pomegranate juice: 1, 3, 5, 8, and 10 ml. All of the tubes were inoculated with three drops of microorganisms to achieve a final concentration of 106 cells/ml. Except for the control tubes (positive) and the uninoculated tubes (negative control), pomegranate juice was added to all of the other tubes (Table 5). To ensure that the medium was suitable for Streptococcus mutans growth, a positive control tube (CT) was used. Before being incubated at 37 °C for 48 hours, each tube was shook (17). The data are presented as the mean of three independent trials[19].

Table1: Determination of MICthrough broth dilution method against *Streptococcus mutans*

Volume of double strength medium (ml)	Tube number	Concentration of pomegranate juice (ml)
5	(UT) a	0.0
5	(CT) b	0.0
5	C	1
5	D	3
5	E	5
5	F	8
5	G	10

* (UT)-uninoculated, (CT)- Control

Minimum bactericidal concentration (MBC)

MIC confirmation test. the minimum concentration of the antimicrobial agents required to kill a specific type of bacteria this is determined using the minimum inhibitory concentration (MIC) broth dilution test. this tests was performed by subculturing broth from the test tube with a visible growth of MIC and are grown on agar plate, which are incubated at 37°c for 1-2 days if there is no growth of bacteria was absorbed in the MIC tube than the growth is observed on agar plate, even if a certain MIC was inhibited, the antibacterial agent would not be killed, confirming the possibility of the growth of the bacteria by spreading the bacteria on the agar. It was done[18, 30].

Determination of antifungal activity

Cup plate method

Preparation of inoculum

The fungal pathogen included in this study was *Candida albicans* ATCC 10231 (obtained from the Department of Biochemistry, Savitribai Phule University). Fungi were grown in subard dextrose medium and incubated at 37°C for 24 hours to obtain the inoculum for testing.

Preparation of Sabouraud dextrose agar media

The medium was prepared by mixing 10 grams of dextrose, 2.5 grams of peptone, 3.75 grams of agar, 250 milliliters of water, and a pH adjustment to 5.8. After that, it was autoclaved at 121°C for 15 minutes under 15 lbs of pressure to sterilize the medium.

Determination of zone of inhibition

The antifungal effects of three different types of greasepaint—plain pomegranate juice, spot teetotaler, and snap teetotaler—were tested in a sterile environment with laminar airflow. The media was placed into a Petri dish and left to sit for three to four minutes. A Petri plate was used to uniformly distribute the midium cultures. Once the agar had solidified, a circle of folklore (Candida albicans) was added to its surface and gently spread with a sterile spreader. After the culture had been stabilized using a sterile cork borer, mugs with a 6 mm perimeter were punched and submerged in the petridish. Separately, 100, 500, 1000, and 1500 milligrams of spot teetotaler greasepaint, snap teetotaler greasepaint, and plain pomegranate juice were added to the cup. Additionally, petridishes were incubated at 37 °C for 24 hours. Using zone anthology, the inhibition zone was assessed after incubation[18].

Broth dilution method

There was a preparation and labeling of nutrient broth (double strength) test tubes. As a control, the first tube (UT) was left uninoculated. As a control, inoculum was supplied to tube b (CT), but pomegranate juice was omitted.In tubes c, d, e, f, g, h pomegranate juice was added in attention of0.5, 1,1.5, 2,2.5, 3 ml independently. Inoculum(3- 4 drops) was added to all tubes to get the final attention of microorganisms of 106 cells/ ml. Pomegranate juice was added in all test tubes except uninoculated(negative control) and control(positive) tube. To ensure the medium was suitable for Candida albicans development, a positive control tube (CT) was used. According to Table 5, all of the test tubes were shaken and then incubated at 37°C for 48 hours. The results were presented as the average of three independent experiments[30].

