REVIEW ARTICLE

Uses of Pomegranate in Dentistry: A Comprehensive Review of Literature

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ABSTRACT

The most prevalent diseases in the oral medicine field have bacterial and fungal origins. Different therapeutic ways are available for oral diseases; however, the most crucial disadvantage of traditional drug therapies is their side effects. As a result, researchers are looking for new herbal medicines to treat oral diseases. Recently *Punica granatum* has received the attention of researchers due to its numerous benefits. This review provides an overview of pomegranate's impacts on oral health and oral microbiome in addition to its anti-inflammatory, antioxidant, and other valuable features. Pomegranate can be used as an herbal cure, and scientists are encouraged to use it in cancer prevention. Moreover, characteristics, chemical composition, bactericidal action, and therapeutic uses are discussed in this paper.
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Keywords: Oral disease, Dental caries, Pomegranate, *Punica granatum*, Oral microbiome

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INTRODUCTION

Oral and dental health have a critical role in general (1, 2) health. Prevalent oro-dental diseases, including dental caries, gingivitis, periodontitis, and pulpitis, are caused by bacterial and fungal agents (3-7). Chlorhexidine and fluorides are chemical products used besides mechanical methods to inhibit bacterial biofilm development, the initial cause of caries and periodontal disorders. The products have potential adverse effects like increasing tooth staining, altering the sense of taste, increasing mineral uptake into the bacterial biofilm and formation of calculus, irritating oral mucosa, and drying the mouth (1, 3, 8-17). A popular form of alternative medicine is using herbal products instead of synthetic ones (18-22). People have used herbal agents more in recent years due to their lower prices, fewer side effects, and broader safety margin than chemical products. Herbal products have antibacterial, antifungal, antiviral, anti-inflammatory, anticaries, antiulcer, and anticancer effects, which makes them a proper candidate in dentistry

(1, 3, 10, 23-34). Pomegranate (*Punica granatum*) is a member of the family *Punicaceae*. It is cultivated in Iran, Mediterranean countries, India, the drier parts of Southeast Asia, the East Indies, Malaysia, California, tropical Africa, and Arizona in the United States, Japan, China, and Russia. The fruit of a pomegranate can be divided into several anatomical compartments, including peel, juice, and seed (35-37). Pomegranate is considered a medicinal plant containing beta-carotene, thiamine, riboflavin, ascorbic acid, magnesium, potassium, albumen, phosphorus, iron, calcium, and significant quantities of antioxidants including polyphenols (flavonoids, tannins) and anthocyanins (the red pigment of juice) (35, 37, 38). Pomegranate has strong antibacterial properties against cariogenic bacteria like *Streptococcus sanguis*, *Streptococcus mutants*, and *Streptococcus mitis*. Therefore, it is safe as a natural mouthwash with no noticeable side effects and has even better activity compared to chemical therapy (39). Pomegranate can be an alternative medicine for periodontal disorders because of its antibacterial activity against dental biofilm and antioxidant activity against reactive oxygen species (ROS). ROS have a role in inflammation-induced destruction of the periodontium (40-44). Pomegranate has a significant role in preventing and treating cancer via modulating multiple pathways (10, 45-47). Pomegranate also has anti-fungal, anti-halitosis, anti-inflammatory, and wound healing activities (43, 48-52). Overall, due to the rich bioactive components of punicalagin (flavonoids) and anthocyanins (delphinidin, cyanidin, pelargonidin), this fruit has a high potential for therapeutic activities, and no systematic review has been done for pomegranate oral health promotion effects so far.

PUNICA GRANATUM

Punica granatum L. (Pomegranate) is a popular fruit from the *Punicaceae* family. The *Punicaceae* family comprises two species, *Punica granatum*, and *Punica protopunica*. The word pomegranate divides into two Latin words, “Pomus" and "granum," which means apple with grains (1). The origination of pomegranate is from a region that now is Iran, Afghanistan, Azerbaijan, etc. Owing to the compatibility of this tree to grow in various climates, nowadays, pomegranate is planted in India, the Mediterranean region, Central and south-east-Asia, Tropical Africa, and parts of California and Arizona (5). However, this tree can be the harm in temperatures below -10 C°. According to these changing cultivation environments, pomegranates can be genetically and bioactive diverse (3). This fruit has a red nonedible peel and a white inner surface. It has many juicy red edible arils and seeds. From long ago in human history, seeds, peels, flowers, and juice has been used as nourishment and treatment (3). Due to pomegranate’s various medical properties, scientists have researched the effects and avail on health and the human body. Phenolic compounds in pomegranate juice, seeds, peel, and pericarp have shown significant antioxidant activity. Antioxidant activity in pomegranate juice is triple compared to that of green tea (23). Compounds such as ellagic acid, punicalagin, and urolithin in pomegranate have made it rich in antioxidant activity (35). Food industries and folks mainly consider pomegranate peel a waste, Although the peel consists of about half of the fruit's weight and is a more powerful antioxidant than the juice (36). The peel contains tannins, which play the most critical role in the antioxidant activity of pomegranate (37).

On the other hand, pomegranate peel showed antimicrobial efficiency against S. aureus and Escherichia coli (38, 39). The higher it contains Punicalagin, the more antimicrobial activity it has (40). Using ethanol in pomegranate extraction resulted in the reduction of methicillin-resistant *Staphylococcus aureus* (41). The peels appeared to have higher antimicrobial activity than flowers, stems, and leaves (42). Moreover, Punicalagin can act effectively against fungi like *Candida albicans* and *Candida parapsilosis* and have synergic interaction with fluconazole (12). On the other hand, pomegranate juice showed inhibition of HIV-1, and punicalagin can block Herpes simplex virus-2 (45, 46, 53).

Cardiovascular health can improve with pomegranate intake by preventing platelet function (48). Different pomegranate elements, including polyphenols and flavonoids, can alleviate nociceptive pain and neuralgia (49). In type 2 diabetes, pomegranate is advantageous through reducing reactive oxygen species (50). Also, supplementary pomegranate in patients with rheumatoid arthritis relieved clinical manifestations (51). Seed oils, juice, and peel have demonstrated anti-cancer roles in breast cancer (52).). It is effective against breast cancer through various mechanisms, e.g., it deters invasion of cancerous cells and apoptosis (37, 54). A study by Lansky et al. showed a positive impact of pomegranate juice, seed oil, and peel on the proliferation and metastasis of prostate cancer cells (Fig. 1. summarizes the clinical applications of pomegranate) (55). It also has vast benefits and applications in dentistry, which will be discussed thoroughly. This manuscript aimed to review the benefits of pomegranate in oral health improvement.

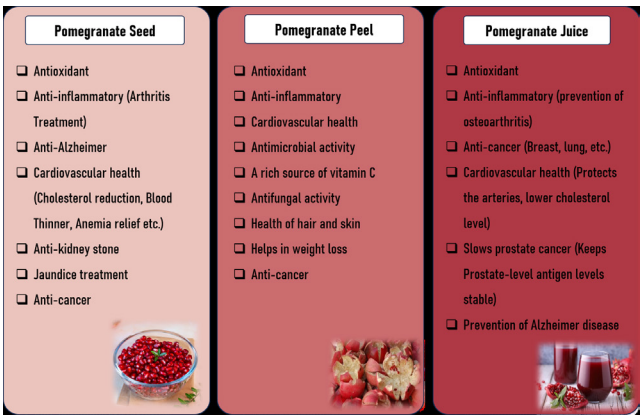

Fig. 1: Clinical application of pomegranate and its active constituents (seed, fruit, peel, etc.)

METHODS

We conducted this study by exploring reliable databases such as SID, Web of Science, Scopus, Science Direct, Google Scholar, and PubMed for English language publications up to 2022 to find all articles related to the use of pomegranate in treating oral diseases. Our priority was recently published articles, and for further information, we identified references cited in these studies.

ORAL HYGIENE AND MOUTHWASH

Several studies have found the efficiency of mouthwashes containing pomegranate extracts since pomegranate has antibacterial and anticaries properties. Flavonoids, anthocyanin, polyphenols, alkaloids, tannins (27, 56-62), punicalagin, and ellagic acid (63) play an essential role in bacteria. The antibacterial function can be associated with several mechanisms, such as altering salivary PH and enzymes, prohibiting bacterial adherence to the dental surface (64-66), inhibitory action in energy metabolism, and the performance of cell membrane and nucleic acid arrangement of bacteria (67). It is also considered an effective prophylactic mouth rinse (68).

