



FORMULATION AND EVALUATION OF POMEGRANATE PEEL LOZENGES FOR MDR

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ABSTRACT

The pomegranate fruit's (Punica granatum) outer skin is the source of pomegranate peel extract (PPE). Thanks to its scientifically proven antibacterial, antioxidant, anticancer, antiulcer, and anti-inflammatory qualities, this extract has become more and more popular. This review concentrates on PPE's antibacterial efficacy against viruses, fungi, bacteria, and parasites that are resistant to many drugs. The serial dilution method and the cup plate method were used to test PSE's antibacterial activity. According to the findings, PPE exhibits a broad range of antibacterial properties against a variety of microorganisms, such as viruses (S. aureus, E. coli, K. pneumoniae, Salmonella, Salmonella enterica, P. aeruginosa, S. marcescens, Brucella spp., and R. glutinis), fungi, and mold (F. sambucinum, P. digitatum, Saccharomyces cerevisiae, Monilinia laxa, M. fructigena, B. coagulans, B. cereus, and B. subtilis).

KEY WORDS: *Pomegranate peel, Multi Drug Resistance, Antimicrobial Activity, Replica plate, Mutant Bacteria, Cytoplasmic membrane disruption.*

INTRODUCTION ^(1,2,3,4)

A condition known as multiple drug resistance, or multidrug resistance, makes it possible for an organism that causes disease to withstand various medications or chemicals with a broad range of structures and functions that are intended to destroy the organism. Antimicrobial resistance is an innate phenomenon resulting from genetic alterations in bacteria over time. Human activity, particularly the excessive and inappropriate use of antimicrobials for the treatment, prevention, or management of human diseases, accelerates the formation and spread of this resistance. Bacteria that have developed a resistance to specific medications are known as multidrug-resistant organisms, meaning that the bacteria can no longer be controlled or killed by the antibiotics.

Based on current projections, drug-resistant illnesses were directly responsible for 1.27 million fatalities worldwide in 2019. AMR was the cause of 297,000 fatalities in India in 2019. As per WHO estimates, illnesses resulting from bacteria resistant to drugs cause 700,000 deaths worldwide, with approximately 200,000 of those deaths occurring in neonates.

The Punicaceae family includes pomegranates (*Punica granatum* L.). The pomegranate fruit is composed of three components: the skins, juice, and seeds.

The pomegranate (*Punica granatum* L.) plant is one of the earliest plants that humans cultivated. It originated in the Mediterranean region, Iran, India, and China. These days, North and South America, as well as tropical Africa, also farm it. Pomegranate fruits are eaten both raw and processed, primarily as juice. It is well known that the fruit and its peel contain high concentrations of several phytochemicals, such as tannins, flavonoids, and phenolic acids. Pomegranate by-products, in particular pomegranate peel extract (PPE), have drawn more attention recently because of their scientifically proven medicinal qualities, which include anti-inflammatory, anti-cancer, antibacterial, and antioxidant effects.

Pomegranate peels' many health benefits have led to their use as folk remedies since ancient times. Bioactive component levels in peels are generally higher than in edible portions. It is also important to remember that the complex bioactive components found in pomegranate peel frequently exist in mixtures, meaning that various physiological activities can be produced by the combined effects of distinct molecules. Peels from pomegranates are rich in many different compounds. Pomegranate peel extract has been found to