Table2: Determination of MIC by broth dilution method against *Candida albicans*

Volume of double strength medium (ml)	Tube number	Concentration of pomegranate juice (ml)
5	(UT) a	0.0
5	(CT) b	0.0
5	C	0.5
5	D	1
5	E	1.5
5	F	2
5	G	2.5
5	H	3

* (UT)-uninoculated, (CT)- Control

Formulation

The study developed a tooth powder using pomegranate juice powders (plain, spray dried, and freeze dried) as antimicrobial agents against dental caries. Pomegranate juice was converted into a free-flowing powder by blending with Neusilin US2 (1:1 ratio). Formulations contained 7.5% pomegranate powder, 90–95% calcium carbonate (abrasive), 0.5–3% sodium lauryl sulphate (foaming agent), and peppermint oil for flavor. Antibacterial efficacy against Streptococcus mutans and Candida albicans was confirmed, with doses based on the MIC and zone of inhibition. The powders were mixed with excipients and triturated to prepare the final tooth powders coded as TJ, TS, and TF.

Table 3: Formulation of tooth powder using *Punica granatum* juice and powder

Formulation code		TJ1	TJ2	TJ3 #	TS1	TS2	TS3 #	TF1	TF2	TF3 #
	PJP (gm)	0.75	0.75	0.75	–	–	–	–	–	–
	SDJ (gm)	–	–	–	0.75	0.75	0.75	–	–	–
	FDJ (gm)	–	–	–	–	–	–	0.75	0.75	0.75
Calcium carbonate (gm)		9.15	9.1	9.05	9.15	9.1	9.05	9.15	9.1	9.05
SLS (gm)		0.1	0.15	0.2	0.1	0.15	0.2	0.1	0.15	0.2
Peppermint oil (gm)		qs	qs	qs	qs	qs	qs	qs	qs	qs
Total (gm)		10	10	10	10	10	10	10	10	10

TJ: Plain pomegranate juice tooth powder
TS: Spray dried tooth powder
TF: Freeze dried tooth powder
#: Based batches

Evaluation tests for tooth powder

Particle shape & size

Particle shape and size were measured under microscope.

pH

The powder was dissolved in distilled water and pH was measured by digital pH meter (Systronics Instruments, India).

Solubility test

To determine the solubility of pomegranate juice, the powder was placed in different test tubes containing different solvents. After addition of each portion of solvent, the test tube was shaken vigorously and visually inspected.

Density

Density determine TJ, TS, TF (bulk density and tapped density)

Flow properties

Flow properties were studied as follows:

Hausner ratio

Angle of repose

Angle of slide

Moisture content

Place 5-10 g of powder sample in a Petri dish and weigh (W1). Place the Petri dish in the oven to dry at 105°-110°C for 1 hour. Remove the Petri dish from the oven, cover and allow to cool, and weigh the Petri dish and lid together after cooling (W2). Clean, dry and weigh the petri dish (W3).

Foaming test

A 1 g powder sample was weighed and poured into a graduated cylinder (100 ml) containing 10 ml of distilled water. After shaking for 2 minutes to generate foam, the graduated cylinder was allowed to stand for 10 minutes and the height of the foam was measured[35].

***In vitro* Antimicrobial study**

Antibacterial study

In a 250 mL Erlenmeyer flask, 100 mL of distilled water was added to dissolve the nutritional agar. 7.0 ± 0.2 was the pH that was set. A 15-pound autoclave was used to sterilize the medium for 15 minutes at 121° C. The sterile Petri plates are filled with the medium after it has cooled to room temperature and has been passed through a laminar flow device. The solidification process begins with a Petri dish filled with medium and placed in a laminar airflow unit. Next, a suspension culture of Streptococcus mutans is diluted in nutrient broth and spread evenly over the surface of solidify agar. The culture is then allowed to stabilize before a sterile cork is used to bore a cup out of the plate. Finally, the plate is incubated at 37°C for 24 hours[18].

Antifungal study

The 250 ml of erlenmeyer fresh was mixed with 100 ml of water to dissolve a sabourand dextrose, and the pH was adjusted to 5.6±0.2. The 15-pound autoclave is set at 121°C for 15 minutes in order to sterilise the medium. The medium is chilled to room temperature after sterilization and then put onto a sterile Petri dish that is being circulated by a laminar airflow machine. Once the medium in a Petri dish has solidified, a loop of suspension culture (Candida albicans) diluted in nutrient broth is deposited on top of the solidified agar using a spreader. The dish is then kept in a laminar airflow device for further solidification. When the culture had stabilized, it was punched out with a sterile cork borer in a uniform distribution. The next step was to incubate the Petri dishes at 37°C for a full day. Measurements were taken of the zone of inhibition after culture[17].