In vitro studies

Table I summarizes the effect of pomegranate on dental caries in in-vitro studies. Shubha et al. used Oro dispersible films with punicalagin against *S. mutans* which are bacteria responsible for dental caries in vitro. Punicalagin is an antibacterial phenolic compound in pomegranate peel. Films released punicalagin by different ratios of polymer-excipient slowly to increase the punicalagin-bacteria interaction time. Disintegration time was reported as 15.49 min and dissolution time as 30 min. It was concluded that punicalagin stays longer in the mouth than chemical mouthwashes, ensuring adequate antibacterial activity and a promising and safe alternative option (69). Hussain et al. conducted a study on consuming hot and cold pomegranate extracts to evaluate their antimicrobial effects compared to chlorhexidine. In this study, *S. mutans* were separated from 55 persons with caries and gingiva inflammation. Conclusions showed that pomegranate extract had a better preventive effect on *S. mutans* colonization than chlorhexidine which made this agent an alternative for mouthwash; on the other hand, it could be made at home so that that note can be its advantage over chlorhexidine (70). Various observations showed the antibacterial effects of pomegranate via different pathways, such as reducing salivary activities of alpha-glucosidase, increasing the actions of ceruloplasmin (an enzyme with the antioxidant feature), and preventing bacterial adherence to the tooth surface (64, 65). In a recent study conducted by Hernawat et al., the impact of *Punica granatum* against bacteria extract mouth rinse on 0.02%, 0.2%, and 2% concentrations was evaluated. The number of bacterial colonies in saliva 24 hours after incubation showed a notable reduction, especially at a concentration of 2% (71). The mentioned property could be correlated to flavonoids, polyphenols, anthocyanin, alkaloids, and tannins (71). The antibacterial function of flavonoids can be associated with their inhibitory activity in arranging nucleic acid, cell membrane performance, and metabolism of energy (67).

Table I: A summary of the in vitro studies on the effect of pomegranate on different dental disorders.

Reference ID	Country	Type of Study	Study Model	Disease or condition	Dose	Duration	Outcome
(68)	India	In vitro	Count of *S.mutans*	Antibacterial activity	1200, 900, 600, 300, 150, 75 mg/ml pomegranate juice	5 days	• Antimicrobial activity against *S. mutans* was observed • Dental caries were prevented
(109)	Iraq	In vitro	antibacterial activity against *Candida tropicalis*, *Candida albicanus*, *Candida dublinensis*, *Candida krusei*, and *Candida glabrata*	Antimicrobial activity	5 g of *Punica granatum* were dissolved in 25 ml solvents. (0.2 gm/ml)	24 hours	Minimum antimicrobial activity was observed when compared to ethanolic extracts
(87)	Indonesia	In vitro	*F. nucleatum* and *S. sanguinis* monospecies biofilm formation	biofilm formation	Pomegranate juice 6.25% and 50%	24 hours	• After treatment with pomegranate juice, biofilm reduction was observed • Inhibition of *F. nucleatum* and *S. sanguinis* was observed
(73)	Baghdad	In vitro	*Streptococcus mutans*, *Candida albicans*, *Lactobacillus acidophilus*	Antimicrobial activity	Different diffusions of pomegranate juice 100%, 75%, 50%, 25%, 10%	48 hours	Pomegranate juice is effective against *Streptococcus mutans* and *Lactobacillus acidophilus* but has no antifungal effect.

CONTINUE

Table I: A summary of the in vitro studies on the effect of pomegranate on different dental disorders. (CONT.)

Reference ID	Country	Type of Study	Study Model	Disease or condition	Dose	Duration	Outcome
(95)	Iran	In vitro	*S. mutans* and *S. sobrinus* biofilm	antimicrobial effects/ attachment of bacteria with strong adhesion/ quantity of biofilm formation by *streptococci*	100µL of pomegranate vinegar mouthwash	158 hours	• Antimicrobial effects of pomegranate on the proliferation of cariogenic *Streptococci* were observed. • Adhesion of these bacteria was decreased
(103)	Brazil	In vitro	three *Streptococci strains, S. mutans, and Candida*.	the antimicrobial effect of a *Punica granatum Linn*	1:1 to 1:1024 *Punica granatum Linn* (pomegranate) phytotherapeutic gel and miconazole	24 hours	• adherence of microorganisms to the oral cavity was controlled • the *Punica granatum L*. gel had a better effect in preventing bacterial adherence than the miconazole
(102)	USA	In vitro	Saliva-Derived Biofilm Model System	The antimicrobial activity	pomegranate polyphenol extract powder 1.25 mg/ml	48 hours	The antibacterial and anti-adherence function of pomegranate extracts was observed.
(199)	Iran	In vitro	*P. aeruginosa*, S. *aureus* and standard strains.	antibacterial activity of ethanolic extracts of pomegranate peels and seeds	200 g of each-Powder of pomegranate peels and seeds were soaked in 70% ethanol solution (1:10 ratio)	24 hours	Antibacterial activity against bacteria was observed; (peel extract had a better antibacterial effect than the seed extract)
(96)	India	In vitro	*S. aureus*	antimicrobic effect of alcoholic extract of peel, seed, fruit, and juice of *Punica granatum*	alcoholic extract of peel, seed, fruit, and juice of *Punica granatum* (10 gr powder in 100ml water)	24 hour	Excellent function against bacteria and fungi was observed.
(83)	India	In vitro	*Candida albicans.*	antifungal impact of *Punica granatum, Acacia nilotica, Cuminum cyminum,* and *Foeniculum vulgare*	10 g Punica and acacia powdered in 100 ml water	18 and 24 hours	The highest inhibition of Candida albicans was observed in pomegranate.
(100)	India	In vitro	*S.mutans* bacteria	Antioxidant and antimutagenic activities	(25, 50, 75, and 100 µg/ml of pomegranate peel extracts	24 hours	Antioxidant and antimutagenic properties due to phosphomolybdenum complex formation and sodium azide antimutagenicity were observed.
(107)	Egypt	In vitro	*S. aureus* growth	inhibition zone of S. aureus growth with pomegranate fatty extract supported by silver nanoparticle (AgNp).	100, 200, 400, and 800 mg of pomegranate fat extract	24 hours	Growth of bacteria was inhibited due to interaction with the sulfur in the bacterial cell wall, which leads to increased permeability of the membrane and death of the bacterial cell
(200)	India	In vitro	against pathogens (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* and *Enterococcus faecalis*)	antibacterial activity of aqueous, ethanol of pomegranate, and acetone extracts	plant material (10 g) was dissolved with 100 ml of distilled water	24 hours	The usage of pomegranate in the curing of urinary tract infections was effective.
(104)	Iraq	In vitro	*Proteus mirabilis* bacteria	Antibacterial effect	and 800, 400, 200, and 1000 mg/ml of alcohol and aqueous extract of pomegranate and *Lantana cammara* leaves	Not mentioned	An inhibitory impact on the growth of bacteria was observed by using Aqueous extracts of pomegranate peel.

CONTINUE

Table I: A summary of the in vitro studies on the effect of pomegranate on different dental disorders. (CONT.)