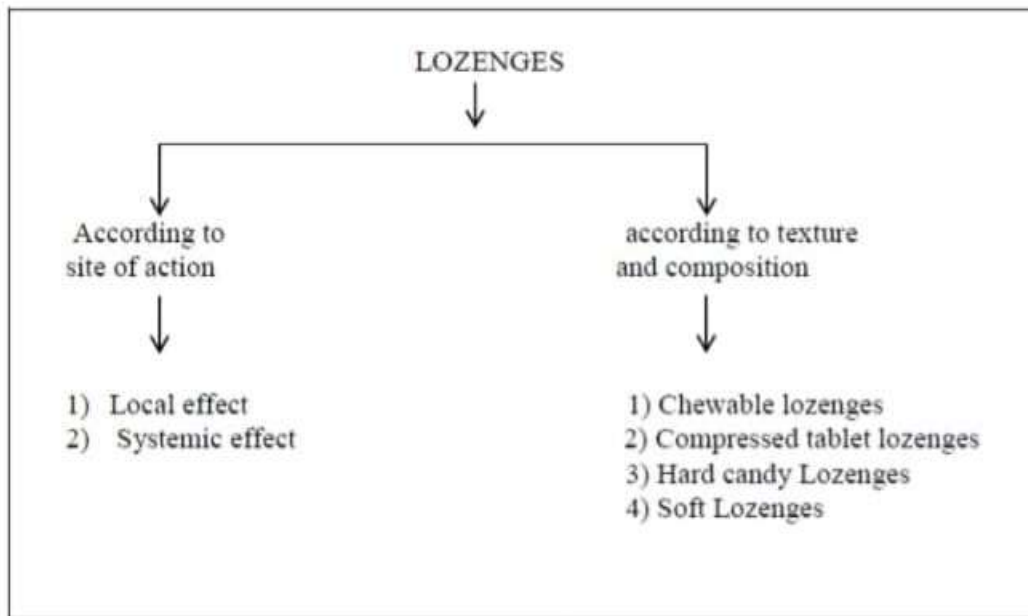
contain a high concentration of bioactive substances, primarily flavonoids, hydrolysable tannins, and phenolic acids. Ellagic acid, gallic acid, caffeic acid, chlorogenic acid, syringic acid, ferulic acid, vanillic acid, p-coumaric acid, and cinnamic acid are the main phenolic acids found in pomegranate peel extract.

The peel colour was shown to be one of the primary factors determining the phenolic acid concentration; types with a deeper red colour were found to have a higher concentration of phenolic acids than those with lighter colours. Extracted pomegranate peel is a great source of flavonoids. Furthermore, PPEs are said to have abundant supplies of tannins. Nearly 49 different substances, mostly flavonoids, phenolic acids, and tannins.

The investigated microorganisms were actively and successfully inhibited from growing by pomegranate peel extract. Conversely, depending on the type of microbe, the inhibition zone varied from 9.6 to 25.7 mm. Antimicrobial phenolic chemicals have the ability to damage membrane proteins, disrupt the cytoplasmic membrane, break down the cell wall, and obstruct enzymes that are incorporated into the membrane, all of which can finally result in cell death.

CLASSIFICATION OF LOZENGES ^(5,6,7)

Lozenges are the flavoured medicated dosage form intended to be sucked and held in mouth or pharynx containing one or more medicaments usually in sweetened base.



According to site of action

- Different methods, such as the place of action, are used to classify lozenges into different types.
- Local effect: e.g. Antiseptic.
- Systemic effect: vitamin, Nicotine.

According to texture and composition

Chewable lozenges: are not intended to disintegrate gradually, but rather to be chewed. Usually composed of a blend of sugar, honey, and gum Arabic, they relieve sore throats, improve breathing, and offer additional health advantages. Chewy or caramel-based medicated lozenges are made of medication mixed into a base of caramel that is chewed rather than dissolved in the mouth.

Compressed tablet lozenges: The manufacture of lozenges containing a heat-sensitive active component is done using this method. These tablets are not like regular tablets in that they have delayed dissolving profiles, non-disintegrating properties, and organoleptic properties. To create a tougher tablet, substantial compression equipment is used in the production of the compressed tablet lozenge. It must gradually dissolve within the mouth. It is extremely uncommon to prepare lozenges using the tablet compression method.



Hard Candy Lozenges: These are solid sugar syrup lozenges. Amorphous or glassy combinations of sugar, other carbohydrates, and herbal excipients are what these are. Hard candy lozenges should have a moisture content of 0.5 to 1.5% and a weight of 1.5 to 4.5g, respectively. Instead of disintegrating, these should dissolve gradually and uniformly over the course of five to ten minutes. Heat-labile ingredients cannot be added to hard candy lozenges since they require a high temperature to be prepared. The heating and congealing process was used to produce these pastilles. This firm lozenge syrup is used to treat mild irritation and painful throat symptoms.

Soft Lozenges: Because they are versatile and easy to prepare on the fly for a wide range of medications, soft lozenges have gained popularity. These soft, translucent lozenges are often made of gelatine, glycerol gelatine, or acacia sucrose as the substrate for the drug. It can be flavoured and coloured, and depending on the intended effect for a specific medicine integrated, it can be chewed or slowly dissolved in the mouth. In order to make lozenges, we can therefore also add a particular herbal excipient. They are composed on sugar-acacia basis including acacia and PEG 1000 or 1450.