RESULTS AND DISCUSSION

Pomegranate juice extraction was standardized based on yield, pigment content, and acceptability. For spray drying, adding β-cyclodextrin with maltodextrin and juice slightly altered the powder's color to yellowish and increased soluble solids, affecting color, moisture, and astringency, which could be managed by adjusting the inlet temperature. Freeze-dried pomegranate powder showed better quality in moisture content, solubility, bulk density, water activity, and flow properties, though solubility gradually decreased over four weeks due to increased bulk density.

Preformulation study of pomegranate juice

Organoleptic Characteristics of PJP, SDJ and FDJ

Table 4: Organoleptic characteristics of PJP, SDJ and FDJ

Powder code	Colour	Odour	Appearance
PJP	Light pink	Bitterness	Light pink crystal

SDJ	Pink	Bitterness	Pink crystal solid
FDJ	Light blue	Bitterness	Blue crystal solid

PJP: Plain pomegranate juice powder,
SDJ: Spray dried juice powder
FDJ: Freeze dried juice powder

Determination of solubility

PJP, SDJ and FDJ are soluble in distilled water, DMSO, acetone, methanol, and ethanol.

Flow properties

Flow properties of pomegranate juice, spray dried and freeze-dried powders were resulted as follows:

Table5: Flow properties of PPJ, SDJ and FDJ

Powders	Bulk density (gm/ml)	Tapped density (gm/ml)	Hausner Ratio	Angle of repose (Degree)	Angle of slide (Degree)
PPJ	0.36	0.42	1.16 (Good)	27.47 Excellent	35.33 (good)
SDJ	0.59	0.60	1.2 (Fair)	30.11 (Good)	34.33 (good)
FDJ	0.8	0.95	1.18 (Good)	32.61 (Good)	35.66 (good)

Determination of anti-bacterial activity

Cup plate method (Spray dried &freeze-dried powder)

In-vitro antibacterial activity was performed against ***Streptococcus mutans***.To measure the antimicrobial efficacy of the spray-dried and freeze-dried powders, the mean zone of inhibition was computed. 'A' bore contains DMSO which showed no inhibitory zone.

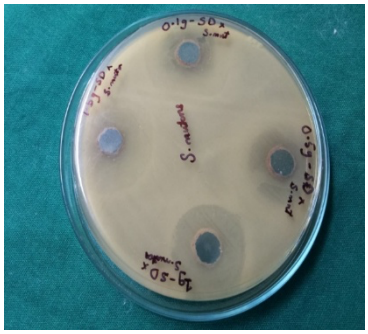


Figure1: Zone of inhibition of Spray dried powder

Table 6: Zone of inhibition of various concentrations of spray dried powder

Concentration (gm/ml)	Diameter of zone, (mm)
0.1	14.2± 0.5
0.5	21.5± 0.5
1	24.1±0.5
1.5	29.8±0.5

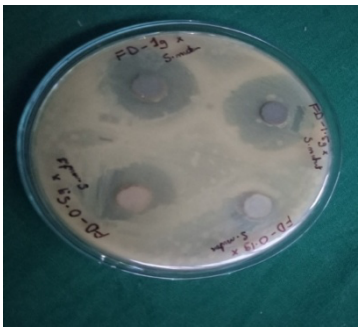


Figure 2: Zone of inhibition of freeze-dried powder

Table7: Zone of inhibition of various concentrations of freeze-dried powder

Concentration (gm/ml)	Diameter of zone, (mm)
0.1	14.2± 0.5
0.5	21.5± 0.5
1	24.1±0.5
1.5	29.8±0.5

Determination of MIC (Pomegranate juice)

The lowest concentration of Pomegranate juice that reduced the observable development of Streptococcus mutans was determined to be the MIC. C (1 ml) demonstrated complete development of Streptococcus mutans, CT (b) served as a control, and UT (a) inoculated meant that the tube had not been inoculated with Streptococcus mutans. No notable death of Streptococcus mutans was seen in test tubes E and D (3, 5 ml). It was observed that the pomegranate juice suppressed the development of Streptococcus mutans in test tubes (8ml) and 10ml, respectively, due to the presence of turbid medium.