Reference ID	Country	Type of Study	Study Model	Disease or condition	Dose	Duration	Outcome
(69)	India	In vitro	colony formation of *Streptococcus mutans, Streptococcus salivarius, Aggregatibacter actinomycetemcomitans*	Dental biofilm formation	75ml, 50ml, 25ml, 10ml and 5 ml per 100 ml water	24 hours	Biofilm formation of organisms was inhibited.
(106)	Brazil	In vitro	*Staphylococcus aureus* FRI 722	Growth of *Staphylocc--cus aureus* and formation of enterotoxin	0.01% and 0.05 and 1 v/v of pomegranate	24 hours	Antibacterial properties also inhibit ion of enterotoxin production was observed.
(105)	India	In vitro	dental plaque bacteria, including *Streptococcus sp, Lactobacillus sp, Staphylococcus sp. and Proteus sp*	the antibacterial function of the antibiotics and pomegranate extracts against dental plaque bacteria	200µl of the pomegranate extracts (500mg/ml)	24 hours	• Doxycycline and clindamycin antibiotics observed the highest range of antibacterial function. • The effectiveness of pomegranate is slow, but it has fewer side effects in long time usage.
(101)	USA	In vitro	*P.aeruginosa, c.* neoformans, *Staphylococcus aureus, Candida-albicans, Escherichia coli, Pseudomonas aeruginosa, Cryptococcus* neoformans	antioxidan, antiplasmo-dial, and antimicrobial activities	Pomegranate juice 1.1,3.2,2.3,1.4 µg/mL.	24 hours	• Antioxidant, antiplasmodial, and antimicrobial activities by daily usage of pomegranate juice was observed due to exhibiting ic 50 values against reactive oxygen species generation.
(90)	India	In vitro	Oral bacteria like *Staphylococcus aureus* , *Candida albicans* and *Pseudomonas aeruginosa*	Polyphenol content and minimum inhibitory concentration of different parts of a pomegranate	805.6 gr/meq-different parts of pomegranate (seed, pith, rind, crude)	24 hours	• All parts of pomegranate contain high levels of Polyphenol • Antibacterial and antifungal effects
(71)	Brazil	In vitro	*Streptococci, Neisseria, Veillonella, Actinomyces*	stabilization of pH of pomegranate peel mouthwash	pomegranate peel mouthwash/ 10 g of pomegranate peel powder is mixed with 640mL water	10 minute	The boiled mix of ingredients, including 1 gm of pomegranate peel powder and 1 gm of turmeric powder, also 100 ml water is the best mouthwash with pH 5.60
(112)	India	In vitro	endodontic bacterial strains	antibacterial activity of three selected fruit juices (Apples, Pomegranate, and Grape) on endodontic bacterial strains	100 µl volume of the fruit extract	24 hours	The Antibacterial impact was observed in all the groups, but apples showed the maximum antibacterial effect.
(201)	Brazil	In vitro	*Candida albicans* and *Enterococcus faecalis* and *Pseudomonas aeruginosa*	Antibacterial activity	Fruit peels of *Punica granatum* 60/80/100mg/ml	24 hours	Growth of mentioned bacteria was inhibited.
(97)	Iran	In vitro	various pathogenic bacteria like *S. aureus*	zone inhibition in S. aureus	Iranian native sour and sweet pomegranate (*Punica granatum*) peel extracts (500 g) were dissolved in 1800 ml methanol	24 hours	• The antibacterial effect was more significant against Gram-positive bacteria compared to that for the Gram-negative bacteria • The bacteriostatic and bactericidal effect of methanolic extracts of pomegranate peels was observed

CONTINUE

Table I: A summary of the in vitro studies on the effect of pomegranate on different dental disorders. (CONT.)

Reference ID	Country	Type of Study	Study Model	Disease or condition	Dose	Duration	Outcome
(58)	Brazil	In vitro	Methicillin-resistant *Staphylococcus aureus*	Infectious diseases	Extracts of pomegranate	2 days	Reduced Methicillin-resistant (MRSA) and -sensitive (MSSA) *S. aureus*
(25)	Brazil	In vitro	Methicillin-resistant *Staphylococcus aureus*	-	240g pomegranate pericarp	24 hours	Reduced Methicillin-resistant (MRSA) *S. aureus*
(166)	Indonesia	In vitro	*Streptococcus sanguis* ATCC 10556	RAS	• 4000 ppm ethanolic extract of pomegranate seeds	An interval of 30, 60 and 90-seconds	• The bacteriostatic effect at 4000 ppm at 30, 60, and 90-second time intervals • The bacteriostatic effect at 8000 ppm at 30 and 60-second time an interval. The bactericidal effect at 8000 ppm at the 90-second time interval
(167)	Indonesia	In vitro	*Streptococcus sanguis* ATCC 10556	RAS			• Minimum inhibitory concentration (MIC) at 500 ppm ethanolic extract of pomegranate seeds • Minimum Bactericidal Concentration (MBC) at 2000 ppm ethanolic extract of pomegranate seeds
(189)	United Kingdom	In vitro	Human primary *Gingival* fibroblasts	Gingival wound	Pomegranate Rind Extract (PRE), *Punicalagin,* alone and in combination with Zn (II)	24 hours, 48 hours, and 72 hours	• Increased antioxidant capacity of PRE by adding *Punicalagin* • No significant change in the antioxidant capacity of PRE by adding Zn (II) • Reduced *Gingival* fibroblast viability and migration at high concentrations of PRE, *Punicalagin*, and Zn (II) • Enhanced fibroblast speed and distance travel during migration by *Punicalagin* with Zn (II) at low concentrations
(192)	India	In vitro	50 orthodontically extracted sound premolar teeth with intact crown closed apex, and healthy periodontal ligament (PDL)	Preserving the vitality of PDL cells of avulsed teeth	Filtered fresh pomegranate juice using filter paper to be suitable for passing through the 0.2 µm filter	45 minutes	• Less number of viable PDL cells in the pomegranate group compared to Propolis, Hank's balanced salt solution (HBSS), and *Aloe vera* groups • Pomegranate juice can be used a the storage medium for avulsed teeth
(193)	Iran	In vitro	PDL fibroblasts obtained from healthy human premolars	Preserving the vitality of PDL cells of avulsed teeth	1%, 2.5%, 5% , and 7.5% pomegranate juice solutions	1 1horr, 3hour, 6hour and 24hr time periods	• The most PDL cell viability amongst all the experimental solutions in the 7.5% pomegranate juice group • The same results were in the 1% pomegranate juice group and HBSS group

El-Sharkawy et al. evaluated the effect of fresh pomegranate juice, pomegranate peel extract, and chlorhexidine mouthwash (0.2%) on salivary *S. mutans* count. A significant decrease in *S.mutans* number was seen in all of these groups, particularly in pomegranate peel extract groups (72). This indicates that pomegranate mouthwashes can be used as an antimicrobial agent. In addition, Nair et al. compared six concentrations (1200, 900, 600, 300, 150, and 75 mg / mL) of pomegranate juice extract with chlorhexidine mouthwash on *S. mutans* cultures. They concluded that the inhibition zone of this bacteria was more significant with the use of pomegranate juice extract (300 and 600 mg / mL) compared to chlorhexidine mouth rinse (73). Also, punicalagin, tannin, and ellagic acid in pomegranate can be effective (63).

Dabholkar et al. evaluated the antimicrobial activity of pomegranate-containing mouthwash against oral-biofilm-forming organisms in 2016. pomegranate juice exhibits antibacterial action against some dental pathogens due to hydrolyzable tannins

termed punicalagin, the most prevalent polyphenols identified in mouth rinse consisting of pomegranate. Chlorhexidine mouthwash, herbal mouthwash, and pomegranate-containing mouthwash were used against *S. mutans, Streptococcus salivarius*, and *Aggregatibacter actinomycetemcomitans*. Although chlorhexidine remains the gold standard, pomegranate-based or herbal mouthwash can readily be substituted for long-term usage, eliminating chlorhexidine's adverse effects (74). Against biofilm-forming organisms, all three forms of mouthwash have antimicrobial action. The significant advantage of natural herbs is that they have no side effects (75). In 2020, Gupta et al. found that it is necessary to develop a mouthwash to decrease oral deranges through Ayurveda. It is advanced by using powder of the pomegranate cover and maintaining its pH. This method can successfully preserve oral hygiene and reduce the possibility of oral diseases. This mouthwash is produced by blending pomegranate peel and turmeric powder. The preparation of mouthwash with this procedure is straightforward and can be used by every socioeconomic stratum (76). Using toothpaste containing pomegranate extract after eating or before bed inhibits the activity of dental pathogens (77). Findings indicate that the potency of pomegranate juice might be utilized to manage germs that cause oral infections (78). On the other hand, studies show that pomegranate and propolis mouth rinses have minimal effect on taste change due to their natural ingredients (79).

Human studies

Table I summarizes the effect of pomegranate on dental caries in human studies. Pinni et al. conducted a randomized clinical trial (RCT) to evaluate pomegranate pericarp extract (80) as an efficient antimicrobial mouth rinse. Counting salivary *Streptococcus mutans (S. mutans)* before and after oral rinsing showed a notable reduction. The presence of tannin can be an influential factor that penetrates the cell wall and deposits proteins, increases the bacteria's lysis, and suppresses enzymes such as glucosyltransferase, which play a crucial role in bacteria adhesion to the tooth surface. Accordingly, PPE mouth rinse was confirmed as an agent with a similar anticaries potential to chlorhexidine. The MIC of the pomegranate extract was 50 mg/ml in the study (81). In another study, the MIC of chlorhexidine ranged from 2.67 to 80.00 microgram/ml (82). This makes the pomegranate extract an efficient alternative to chlorhexidine for long-term use (83). It is also according to the findings of Umar et al. and Champaneri et al. studies which compared the pomegranate mouthwash with chlorhexidine (84, 85). This might be due to the increase of ceruloplasmin activity in saliva and the decrease of alpha-glucosidase enzyme activity, which leads to sucrose breakdown (84). Pomegranate mouthwash was applied two times daily for fifteen days. It appeared more efficient in decreasing gingival bleeding on probing points than chlorhexidine (86). The effect can be due to the polyphenols, which can destroy pathogenic bacteria (87). *Punica granatum* peel extract has been proven to have plentiful supplies of phenols and tannin phenols compared to different portions of the *Punica granatum* plant (88). According to the findings of the cited study, pomegranate is helpful as a pre-procedural rinse in patients visiting dental clinics for various procedures. It is also biocompatible, meaning it has no adverse effects on the body. As a result, it can be used as an alternate mouthwash in oral prophylaxis protocols (68). Additionally, Kritivasan et al. demonstrated the prophylactic antimicrobial activity of pomegranate juice on oral bacteria as a pre-procedural rinse (68). El Naggar et al. evaluated pomegranate, propolis, and chlorhexidine mouthwashes regarding salivary pH alteration. They found that salivary pH values increased immediately after consuming the mouthwashes containing pomegranate, propolis, or chlorhexidine (89). Flavonoids and pomegranate polyphenols account for instant salivary PH enhancement (65). However, after a week, the oral pH was the highest for chlorhexidine (7.42±0.45) and lowest for propolis (6.93±0.45), denoting the long-lasting effect of chlorhexidine in raising pH levels compared to pomegranate and propolis. Regarding the antibacterial effect, observations showcased similar results for all propolis, pomegranate, and chlorhexidine mouth rinses (89).