List of Instruments and Equipments⁽¹⁰⁾

Following instrument and equipment were used in the work.

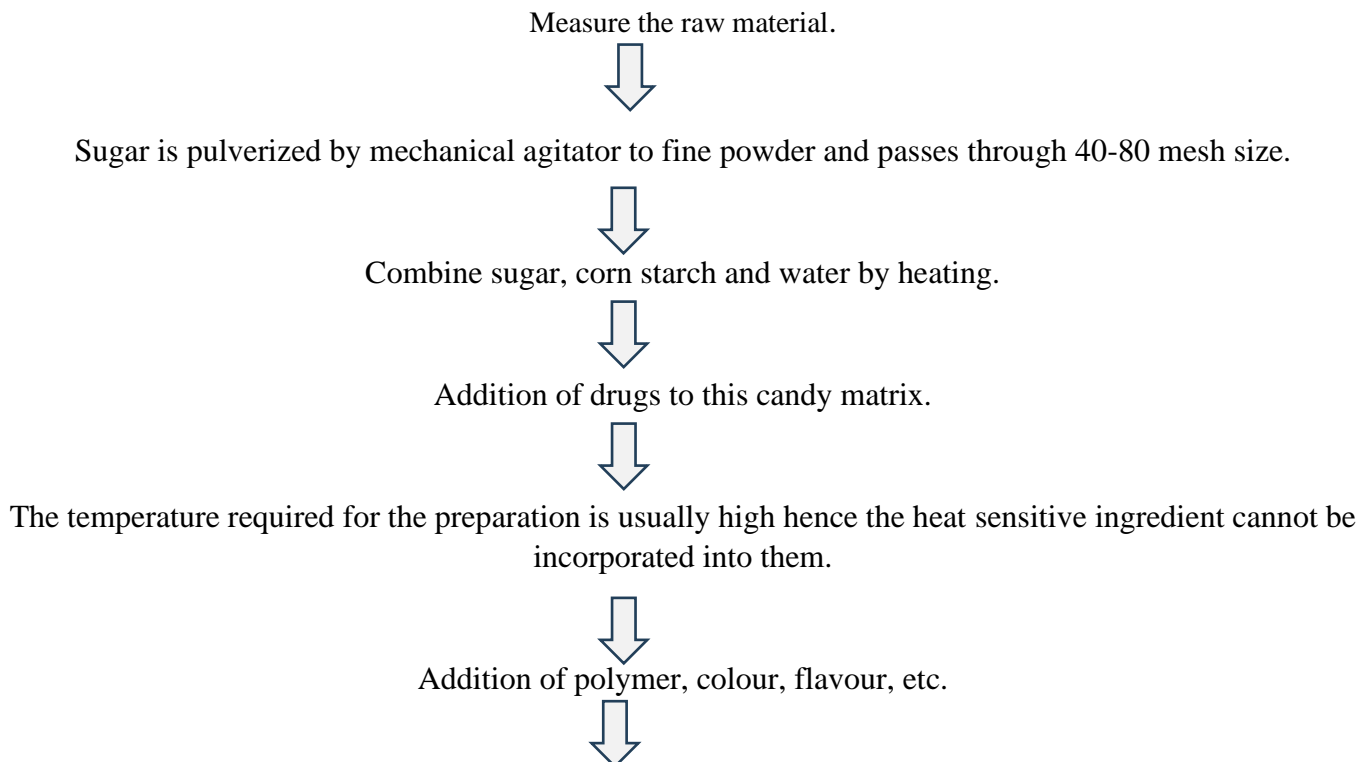
Sr no.	Equipment
1.	Digital weigh balance
2.	Hardness tester
3.	Disintegration apparatus
4.	Digital Friability test apparatus
5.	Incubator
6.	Autoclave

Material and Method

➤ Material^(8,9)

Pomegranate peel extract, Candy sugar, Corn starch, Lactose, Mannitol, Talc and Peppermint oil

➤ Method of preparation of Hard candy lozenges^(11,12)





Continue agitating the mixture and add the required amount of peppermint oil drop by drop.



Keep the mould ready.



Poured into mould of desired shape and size to form a candy.



Until it becomes harder, keep it at room temperature.



Sealing and wrapping of candy in polyethylene wrapping.