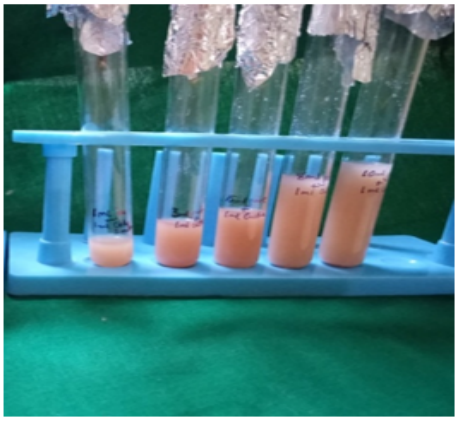


Figure3: Determination of MIC by pomegranate juice against (Streptococcus mutans)

Table 8: Determination of MIC by broth dilution methodMBC

Tube number	Visual results
(UT) a	Clear
(CT) b	Turbid
C	Turbid
D	Turbid
E	Turbid
F	Turbid
G	Slightly turbid

The no growth in MIC tube is shown growth on agar plate which means that even if the particular MLC show inhibition plating and the bacteria are into agar might still result in organised profitability because of the antimicrobial do not cause death. Then to determine the dose of pomegranate juice MBC was performed. The test tubes were labelled as UT (a) for uninoculated, CT (b) for control and C, D, E, F, G for pomegranate juice of 1, 3, 5, 8, 10ml respectively. All the test tubes C, D, E, F, G have been found to be clear. Which means than there was no growth in the test tubes and MBC of pomegranate juice against *S. mutans* was found 1ml.

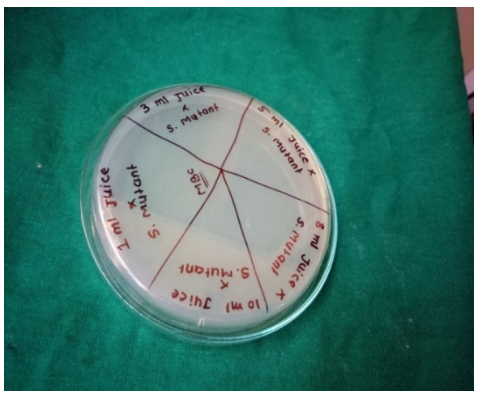


Figure 4: Minimum bactericidal concentration (MBC)

Table 9: Determination of MBCby broth dilution method

Tube number	Visual results
(UT) a	Clear
(CT) b	Turbid
C	Clear
D	Clear
E	Clear
F	Clear
G	Clear

Minimum bacterial concentration of pomegranate juice against *S. mutans*is 1 ml analysed.

Determination of anti-fungal activity

Cup plate method (Spray dried &freeze-dried powder)

In-vitro antifungal activity was performed against *C. Albicans* by zone of inhibition using cup plate method. The spray dried powder of pomegranate juice (gm/ml) showed zone of inhibition of 29.5± 0.5mm. Freeze dried powder (gm/ml) showed 28 ± 0.5 mm of zone of inhibition. Increase in concentration of spray dried powder or freeze dried powder showed increase in zone of inhibiton. Spray dried powder showed good antifungal activity as compared to freeze powder.

(a): Spray sried powder (b): Freeze dried powder

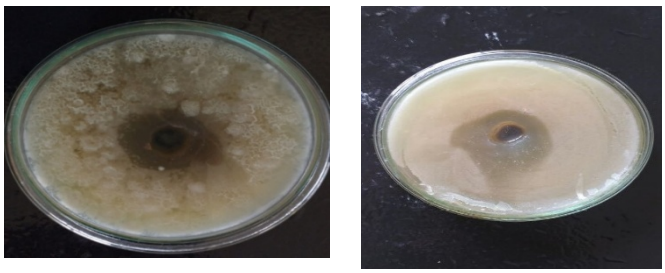


Figure 5: Antifungal activity of spray dried and freeze dried powder against *Candida albicans*

Table 10: Zone of inhibition of various concentrations of spray dried and freeze-dried powder

Test microorganisms	Sample name	Concentration (gm/ml)	Diameter of zone(mm)
C. albicans	Spray dried powder	1.5	29.5± 0.5
C. albicans	Freeze dried powder	1.5	28 ± 0.5

Determination of minimum inhibitory concentration of pomegranate juice

At what concentration of pomegranate juice does the observable inhibition of Candida albicans development begin to diminish? This is known as the minimum inhibitory concentration (MIC). One set of tubes was designated as CT (control) and the other as UT (a), meaning that Candida albicans was not introduced into either of them. c) Candida albicans grew to full size in 0.5 ml. No significant death of Candida albicans was observed in the D and E test tubes (1, 1.5 ml). The medium was obviously clear in F (2 ml).G & H (2.5 to 3ml) showed clear media which indicated that pomegranate juice inhibited the growth of *Candida albicans* at concentration of 2ml. Hence, the pomegranate juice showed antifungal activity at MIC value of 2ml.