ORAL MICROBIOME

From cited studies, it is documented that different forms of pomegranate extracts are comparatively effective in controlling oral microbiome due to their antibacterial and antifungal activities. The following studies have found that ingredients like flavonoids, ellagitannin, and polyphenols (31, 90-94) are associated with antimicrobial activity.

ANTI-BACTERIAL ACTIVITY

***In vitro* studies**

Evaluating the antibacterial activity of pomegranate glycolic extract (PGE) and methanolic extract of Punica granatum peel (MEPGP) revealed a relation between this property and substances such as tannins and polyphenols. Tannins affect bacteria's cell membrane and can cross it, precipitate proteins, and suppress some enzymes like glycosyltransferases. Polyphenols affect bacteria's cell walls, inhibit enzymes using oxidized agents, interact with proteins, and disorder microorganisms' co-aggregation (90, 91). In a study by Janani et al., the polyphenol content of extracts in different parts of pomegranate and its minimum inhibitory concentration (MIC) against four types of bacteria and one fungus were assessed. They found that all parts of pomegranate comprise high amounts of polyphenol in pomegranate. The highest MIC for *Staphylococcus aureus (S. aureus)* was for methanolic and aqueous seed extracts. The crude extract was efficient against *Staphylococcus epidermis*, while methanolic and ethanolic rind extracts were beneficial against *Pseudomonas aeruginosa* and

Candida albicans (95). Caries and periodontal disorders, denture-associated stomatitis, and other infections can be caused by *S. mutans* (96).

Another study reported that ethanolic and methanolic extracts of pomegranate have a considerable antimicrobial effect on both Gram-negative and Gram-positive non-oral bacteria (97). Pramadita et al. studied how pomegranate juice affects both monospecies and multispecies of *F. nucleatum* and *S. sanguinis* biofilm growth during 1-hour, 3 and 6 hours, and 24-hour periods in BHI broth cultures (92). It is assumed that pomegranate fruit extract contains compounds with antibacterial, virucidal, and antifungal potentials (98). A notable decrease in the biofilm formation of *F. nucleatum* and *S. sanguinis* in cultures treated with pomegranate juice was observed, determining its anti-inflammatory benefits (92). Furthermore, pomegranate liquor has decrement in the total number of colony-forming groups of *Lactobacilli* (46%) and *Streptococci* (23%) (99). In 2015, Ramezanalizadeh et al. evaluated the effects of rose aqua and pomegranate vinegar compared to Persica mouthwash on two oral bacteria, *S. mutans* and *Streptococcus sobrinus*, which are the cause of tooth decomposition. Pomegranate vinegar, rose water, and Persica reduced plaque organization by *S. mutans* by 93%, 80%, and 68%, respectively. For *Streptococcus sobrinus*, the results were 92%, 57%, and 48%, respectively. So, pomegranate vinegar and Persica mouthwash prohibited the increase of bacteria (100). Based on Nozohour et al. investigations, the extract of *Punica granatum* peel containing ethanol and seed demonstrated inhibitory impacts on clinical separates of *S. aureus* and *P. aeruginosa*. The minimum repressive concentrations of *Punica granatum* seed and peel extracts were 12.5 and 25.0 mg/mL sequentially. The average inhibition zones and the minimum repressive concentrations values demonstrated that the activity of pomegranate peel extract against bacteria significantly affected mentioned bacteria more than the extract of pomegranate seed (100). Dahham et al. evaluated the antimicrobic impact of alcoholic extract of seed, fruit, peel, and juice of *Punica granatum* on specified bacteria, including *S. aureus*. It showed that the *Punica granatum* peel extract is effective against bacteria (101). Naziri et al. showed that peel extracts of sweet and sour *Punica granatum* result in zone inhibition of 16.0 to 25.3 mm and 15.3 to 25.7 mm, respectively, in the case of *S. aureus* at 1 to 8 mg/disc (102). Melendez and Capriles showed that *Punica granatum* extract created inhibition zone sizes of 22 mm against *S. aureus* (103). Mathabe et al. demonstrated that ethanol, methanol, acetone, and water extracts from pomegranate functioned versus *S. aureus*, displaying inhibition zones of 27.0 to 29.3 mm (104). Braga et al. demonstrated that *Punica granatum* extracts prohibit *S. aureus* growth at the concentration of 0.01 to 1% v/w (105). As concluded, water, butanoic and ethanolic extracts of Punica can affect *P.aeruginosa, Escherichia coli,* and methicillin-resistant *S. aureus* (106). Li et al. evaluated the antibacterial properties of pomegranate polyphenol extract lozenges on *S. mutans* in a biofilm model derived from saliva. In this study, The MIC of pomegranate extract against the bacteria was 1.25 mg/ml. The extract had an anti-adherence effect against the bacteria (107). Bacteria's tooth surface adhesion is necessary for biofilm formation. Vasconcelos et al. assessed the antimicrobial effect of pomegranate extracts. They concluded that the lowest inhibitory concentration levels are at 5% and 10% against *Candida albicans, Streptococcus mitis,* and *S. mutans* (108). In a study by Sharma et al., it was concluded that the 5% concentration of aqueous extract of pomegranate versus resistant clinical strains of *Escherichia coli, E .faecalis, Pseudomonas aeruginosa,* and *Klebsiella pneumonia* is related to the antibacterial activity of ethanolic, acetic and pomegranate. Therefore, the pomegranate tincture utilized at a concentration of 5% is shown in the control of root canal microorganisms (96). In 2021, Shaymaa et al. evaluated the aqueous and alcohol extract of Cammara leaves and *Punica granatum* peels against the bacteria Proteus mirabilis. The results exhibited that the pomegranate peel extract has significant dose-dependent activity. The largest inhibitory zones appear at 1600 mg/ml. *Lantana cammara* leaf extract did not exhibit substantial inhibition, and the bacteria were resistant to the extract (109).

Devi et al. compared the effect of antibiotics and pomegranate extracts against dental plaque bacteria and found that Doxycycline and Clindamycin presented the highest range of antibacterial activities. Nevertheless, pomegranate does not have many side effects and could be used as a long-term treatment for particular groups of patients (110). At the same time, pomegranate extract improved the impact of several antibiotics comprising tetracycline, gentamicin, ampicillin, oxacillin, and chloramphenicol versus 30 clinical S. aureus isolation (111).

Besides, Hassan et al. measured the inhibition zone of *S. aureus* growth with pomegranate fatty extract supported by silver nanoparticles (AgNp). They found it effectively inhibited bacterial isolation at 100, 200, 400, and 800 concentrations. Silver nanoparticles interact with the sulfur in the cell wall of bacteria, which leads to increased permeability of the cell wall and death of the bacterial cell. The aqueous extracts of the pomegranate grease plant used in this study are vital antagonists in preventing the growth of *S. aureus* bacteria. The efficiency of the extract of pomegranate fortified by Silver nanoparticles intensifies the inhibition of bacterial proliferation (112). Abd-El-Aziz et al. studied the antibacterial function of P*unica granatum* peel extracts and green tea on *S. mutans* of patients who received orthodontic treatment and its adhesiveness to the dental surface. The *Punica granatum* extract, entire phenol contents, and its antibacterial effect have been evaluated (113). The antibacterial effect of the extract differs based on the

concentrations of the effective secondary metabolites in the extracts, the chemical constituents of the plant, the extraction procedures, and the utility of bacterial strains (114). The maximum antibacterial effects of the ethanolic extraction of green tea and *Punica granatum* peel are because of saponin, tannin, and glycosides. Gamma radiation up to 10 kGy is an efficient technique for improving extract obtained from *Punica granatum* peels and green tea leaves. Gamma-irradiated (10kGy) ethanol extracts of *Punica granatum* peels and green tea included the most considerable total phenol contents. They had an elevated impediment activity on *S. mutans* and diminished their bonding to the dental surfaces (113). Qader et al. conducted that the antibacterial effect of plant extracts changes due to the concentrations of the effective secondary metabolites that exist in the extracts, the chemical constituents of the plant, the extraction technique, and the bacterial strains used (115). The antimicrobial agents available in pomegranate (the hydrolyzable tannins) can create high molecular weight complexes with soluble proteins, increase bacterial lysis, and inhibit bacterial adherence to teeth surface (116). In 2017, Behera et al. described the antibacterial efficacy of three chosen fruit juices (Pomegranate, Apple, and Grape) on endodontic bacterial strains. The results accomplished in this study clearly showed a remarkable antimicrobial activity of apple fruit juice against *S. mutans* and *E. faecalis*. However, when the antibacterial efficacy diameter of the inhibition zone of grapes and pomegranate was compared, a remarkable value of $P > 0.05$ was observed, which makes it insignificant (117).