➤ **Microbial evaluation of pomegranate peel powder⁽¹³⁾**

Replica plate method

To mount the piece of sterile velvet, stretch it onto a cylindrical metal block slightly smaller than the Petri dish. Place the block with the velvet side facing up. Turn the Petri dish over with the lawn of bacteria (master plate) gently pressed against the velvet. The number of projecting fibres of the velvet (roughly 1000/sq. Inch) act like inoculating needles sampling each clone of the cell in the lawn. Remove the Petri dish Press two or more Phage coated Agar against the Velvet in turn. Save original master plate. Incubate Phage Coated Plates. A few colonies appear on Phage Coated Plate. Some of these colonies may represent mutations that occurred during the cell division that occurred following replica plating. Colonies found at the same positions on each replica plate can be assumed to have arisen from the inoculum of Phage resistant ones transferred through the velvet from the Phage resistant clone on master plate.



Fig : Agar plate with antibiotic

**Formulation Table⁽¹⁴⁾**

Sr no.	Materials	Quantity
1.	Pomegranate peel extract	0.04 gm
2.	Candy Sugar	1.23 gm
3.	Corn Starch	0.2 gm
4.	Maltose	0.2 gm
5.	Mannitol	0.2 gm
6.	Talc	0.2 gm
7.	Peppermint Oil	q.s.

Rationale

Reason behind developing herbal formulation is to get the lesser side effect and can have multiple benefits.

1.27 million fatalities worldwide in 2019 were directly linked to drug-resistant illnesses, according to current estimates.

In India in 2019, there were 2,97,000 deaths attributable to drug resistant.

Now a days the use of antibiotic is very common so, the resistance is frequently developed due to low immunity, especially in children.

Antibiotic resistance is a public health threat of almost importance, especially when it comes to children.

According to WHO data, infections caused by multidrug resistant bacteria produce 7,00,000 deaths across all ages, of which around 2,00,000 are children.

The problematic overuse and misuse of antibiotics for wrong diagnosis and indications or at wrong dosage leads to the resistance.

To prevent this problem, we formulate the lozenges of pomegranate peel extract which is actively effective against MDR.

A substantial amount of phenolic components, including flavonoids (anthocyanins and catechins), hydrolysable tannins (punicalin, punicalagin, ellagic acid, and gallic acid), and tannins, are present in pomegranate peel extract and are what give it its biological action. These substances have the potential to cause cell death by damaging membrane proteins, rupturing the cytoplasmic membrane, destroying the cell wall, and interfering with enzymes that are integrated into the membrane.

These lozenges help to prevent the development of mutant bacteria against antibiotic drug.

It will also help to improve the efficacy of antibiotic drug.

It enhances immunity in children and also having many health benefits.

Pre formulation Studies^(15,16,17)

An examination of the physical and chemical characteristics of the drug material both by itself and in combination with excipients is known as a preformulation study. Preformulation testing overarching goal is to produce data that will assist the formulator in creating a stable, bioavailable dosage form that can be mass manufactured. The preformulation parameters that are frequently studied are the Hausner ratio, Carr's compressibility index, bulk density, tapped density, pour density, and angle of repose.

Bulk Density

Unless otherwise instructed, pass through a 1.00 mm (no.18) screen a sufficient amount of material to finish the test in order to break up any agglomerates that may have formed during storage. Enter around 100 g of the test sample (M), which was precisely weighed to within 0.1% of its capacity, into a dry 250 ml cylinder without compacting it. The amount of the test sample and the cylinder's volume may be changed if using 100 g is not feasible. Choose a sample mass that has a 150–250 ml apparent untapped volume. For apparent volumes ranging from 50 to 100 ml, a 100 ml cylinder is utilized. Carefully fill the cylinder. Level the powder carefully, taking care not to compact it, and measure the unsettled apparent volume (Vo). Utilizing the following formula, find the bulk density in g/ml:

Bulk Density = Sample Weight / Sample Volume.

Tapped Density

A measured amount of powder is added to a measuring cylinder with precision. Use an appropriate mechanical tapped density tester to mechanically tap the sample-containing cylinder by elevating it and letting it fall under its own weight at a nominal rate of 300 drops/min. After 500 taps on the cylinder, calculate the tapped volume (Va). After 750 more tapings, repeat the process and measure the tapped volume once more as (Vb).

Vb is the final tapped volume (Vf) if there is a less than 2% difference between Va and Vb. The following calculation can be used to determine the tapped density if there is a greater change after 1,250 more tapings (United States Pharmacopoeia, 2004).