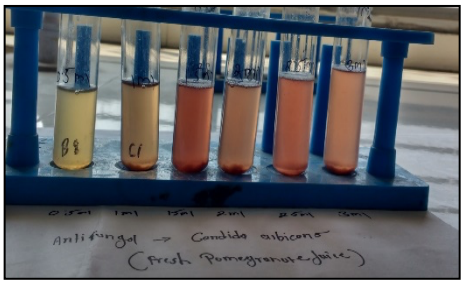


Figure 6: Estimation of MIC by broth dilution method

Table 11: Determination of MIC by broth dilution method

Tube number	Visual results
(UT) a	Clear
(CT) b	Turbid
C	Turbid
D	Turbid
E	Slightly turbid
F	Clear
G	Clear
H	Clear

Minimum inhibitory concentration of pomegranate juice against *C. albicans*2ml analyzed.

Formulation & development

Method of formulation of plain pomegranate juice powder

The proper ratio of the Neusilin US2 as an adsorbent was added to pomegranate juice to produce the free-flowing powder of pomegranate juice. The equal amount of Neusilin US2 and pomegranate juice (1:1w/w) produced free flowing powder.

Formulation of tooth powder

Dose calculation:

For zone of inhibition (ZOI) test, 1500 mg of pomegranate juice powder was dissolved in 1 mL DMSO, and 0.1 mL (150 mg) was used, yielding a 29 mm ZOI. Since 2 g of formulation required 150 mg powder, dose for a 10 g tooth powder formulation was calculated as 0.75 g.

Preliminary trial batches

Selection of pomegranate juice dose

Pomegranate juice showed antimicrobial action against bacteria *Streptococcus mutans* and fungi *Candida albicans.* The MIC against bacteria and fungus was found to be 1ml. Thus, it was considered as final dose.

Selection of plain pomegranate juice powder

The plain pomegranate juice powder showed antimicrobial action against bacteria *Streptococcus mutans* and fungi *Candida albicans.* The zone of inhibition to against bacteria and fungus was found to be 1.5gm/ml and was considered as final dose.

Selection of spray dried powder

Powder was tested for its antimicrobial activity against bacteria *Streptococcus mutans* and fungi *Candida albicans.* Spray dried powder showed good antimicrobial activity against bacteria as well as fungus at the concentration of 1.5gm/ml and it was considered as final dose.

Selection of freeze-dried powder

The antibacterial activity of the freeze-dried powder was further evaluated against *Streptococcus mutans* and *Candida albicans*, two bacteria and fungus, respectively. At a concentration of 1.5 gm/ml, the freeze-dried powder demonstrated a zone of inhibition against fungus and was deemed the final dosage.

Formulation of tooth powder using *Punica granatum* juice and juice powder

A tooth powder was formulated by using plain fruit juice powder, spray dried powder and freeze-dried powder of *Punica granatum* as an antimicrobial agent separately. Formulated tooth powder was coded as TJ3 (plain pomegranate tooth powder), TS3 (Spray dried tooth powder) and TF3(Freeze dried tooth powder) respectively.

Evaluation test for tooth powder

Particle size & shape

Particle size of formulated tooth powder found to be in the range 74 to 210 microns.Particle shape of formulated tooth powder was determined by under microscope. Particle shape of formulated tooth powder found to be the spherical in shape.