In vivo studies

Table I summarizes the effect of pomegranate on dental caries in in vivo studies. In 2018, López-Rios et al. evaluated the toxicity of Xanthigen®, a nutraceutical mixture for weight management containing pomegranate seed oil and some other ingredients, genotoxicity, and 90-day repeated oral toxicity. Based on the observations and analysis, Xanthigen® did not present either clastogenic activity or genotoxic using the chromosome aberration test, the Ames test, and In Vivo micronucleus assay. No safety issues were conducted in any of the dosages used in the 14-day and 90-day (118). Research conducted in 2016 evaluated the subchronic toxicity of the mouth of Fitnox in Wistar albino rats. Fitnox is a recently developed dietary component for physical endurance made of the extracts of *Punica granatum* peel (25-30%), *Moringa oleifera* leaf (45-50%), and *Kaempferia parviflora* (black ginger) root (15-20%). Consequences revealed no remarkable alteration between the Fitnox and the control. Due to the consequences, the conclusion showed Fitnox delivered via oral cavity to rats (dose of 1000 mg/kg per rat, orally-90 days) is secure with no drug-correlated toxicity perceived at the time of the research (119).

ANTIFUNGAL ACTIVITY

Candida albicans cause oral candidiasis, the most common opportunistic infection of the oral mucosa (120). *Candida albicans* is commonly found on dental biofilm, and its capacity to generate collagenolytic enzymes and organic acids shows its function in developing caries (121). The emergence of antifungal resistance highlights the need to use natural drugs rather than synthetic medications, which have fewer side effects (90). In the following, we consider the antifungal activity of the pomegranate.

In vitro studies

An in vitro study was carried out to discover the antifungal effectiveness of pomegranate peel extract compared to clotrimazole against *Candida*. The antifungal effect of pomegranate and clotrimazole was statistically significant and had analogous effectiveness. Punicalagin and polyphenols are substances exhibiting antifungal activity. The ethanolic peel extract also has antifungal effects, specifically against *Candida* species (122). Therefore, *Punica granatum* peel extract can be an efficient natural replacement for synthetic antifungal drugs (123). Several in vitro studies have manifested that punicalagin isolated from the pomegranate peel extract can precipitate proteins on cell surfaces, while polyphenols are recognized to interact with proteins and prevent the growth of microbial co-aggregation; so the extract showed antifungal properties by deprivation of substrates and direct effect on microbial metabolism (124, 125).

In vivo studies

In vivo investigations have shown that Phyto therapeutic compounds produced from *Punica granatum Linn* extract have antibacterial (126) and antifungal (127) properties. The adhesion of various bacterial strains and one yeast usually seen in the oral cavity; was inhibited by the *Punica granatum Linn* (Pomegranate) gel rich in tannin and polyphenolics. The outcomes of this study suggest the feasibility of using Punica granatum Linn (Pomegranate) gel to manage yeasts and bacteria. It also had greater effectiveness than miconazole in prohibiting microbial adherence. The results showcased that the synthesis of glucan and its antimicrobial activity make this gel effective in controlling the formed biofilm (108).

Human studies

The application of the gel, including the extract of *Punica granatum* as an antifungal agent versus candidiasis related to denture stomatitis, resulted in the clinical negativity of fungus in most patients. Thus, this extract can be utilized as a topical antifungal agent for treating candidiasis associated with denture stomatitis. *Punica granatum* contains tannins, the compound

acting on the cell membrane based on the ability of precipitation proteins (127). The findings indicated that different forms of *Punica granatum* might be used as an inexpensive and easy adjuvant to prescription antifungal medications (88).

PERIODONTIUM & PLAQUES

Following studies focus on pomegranate extracts' effectiveness on periodontium health. Administrating different pomegranate products revealed a significant reduction in plaque index, gingival index, depth of probing depth, relative attachment level, sulcus bleeding, and also percentage of microorganisms in the pocket (128-130). These results are associated with antibacterial activity and inhibitory function against microorganisms' adhesion to the dental surface (66, 97, 131-134).

In vitro studies

The presence of antibacterial factors like hydrolyzable tannins can enhance bacterial lysis and prevent bacterial adhesion to the tooth (63, 135-137). Moreover, immunoregulatory function over B- and T- lymphocyte subgroups and macrophages can prove Pomegranate`s anti-inflammatory features (137).

Human studies

Research by Tyagi et al. in 2021 illustrated the benefits of herbal gel and chip extracted from pomegranate and used as subgingival supplementary after scaling and root planning procedure. This clinical study was performed on three groups of adult patients with periodontitis. Their findings verified a considerable reduction in gingival index, plaque index, relative attachment levels, and depth of probing pocket (128). These results were under the medical properties of pomegranate, for instance, antibacterial activity (83, 131-133) or preventing effects on the adherence of microorganisms to the teeth (97). In addition, the study of Abullais et al. evaluated the effect of herbal extracts (HE) containing pomegranate and some other compounds as subgingival irrigation on periodontal health status (by analyzing sulcus bleeding and plaque indexes, probing depth and percentage of pocket microorganisms). The results revealed a considerable reduction of these indexes in both groups of pomegranate and chlorhexidine. Comparing intergroup results showed no meaningful difference between parameters except plaque index, in which the trial group displayed more reduction (129). Batista et al. compared the reduction of gingival bleeding among patients who used chlorhexidine 0.12% and those who used pomegranate and chamomile plant extract as a mouth rinse in 2014. Pomegranate and chamomile extract mouthwashes efficiently decreased gingival bleeding, indicating that they both have antimicrobial and anti-inflammatory actions resembling chlorhexidine 0.12% (130).

EFFECT ON BONE MASS

Following studies work on the effect of different forms of herbal extracts and materials on bone regeneration. Administrating pomegranate seed oil promotes bone formation by increasing insulin-like growth factor type 1 gene expression (59, 138-143). Pomegranate peel extract can enhance restorative procedures by raising the absorption of vitamin D (144).

In vivo studies

A study was conducted by Bachagol et al. to assess the bone-sparing effect of pomegranate seed oil (PSO) oral lavage on ovariectomized mice. Serum insulin-like growth factor type 1 (IGF-1) level was evaluated. Histone acetylation levels in the liver during the IGF-1 synthesis process were analyzed. The results indicated peak bone mass, bone length, and bone formation promotion due to increased IGF-1 gene expression in the liver after getting PSO in mice (138).

Baban et al. evaluated the effects of oral supplementation of *Punica granatum L.* peel extract on surgically created bone defects in rabbits. Serum vitamin D level performs dominant gain in whole time intervals reaching the maximum value. On day three, no change was seen in rabbits getting water Supplementation. *Punica granatum* peel extract could rise, absorbing vitamin D. Therefore, it might boost the bone restorative procedure (144).

PERIODONTITIS

Pomegranate extract has an antibacterial function versus periodontal microorganisms by different oxidizing enzymes in pathogens and reacting with sulfhydryl groups (1). Pomegranate has antiviral traits (145) and is effective in treating periodontitis because Herpes viruses can speed up the progression of this disease by stimulating the release of cytokine from host cells (146, 147). Pomegranate has a preventive impact on the expression of matrix metalloproteinases and IL–1β, which cause tissue damage. Also, it can improve antibiotic activity, which is a sign of its capacity to treat periodontitis (148). Daily usage of pomegranate mouthwash can decrease the function of aspartate aminotransferase, which is increased in periodontitis (131).