Tapped Density = Weight of sample / Tapped Volume of Sample

Hausner's ratio (H)

By calculating the ratio of tapped density (TD) to bulk density (BD), one may express the flow parameters of the powder. Equation following is used to compute it:

Hausner's ratio = Tapped density/Bulk density

Carr's index

Using Carr's compressibility index, the compressibility index of granules can be calculated using the following formula:

Carr's index (%) = Tapped density - Bulk density/Tapped density x 100

Angle of repose

A powder is let to freely fall onto a surface and pass through a funnel to ascertain it. As soon as the pile reaches the funnel's tip, more powder is not added. Without upsetting the pile, a circle is drawn around it. The resulting cone's height and diameter are measured. After three iterations of the identical process, the average value is determined. Equation for calculating angle of repose is as follows:

Tan θ = h/r

Where, h = Height of the powder cone

r = Radius of the powder

Results of Preformulation Study

Sr. No.	Parameter	Observation
1	Organoleptic Characteristics a. Colour b. Odor c. Taste d. Texture e. Shape	Brown Odorless Sweet Smooth Spherical
2	Bulk Density	0.4 gm/ml
3	Tapped Density	0.5 gm/ml
4	Hausner's Ratio	1.25 %
5	Carr's Index	20 %
6	Angle of Repose	39.99
7	Ash Value	0.56 %

Evaluation of Herbal Lozenges^(18,19,20)

- Organoleptic parameters:** Organoleptic characteristics of manufactured hard candy lozenges containing pomegranate peel extract were assessed by hand.
- Diameter and Thickness:** A vernier caliper was used to measure the thickness and diameter. The dimensions of the hard candy lozenges play a crucial role in their production. After selecting the three lozenges at random from the formulation, the diameter and thickness were measured.
- Hardness:** Ten hard lozenges were tested for hardness using a Monsanto hardness tester. A computed and reported mean and standard deviation were obtained. It has the unit of kg/cm³.
- Weight Variation Test:** Following a random selection of twenty lozenges from the formulation, each lozenge was weighed separately. If no more than two of the individual lozenge weights differ from the average weight by a greater percentage than the IP limitations indicated in the table, the batch passes the weight variation test.
% Weight Variation = Initial weight – Average weight / Average weight x 100
- Friability Test:** Roche Friabilator was utilized to ascertain friability. All of the friabilator's parameters were established after ten lozenges were weighed and put inside. For four minutes, the device was turned at a speed of 25 rpm. The lozenges were taken out and weighed once again following the revolution. Samples with a maximum mean weight decrease of 1.0% are not allowed.
% Friability = Initial weight - Final weight / Initial weight x 100
- Mouth Dissolving Time:** The USP disintegration device was utilized to ascertain the duration required for the lozenges to dissolve entirely.



Result of the evaluation parameters of lozenges are given below

Sr. No.	Parameters	Observation
1	Organoleptic Characteristics a. Colour b. Odor c. Taste d. Texture e. Shape	Brown Odorless Sweet Smooth Oval
2	Diameter	16.1 mm
3	Thickness	7.5 mm
4	Hardness	9.5 kg/cm ³
5	Weight Variation Test	± 0.48 – 0.97 %
6	Friability	0.3 %
7	Mouth Dissolving time	9 min

Phytochemical Evaluation of Extract ⁽²¹⁾

Test for phytochemicals	Chemical test	Result
Alkaloids	Dragendorff's Test	+
	Mayer's Test	+
	Hager's Test	+
	Wagner's Test	+
Tannins and phenolic compounds	Ferric Chloride Test	+
	Lead Acetate Test	+
Flavonoids	Ferric Chloride Test	-
	Alkaline Test	-
Saponins	Foam Test	+
Carbohydrate	Molisch's Test	-
	Benedict's Test	-
Anthraquinone glycosides	Borntrager's Test	+
Protein	Biuret Test	-
Amino acid	Ninhydrin Test	-
Glycoside	Killer Killani Test	-
Steroid	Salkowski Test	+

CONCLUSION

The text discusses the issue of multidrug resistance in diseases, particularly focusing on bacteria and highlights the antibacterial activity of pomegranate peel against multidrug-resistant bacteria. It also delves into the scientific classification, chemistry and uses of pomegranate peel. Additionally, it provides an overview of lozenges as a dosage form, their advantages, disadvantages, classification and method of preparation. The objective is to explore the potential health benefits of herbal lozenges.

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