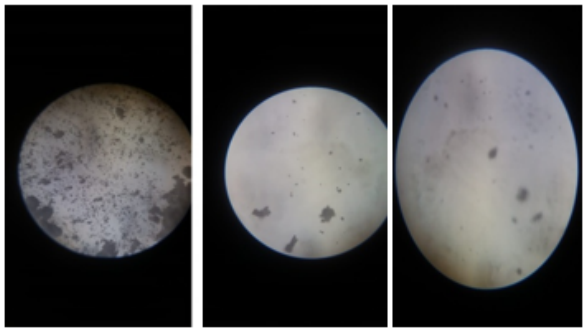


Figure 7: Particle shape of formulated tooth powder (a) plain pomegranate juice tooth powder,(b) spray dried tooth powder (c) freezes dried tooth powder

pH

Using a digital pH meter, we found out what the pH of the teeth powders we made were.The pH of formulation found to be in the range 4.2±0.30 to 9.2±0.30.

Solubility test

Solubility of formulated tooth powder formulation was found to be in distilled water, DMSO, acetone, methanol, and ethanol

Density

Density (bulk density and tap density) of formulated tooth powder was determined. The bulk density of formulated tooth powder was found to be 0.8, 0.36, 066, 0.5, 0.71, 0.5, 0.96, 0.66, 0.71 gm/ml and tap density of batches formulated tooth powder was found to be 0.95, 0.42, 0.78, 0.60, 0.85, 0.71, 0.78, 0.85, 0.96 gm/ml respectively.

Flow properties

Flow properties of formulated tooth powder were determined. Hausner's ratio of tooth powder containing plain pomegranate juice powder (TJ3) was fair, angle of repose was excellent, and angle of slide was good. The tooth powder containing spray dried pomegranate powder (TS3) showed fair Hausner's ratio, excellent angle of repose and good angle of slide. The tooth powder containing freeze dried pomegranate juice powder (TF3) showed passable Hausner's ratio, fair angle of repose and angle of slide.

Moisture content

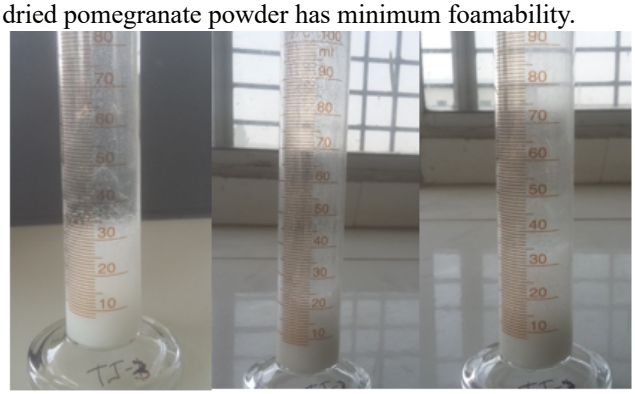
For all three batches, the oven drying technique was used to determine the moisture content.Formulated tooth powder showed moisture content of 0.40, 0.40, and 0.60% respectively.

Table 12: Moisture content & flow properties of formulated tooth powder

Code	TJ1	TJ2	TJ3	TS1	TS2	TS3	TF1	TF2	TF3
Moisture content (%)	1.13	0.89	0.40	0.95	1.1	0.40	0.76	0.99	0.60
Hausner's ratio(gm/ml)	1.18	1.16	1.18	1.2	1.19	1.17	1.18	1.19	1.18
Angle of repose (°)	32.6	27.47	25.17	30.11	34.33	27.45	33.66	38.43	30.21
Angle of slide (°)	35.66	35.33	34.33	33.66	34.36	30.96	33.66	35.21	33.66
Bulk density (gm/ml)	0.8	0.36	0.66	0.5	0.71	0.5	0.96	0.66	0.71
Tapped density (gm/ml)	0.95	0.42	0.78	0.60	0.85	0.71	0.78	0.85	0.96

Foaming index

The maximum height of foam that occurs during measurement is a direct indication of the foaming index of the sample. The batches TJ3, TS3, TF3 showed foam height of 3.1, 2.9, 3.3 cm respectively. The test revealed that tooth powder of freeze-dried pomegranate powder has maximum foamability than plain pomegranate juice powder and spray dried pomegranate powder has minimum foamability.