In vitro studies

Widyarman et al. analyzed the impact of *Punica granatum* juice on the *Aggregatibacter actinomycetemcomitans, Porphyromonasgingivalis*, and *Treponema denticola*. The biofilm mass was significantly reduced after treatments with pomegranate juice over all concentrations and

incubation times for both monospecies and multispecies biofilm. Pomegranate juice can be an alternative cure for periodontal disease prevention (149).

Human studies

A clinical study conducted by Sastravaha et al. examined the effect of the mixed extracts of the *Centella asiatica*, commonly recognized as Asiatic Pennywort, and *Punica granatum* on soft tissue wound healing after scaling and root planning in periodontitis patients. The consequences show that scaling and root planning, alongside local delivery with *Punica granatum* and *C. asiatica,* significantly decreased the clinical symptoms of chronic periodontitis (150).

GINGIVITIS

Various studies stated the pomegranate extracts' efficacy on gingivitis due to the reduction of gingival bleeding (126, 151), plaque index, gingival index, bleeding on probing, and salivary levels of IL-1β (152). polyphenols isolated from pomegranate inhibit the expression of IL-6 and IL-8 (126). Pomegranate is also considered an anti-inflammation agent by prohibiting the formation of leukotriene and prostaglandin (153).

***In vitro* studies**

The research done by Pereira et al. demonstrated a remarkable decrease in gingiva bleeding after using pomegranate dentifrice. Also, this study showed that the polyphenols derived from pomegranate have a preventive influence on the expression of IL-6 and IL-8 (126).

Pomegranate inhibits the eicosanoids, cyclooxygenase, and lipoxygenase enzymes so that extract can inhibit the formation of prostaglandin and leukotriene. It also acts as an anti-inflammation agent. Hence, they concluded that pomegranate mouthwash was effectively enhanced (153).

Human studies

Somu et al. designed a clinical study in 2011 to identify pomegranate extract gel's preventive and therapeutic properties in gingivitis. By collecting plaque samples and evaluating recorded parameters such as the papillary bleeding index, gingival index, and plaque index, considerable progress was observed in the first group using pomegranate gel and mechanical debridement compared with the rest of the groups (154).

Kiany et al. evaluated the effectiveness of pomegranate on gingival inflammation and dental plaque in a double-blind clinical trial. One hundred and four patients with mild to moderate gingivitis participated in this study and were divided into four groups. Group 1 used Persica, Group 2 used Matrica, Group 3 used pomegranate mouthwashes, and Group 4 used a placebo. In each group, they used oral rinses two times a day for one month. It was concluded that there was no remarkable difference in decreasing plaque in the four groups. Also, all herbal mouthwashes decreased gingival bleeding more than the placebo (151). Comparing the effectiveness of pomegranate peel extract (6.25% and 12.5% concentration) as a mouthwash with standard chlorhexidine (0.12%) in type-2 diabetes mellitus patients with moderate gingivitis showed that pomegranate mouthwash caused no side effects for either of the groups after 14 days of study. On the contrary, displeasing side effects such as irritation, unpleasant taste, and teeth staining were observed with using chlorhexidine (0.12%) mouthwash. Biochemical and clinical factors such as Plaque index, Bleeding on probing (BOP%), Gingival index, salivary levels of IL-1β, and AST were assessed in the study. These factors were reduced in all groups within a fortnight except for salivary IL-1β in the chlorhexidine group (152). This can result from chlorhexidine irritation side effects that may evoke an inflammatory response (153). There was no statistical difference between the chlorhexidine and 6.25% pomegranate mouthwashes. The group with 12.5% pomegranate peel extract showed the highest results regarding the reduction of the parameters. However, it was not significant (152). Rahimabadi et al. reported that pomegranate flower extract (mixed with vinegar) reduced the same clinical parameters in gingivitis (155). These can be the inhibitory effects of the polyphenolic-rich component of the peel extract (103). Moreover, Herbal biodegradable chips of *Punica granatum* extract have decreased IL-1β in gingival crevicular fluid (156). In 2009, DiSilvestro et al. evaluated the effects of pomegranate excerpt on gingivitis and could benefit oral healthiness. This analysis showed that pomegranate excerpt reduced the amount of protein, reduced actions of aspartate aminotransferase, decreased alpha-glucosidase activity, heightened measures of the antioxidant enzyme ceruloplasmin, and heightened radical scavenging space. This information raises the probability of using pomegranate extracts in oral health products such as toothpaste and mouthwash (157). In 2018, Jeevika CH.D. et al. detected the competence of pomegranate mouth rinse on plaque accretion, gingivitis, and all salivary proteins. Pomegranate mouth rinse decreased the mean plaque and gingival indicator account significantly at 3, 6, and 9 months and it significantly reduced all salivary proteins from criterion until 90 days analyzed contrasted to placebo (158). Another clinical study was done by Salgado et al. to assess the antigingivitic and antiplaque influence of the gel, including 10% pomegranate. It resulted in 10% of this plant being ineffective in inhibiting supragingival dental plaque forming and gingivitis (159).

DENTAL CARIES

Pomegranate has antibacterial activity against *S. mutants*, the primary cause of dental caries. Carbohydrate fermentation by the bacteria leads to acid formation,

which causes tooth demineralization and caries (107, 160). In the following, we consider the effects of pomegranate in controlling dental caries.

In vitro studies

Gulube et al. investigated the influence of the P*unica granatum* on the formation of biofilm and the production of acid and extracellular polysaccharides by *S. mutans.* At high concentrations, this extract of pomegranate could kill *S. mutans*. The extract at lower concentrations could reduce the formation of biofilm and the production of acid and extracellular polysaccharides. Based on the results, the lowest minimum bactericidal concentration (MBC) was 6.25 mg/mL. So they concluded that *Punica granatum* extract prevents dental caries (161). Pomegranate polyphenols (like tannins) have anticaries properties through antibacterial and anti-adherence activities. Tannins like punicalagin cross the bacterial cell wall and affect the proteins, leading to bacteria lysis. Tannins also suppress glucosyltransferase, an essential enzyme in the attachment of the bacteria to tooth surfaces (107, 160, 162). Benslimane et al. designed a study to evoke polyphenols from the peel extract of pomegranate with three solvents, including acetone 70%, ethanol 70%, and methanol 70% (v/v). The potential of anti-oxidant content was manifested by the cleaning acting of free radicals and ferric lessening power assays (by giving electrons or hydrogen). The antimicrobial function of pomegranate was figured out versus six oral bacteria separated from caries and plaques, including *S. mutans* and *Enterococcus faecalis*. The greatest phenolic and flavonoid amount also scavenging functions were observed with ethanolic pomegranate. Also, the greatest proanthocyanin and lessoning power assay were exhibited with the acetonic extract. Investigating the antibacterial activity showed that mentioned bacteria were susceptible to pomegranate, and the most vulnerable bacteria were gram-positive ones. This study showed that using pomegranate as an antioxidant and antibacterial agent was influential in treating caries (163). Loading *Punica granatum* peel extract into polyethyleneimine dextran sulfate nanoparticles (PDNPs) for caries prevention and oral malodor decrement seems to be an excellent mucoadhesive drug delivery system. In vitro dissolution studies acknowledged that PGE-loaded PDNPs manifested an extended-release characteristic with a burst delivery within 5 min. Furthermore, they remained effective against oral bacteria (164).

Human studies

The pH level of dental plaque after drinking sugar-free pomegranate juice and orange juice by crossover design Complete prophylaxis decreased after drinking both fruit juices. The decrease is similar for the two fruit juices (165). Additionally, previous research showed a reduction in the pH of dental plaque after drinking apple juice compared to consuming Guava, lemon, and pomegranate juice (166). Jacob et al. designed a triple-blind randomized clinical trial to evaluate the antibacterial efficiency of two different types of mouthwash, which contained chlorhexidine and pomegranate peel extract, on other bacteria like *Veillonella, S. mutans,* and *Lactobacilli* by evaluating samples of saliva in 60 patients with advanced dental caries. After two and four weeks of consuming mouthwash, pomegranate peel extract was efficient in disturbing all bacteria tested in this study; however, chlorhexidine still was the most effective agent against *S. mutans* (167). In 2021, Santos et al. designed a randomized study lasting 28 days to make biscuits with pomegranate and propolis to evaluate their efficiency on dog oral health compared to sodium hexametaphosphate, which is a good agent in dental remineralizations by binding with calcium in saliva. To this aim, five groups of biscuits were tested: without any additive, with sodium hexametaphosphate, with propolis, with Pomegranate, and a mixture of pomegranate and propolis. Curing with sodium hexametaphosphate and Pomegranate exhibited the best decrease in dental calculus because pomegranate can interfere with the poly glucan, essential in bacterial connection to the tooth (135). Also, treatment with other groups was approximately the same (168).

RECURRENT APHTHOUS STOMATITIS

Recurrent aphthous stomatitis (RAS) is a prevalent oral disorder affecting almost 20% of the population (169). RAS is recognized by ulcers with red margins on the oral mucosa, which are self-limiting in one or two weeks (170). It has unknown etiologies, but some risk factors are trauma, bacteria, genetics, and immune system factors (170). The topical use of pomegranate seed, peel, and flower extract effectively controlled RAS symptoms (169-171). In the following, we consider the effects of the pomegranate in managing RAS symptoms.

In vitro studies

High amounts of polyphenol anti-oxidants such as tannins and flavonoids can be extracted from pomegranate seeds that have antibacterial effects. Therefore, Setiadhi et al. examined the possible antibacterial effect of pomegranate seed against *Streptococcus sanguis* since it has been known as a predisposing factor for RAS. They measured MIC and MBC through the microdilution method and in 30, 60, and 90-second intervals. They found that it has a growth inhibitory activity against the bacteria as a possible cause for RAS, and its antibacterial result depends on the concentration (171, 172).

Human studies

Darakhshan et al. found that in two groups of 28 patients each, using pomegranate peel extract as a topical gel can lessen the healing duration, improve the pain, and reduce the lesion size compared to a placebo gel (169). In patients with RAS, the antioxidant capacity is decreased, but the lipid peroxidation is increased. Pomegranate has anti-oxidant and antibacterial effects

because it contains tannins, making it a good choice for managing RAS (169).

BIOLOGICAL EFFECTS (SYSTEMIC DISEASE)

Fruit extracts have been shown to have antitumor, antidiarrheal, antibacterial, antifungal, and anti-ulcer properties. The fruit part is responsible for the species' observed antibacterial action, particularly against Clostridium perfringens, *Staphylococcus aureus,* Salmonella sp., and Serratia sp (160). In the following, we consider the biological effects of the pomegranate.

In vitro studies

In 2010, Viana et al. found that the pharmacological functions of *Punica granatum* chemical ingredients propose a broad range of potential clinical use for treating many diseases. It has been reported that *Punica granatum* juice interdicted the advance of atherosclerotic lesions, ameliorated stress-induced ischemia in illnesses with coronary heart illness, and ameliorated postprandial hyperglycemia and lipid index in diabetic diseases. *Punica granatum* has a broad spectrum of antibacterial, antiviral, and antihelminthic attributes. A gel comprising an exploit of *Punica granatum* was efficient in dental illnesses. The intake of pomegranate has been shown to promote health and prevent diseases, composed with a not much toxicity index (160).

In vivo studies

In 2013, Kheibari et al. conducted the present study to evaluate the clinical activity of the concentrated extract of pomegranate in animals as a model for human medicine. The recovery amount of the oral lesions was observed daily by visualization. As fibrin deposition and re-growth of the epithelium were observed, the healing progress was considered an excellent response to treatment. Daily observation of the healing progress of the oral lesions significantly exhibited faster healing of the oral lesions in the concentrated extract of the pomegranate ($p=0.001$). It could be conducted that the concentrated extract of the pomegranate might be considered a herbal drug for the healing and treatment of oral foot and mouth disease lesions in cattle, and it may be successful in similar lesions in humans (173).

Kumar et al. Evaluated pomegranate juice pretreatment on the bioavailability of buspirone rabbits in 2011. The results showed a significant difference in the bioavailabilities of buspirone after pretreatment with pomegranate juice, possibly due to the inhibition of CYP3A4 (174).

Human studies

In 2012, Hanley et al. reported that pomegranate juice and an a1-g capsule containing pomegranate extract were evaluated in vitro and in vivo as inhibitors of cytochrome P450 2C9(CYP2C9), with flurbiprofen serving as the index substrate. CYP2C9 is the essential enzyme for the clearance of S-warfarin (175).

ANTI-INFLAMMATORY AND ANTI-OXIDANT EFFECTS

The anti-inflammatory and anti-oxidant effects of the pomegranate are associated with its polyphenols, including ellagitannins (ellagic acid, gallic acid, and punicalagin), anthocyanins, gallotannins, gallery esters, dihydro flavonol, and hydroxycinnamic acids (176, 177). *Punica granatum* significantly prevents and fights various diseases, highlighting its antimicrobial and anti-inflammatory features (178). In the following, we consider the anti-inflammatory and antioxidant effects of pomegranate.

In vitro studies

Acute inflammation is a helpful host response for preventing harm to tissue, but it may also lead to immune-associated diseases such as rheumatoid arthritis, inflammatory bowel disease, and cancers. Pomegranate has been shown to prevent inflammation by various mechanisms. Cyclooxygenase (COX) and lipooxygenase (LOX), clef enzymes transforming arachidonic acid to prostaglandins and leukotrienes, are subsequently interdicted by *P. granatum*. Also, pomegranate has a considerable inhibitory result on osteoarthritis (OA) by repressing the statement of MMPs in OA chondrocyte cultures and forbidding collagen decay. IL-1β persuades the explanation of MMPs, especially MMP-1, and MMP-13, which depend on the immutable separation of cartilage matrix through digesting type-II collagen and the resultful liberation of matrix proteoglycan from the cartilage. These scrutinies show that pomegranate prevents the p38-mitogen-activated protein kinase passage and copy factor, NF-kB. Dominion of 50 mg/kg of Pg juice for 28 days causes a reduction in malondialdehyde (MDA), TNF-α, and IL-1β levels in mice with liver fibrosis. Pretreatment with 13.6 mg/kg of Pg juice declined the arthritis occurrence and less IL-6 and IL-1β levels in the animal pattern of rheumatoid arthritis (179). Fig. 2 summarizes the anti-inflammatory effect of pomegranate. Another study indicated the dependence of pomegranate juice's antibacterial activity on the contents of pigments, citric acid, and phenolic compounds and discovered that anti-oxidant activities in vitro with punicalagin, ellagic acid, total pomegranate tannin (polyphenol extracts from whole pomegranate juice), and pomegranate juice has more antioxidant activity than any of its constituents (23).

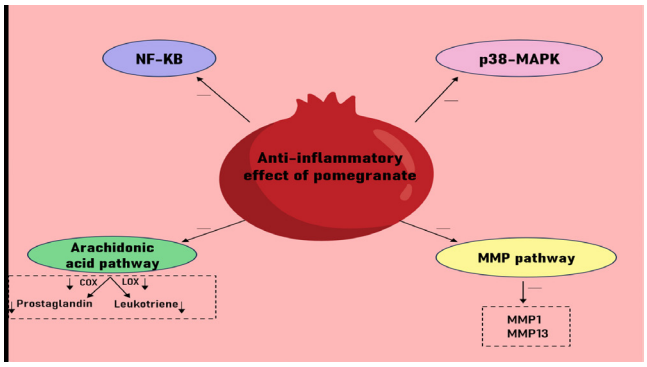

Fig. 2: Pomegranate inhibits cyclooxygenase and consequently inhibits the conversion of arachidonic acid to prostaglandins. In addition, this substance inhibits lipoxygenase and finally inhibits leukotriene formation from arachidonic acid. It also inhibits MMP expression and inhibits the p38-mitogen-activated protein kinase pathway. Another anti-inflammatory effect is inhibiting NF-kB.

In vivo studies

Omer et al. studied the anti-inflammatory and antioxidant properties of the ethanolic extract of pomegranate seed used as an oral supplement in rabbits with buccal gingival wounds. They measured alkaline phosphatase (138) activity, total proteins (41), and uric acid (UA) in serum by collecting blood samples at times 0, 3 hours, one day, three days, and seven days after the creation of wounds. ALP, an enzyme that is increased in inflammatory processes, was used as a marker of inflammation. Increasing TP of serum shows the generation of free radicals. UA is a scavenger of free radicals in the blood (180). TP and UA in serum were used as free radical production and oxidative stress markers. ALP, TP, and UA increased in all intervals in rabbits that did not receive the pomegranate extract. In rabbits that received the pomegranate extract, ALP, TP, and UA increased only 3 hours and one day after the wounds, showing pomegranate's anti-inflammatory and antioxidant effects (176).