(a) (b) (c)

Figure 8: Foaming test of formulated tooth powder (a) plain pomegranate juice tooth powder(TJ3), (b) spray dried tooth powder (TS3), (c) freeze dried tooth powder (TF3)

Table13: Determination of foaming character

Code	TJ1	TJ2	TJ3	TS1	TS2	TS3	TF1	TF2	TF3
SLS (gm)	0.1	0.15	0.2	0.1	0.15	0.2	0.1	0.15	0.2
Foaming height (cm)	1.6	2.3	3.1	2.2	2.5	2.9	2.7	3.0	3.3

Selection of best batch in formulation:

Batch TJ3, TS3, TF3 was selected best batches due to good flow properties, moisture content and foamability.

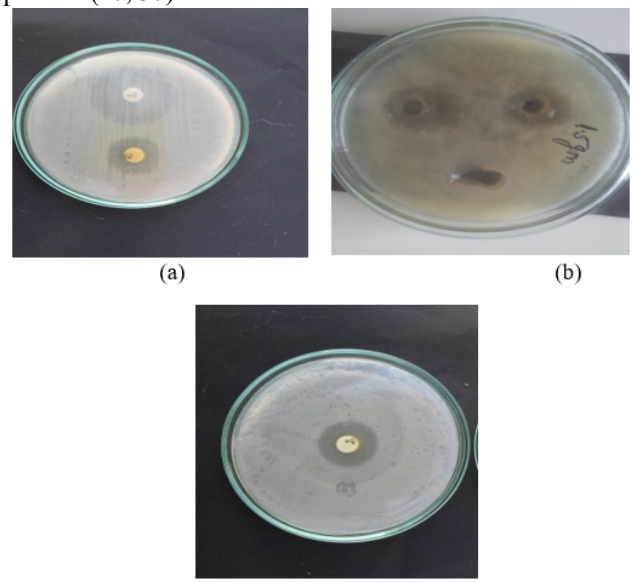
Table 14: Determination of flow properties, moisture content and foamability

Code	Flow properties			Moisture content (%)	Foaming height(cm)
	Hausner's ratio	Angle of repose (°)	Angle of slide (°)		
TJ3	1.18	25.17	34.33	0.40	3.1
TS3	1.17	27.45	30.96	0.40	2.9
TF3	1.18	30.21	33.66	0.60	3.3

***In vitro* antimicrobial study**

Antibacterial study

Antibacterial activity of plain pomegranate tooth powder (TJ3), spray dried tooth powder (TS3) and freeze-dried tooth powder (TF3) was evaluated by cup plate method are as shown in table.27. (5) Zone of inhibition of TJ3, TS3 and TF3 against was found to be 28.5 ± 0.5, 28.3±0.5 and 29.9± 0.5 mm, respectively as shown in Figure. 29. The test showed that pomegranate juice tooth powder and spray dried tooth powder have nearly same zone of inhibition and slightly increased zone of inhibition of freeze-dried tooth powder (17, 37).



(a) (b)

(c)

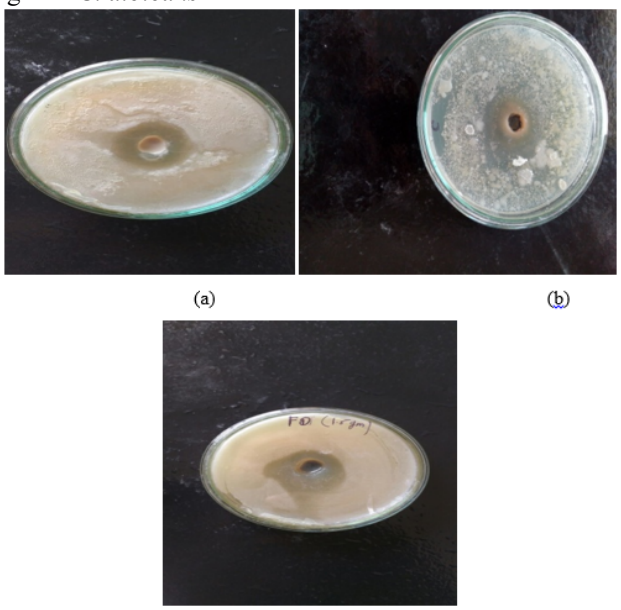
Figure 9: Antibacterial activity of formulated tooth powder TJ3 (a), TS3 (b) and TF3 (c)against *Streptococcus mutans*

Table15: Zone of inhibition shown by plain pomegranate tooth powder (TJ3), spray dried tooth powder (TS3) and freeze-dried tooth powder (TF3)for *Streptococcus mutans*