Nirwana et al. evaluated the activity of pomegranate distillate on rat dental pulp mechanically and showed the phase of NF-kB. Pomegranate fruit distillate as a pulp cover material was found to prevent the translocation of nuclear factor kappa B (NF-kB) into the cell's core. It could be demonstrated by lowering the NF-kB phrase. That pomegranate fruit distillate reduced NF-KB expression and the manufacture of the proinflammatory cytokine and sped up the inflammation procedure (181). Hana et al. used intramuscular injection into the periodontium of rabbits to cause inflammation and oxidative damage. ALP and acid phosphatase (ACP) were used as inflammation markers. ACP is an enzyme that raises inflammation. Malondialdehyde (MDA) and TP were used as oxidative process markers. MDA is the final product of polyunsaturated fatty acids peroxidation. The dose of pomegranate injected in the study group was 50µl/kg of 0.25g/dl in saline solution. In rabbits injected with the pomegranate solution, ALP and ACP increased on days one and three after the injury; however, they decreased on days seven and 14 after the injection. MDA and TP in the rabbits decreased seven days after the pomegranate injection. It was concluded that pomegranate has anti-inflammatory and antioxidant effects, making it a good choice for treating periodontal disorders (182).

Human studies

Rose et al. evaluated the anti-inflammatory effect of pomegranate on oral mucositis following cancer chemotherapy treatment. The experimental group received the pomegranate popsicles, and the control group followed the mouth routine with fresh water thrice daily. After seven days, patients who received pomegranate popsicles were 20% less likely to develop oral mucositis. So pomegranate is a natural and cost-effective choice to decrease oral mucositis in cancer patients undergoing chemotherapy (183). Oxidative stress (OS) makes toxic metabolites that can start and develop cancers. The use of polyphenols and flavonoids is helpful for the prohibition of cardiovascular, inflammatory, and other diseases by prohibiting OS that persuades lipid peroxidation in arterial macrophages and lipoproteins. Pomegranate contains flavonoids and anthocyanidins in its kernel oil and syrup. Pomegranate fruit extracts display scavenging activity versus hydroxyl radicals and superoxide anions, which could be relevant to anthocyanidins. Studies have indicated that methanolic juice from the pomegranate shell has a broad spectrum of antioxidant operations (184).

WOUND HEALING

The peel of pomegranate fruit is a potent astringent used to treat traumatic bleeding, ulcers, and diarrhea. In the Indian subcontinent, dried pomegranate peel is used to cure nose bleeds, aphthae, and intestinal worms. Ellagitannins, represented by ellagic acid (EA), gallic acid, and punicalagin, are thought to be responsible for the medicinal advantages of pomegranate peel (185-190). In the following, we consider the wound-healing effects of the pomegranate.

In vitro studies

Another practice was an in vitro study conducted by Celiksoy et al. in 2020. They investigated the wound healing potential of pomegranate rind extract (PRE) and an anti-oxidant element known as punicalagin, alone or combined with Zn (1). Many previous studies recognized the wound-healing ability of punicalagin and PRE in the oral cavity (181, 185, 191-194). Still, this practice notably reported wound healing advantages of the punicalagin and Zn (1) combination. The mentioned effect was based on evidence, such as wound repopulation reactions and fibroblast migration in the gingiva. However, no prompting impact on the proliferation of fibroblast cells was observed (195-197). Many essential components required to maintain cell viability and proliferation are present in pomegranate

juice. These characteristics suggest that pomegranate juice is a suitable storage medium for avulsed teeth (198). Babaji et al. conducted a study in 2017 to assess the potential of Hank's balanced salt solution (HBSS)-considered a standard agent- propolis, Aloe vera, and pomegranate in sustaining periodontal ligament (PDL) viability of teeth under avulsion. The data from the analysis of 50 sound-extracted teeth revealed that we could use propolis, Aloe vera, and pomegranate as storage media (198). Similar results were obtained in a study performed by Tavassoli-Hojjati et al. in 2014, in which 7.5% pomegranate juice (PJ) was suggested as an effective agent in preserving the vitality of PDL cells. Regarding mentioned effects, surveys showed similar results for 1% PJ and HBSS (199). Punicalagin as an anti-oxidant agent in pomegranate can explain the findings (200). Another report showcased pomegranate`s function against collagenase and elastase enzymes (201). In addition, the serine protease inhibitor presence can also stop the activity of collagenase and trypsin (202). Based on the above, the capacity of pomegranate for solid cell binding can be proved.

In vivo studies

Nirwana et al. performed a practice in 2017 to evaluate pomegranate extract efficacy on wound healing procedures. They divided 12 Cavia cobaya into control and treatment groups. The trial group, subjected to 2.5% pomegranate fruit extract gel, revealed notable progress in wound healing. Immunohistochemical methods displayed increased expression of Vascular endothelial growth factor (VEGF) and Platelet-derived growth factor (PDGF), which are critical in angiogenesis through wound healing (181).

ANTICANCER EFFECTS

Punica granatum (Pg) fruit has long been used as a traditional treatment for respiratory diseases, acidosis, dysentery, diarrhea, microbiological infections, and helminth infections. Additionally, it has been demonstrated that pg seeds contain the estrogenic substances estrone and estradiol. The dried pericarp and fruit juice also help treat dental disorders, colic, menorrhagia, colitis, oxyuriasis, headaches, acne, diuretics, piles, and allergic dermatitis. New scientific research for the conventional applications of PG has emerged in recent studies. *Punica granatum* inhibits various neoplasms, such as prostate, colon, breast, and lung cancers. In the following, we consider the anticancer effects of pomegranate (203).

In vitro studies

In 2012, Rahimi. et al. found that *Punica granatum* and its chemical ingredients have different pharmacological and toxicological virtues containing anti-oxidant, anti-inflammatory, anticancer, and anti-angiogenesis processes. They also display inhibitory factors on invasion/motility, cell course, apoptosis, and biotic enzymes. Also, they motivate cell separation and have antimutagenic factors. These virtues forcefully offer a broad use of pomegranate for clinical usage (179). *Punica granatum* inhibits various neoplasms, such as prostate, breast, colon, and lung cancers. Pomegranate prevents NF-kB and cell livability of prostate cancer cell lines in a dose-dependent method in the LAPC4 xenograft model, *in-vitro*. Pomegranate polyphenols, ellagitannin-rich juice, and whole juice extract inhibited gene expression of HSD3B2, AKR1C3, and SRD5A1, which are clef androgen-synthesizing enzymes in LNCaP, LNCaP-AR, and DU-145 human prostate cancer cells. Therapy with pomegranate fruit juice for 72 h was found to result in a considerable deterrence of lung cancer, with dose-dependent detention of cells in the G0/G1 stage of the cell cycle, infusion of WAF1/p21 and KIP1/p27, reduction in the protein explanations of cyclins D1, D2, and E, reduction in cyclin-dependent kinase (CDK) 2, cdk4 and cdk6 explanation, phosphorylation of MAPK proteins, deterrence of PI3K, phosphorylation of Akt at Thr308, NF-kB and IKK alpha, decay and phosphorylation of IκB, Ki-67, and PCNA.

In vivo studies

In 2013 Hernawati S. designed a study to evaluate and compare ellagic acid, an ingredient gathered from pomegranate, with an extract from pomegranate fruit in decreasing the expression of a proto-oncogene called BCL2. This gene prevents the procedure of apoptosis. In the research, 32 mice were used. They were separated into four groups: two control and two treatment groups. One of the treatment groups was treated with an extract of pomegranate, and the other was treated with ellagic acid. Results demonstrated that using pomegranate is remarkably better than ellagic acid in decreasing the expression of mentioned proto-oncogene in malign cells of the oral mucosa in the mice (204-207).

CONCLUSION

In this study, we assessed the beneficial properties of the *Punica granatum*, including its antimicrobial, anti-oxidative, anticaries, wound healing, and anti-inflammatory effects, which can effectively prevent and treat inflammatory diseases like periodontal disease. The maximum antibacterial activity of the pomegranate is because of the presence of saponin, tannin, flavonoid, polyphenols, and glycosides, which are noted as potential antimicrobial agents. Polyphenols have anticaries properties through antibacterial and anti-adherence activities by suppressing glucosyltransferase, a critical enzyme in the attachment of the bacteria to tooth surfaces. Tannins affect the cell membrane of the bacteria, while polyphenols are recognized to interact with proteins and have antifungal and antimicrobial activity by an effect on metabolism. Tannins and flavonoids have a considerable inhibitory impact versus *Streptococcus sanguis*, which is the reason for recurrent aphthous stomatitis. The glycolic extract strongly affects

Porphyromonas gingivalis, the primary pathogen in periodontitis. Polyphenols have a preventive influence on the expression of prostaglandins and leukotrienes, so the pomegranate extract can be an anti-inflammation agent. Pomegranate decreases the expression of a proto-oncogene called BCL2. This gene prevents the procedure of apoptosis. Pomegranate can be a useful treatment option for many oral diseases due to its appropriate therapeutic effects and the absence of side effects. However, more in-vitro, in-vivo, and controlled trial studies are needed to demonstrate the exact mechanism of pomegranate action and its influence on human models.

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