Sample Name	**Zone of inhibition, mm, mean±SD (n=3)**
Plain pomegranate tooth powder (TJ3)	28.5±0.5
Spray dried tooth powder (TS3)	28.3±1.0
Freeze dried tooth powder (TF3)	28.9± 0.5

Antifungal study

Antifungal activity of plain pomegranate tooth powder (TJ3), spray dried tooth powder (TS3) and freeze-dried tooth powder (TF3) was evaluated by cup plate method are as shown in table.29. Zone of inhibition of TJ3, TS3 and TF3 against *C. albicans* was found to be 27.05 ± 1.0, 29.5±0.5 and 28± 0.5 mm, respectively as shown in Figure. 28. The spray dried tooth powder showed MIC against *C. albicans* as compared to freeze dried tooth powder. Plain pomegranate juice powder showed least zone of inhibition against *C. albicans*.



(a) (b)

(c)

Figure 10: Antifungal activity formulated tooth powder TJ3 (a), TS3 (b) and TF3 (c) against *Candida albicans*

Table16: Zone of inhibition shown by plain pomegranate tooth powder (TJ3), spray dried tooth powder (TS3) and freeze-dried tooth powder (TF3) for *C. albicans.*

Sample Name	**Zone of inhibition, mm, mean±SD (n=3)**
Plain pomegranate tooth powder (TJ3)	27.05±1.0
Spray dried tooth powder (TS3)	29.5±0.5
Freeze dried tooth powder (TF3)	28± 0.5

Comparative study of TJ3, TS3 and TF3

Formulated tooth powder TJ3, TS3 and TF3 are comparison TS3 is highest antimicrobial activity than TJ3 and TF3.

Table17:Comparative study of TJ3, TS3 and TF3

Code	**Moisture content (%)**	**Foaming height(cm)**	**Zone of inhibition (mm)**	
			S.mutans	***C. albicans***
TJ3	0.40	3.1	28.5± 0.5	27.05 ±1.0
TS3	0.40	2.9	28.3± 1.0	29.5± 0.05
TF3	0.60	3.3	28.9± 0.48	28±0. 03

CONCLUSION

This study aimed to develop an effective antibacterial toothpaste formulation using Punica granatum (pomegranate) juice to address common oral conditions such as dental caries, plaque, and root infections. Three forms of pomegranate—plain juice, spray-dried juice, and freeze-dried juice—were evaluated for their antimicrobial efficacy against Streptococcus mutans and Candida albicans. The juice powders were incorporated into toothpaste formulations with standard excipients including calcium carbonate, sodium lauryl sulfate (SLS), and peppermint oil. Preformulation studies confirmed compatibility between pomegranate extracts and excipients without compromising antimicrobial activity.

Among the three forms, the spray-dried pomegranate juice powder demonstrated superior antibacterial and antifungal properties, followed by freeze-dried and plain juice powders. The formulated toothpaste showed desirable physical characteristics such as fine particle size, good flow properties, acceptable pH, and foaming capacity. The optimized formulation contained 7.5% pomegranate extract and passed stability testing under ICH guidelines, with no significant changes observed in appearance or performance. In vitro evaluation confirmed the efficacy of the spray-dried pomegranate toothpaste in inhibiting the growth of tested oral pathogens. These findings suggest that toothpaste containing spray-dried Punica granatum extract offers a promising natural alternative for managing oral microbial infections. The formulation is not only effective but also supports the integration of herbal ingredients into dental care products with minimal risk of side effects.

So, the developed pomegranate-based toothpaste—especially the spray-dried variant—demonstrates potential as a natural, patient-compliant, and effective approach for preventing and treating dental caries, plaque, and related oral infections.

Future perspective

Most antibacterial dental infections are treated with toothpaste and mouthwash. Toothpaste is an innovative dosage form used to treat bacterial and fungal dental infections such as plaque and cavities using antibacterial agents.

Toothpaste made with natural antibacterial agents extracted from herbs can also soothe teeth. Natural antimicrobials minimize side effects and toothpaste costs over synthetic toothpastes.

Toothpaste can be further explored to treat a variety of antibacterial infections as an alternative to topical toothpaste and mouthwash.

Conflict of interest

No conflicts of interest.

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