

Study of Antimicrobial Activity to Pomegranate Juice Against Some of Dental Pathogenes

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ABSTRACT

Dental caries is a localized, transmissible infectious process that ends up in the destruction of hard dental tissue. *Streptococcus mutans* is one of the main opportunist pathogen of dental caries which plays a central role in fermenting carbohydrates that result in acid production, leading to the corrosion of tooth enamel. In addition, other microflora like *Lactobacillus* species are also associated with active caries lesions. *Punica granatum*, which belongs to the family of Punicaceae has been used extensively as a traditional medicine in many countries for the treatment of dysentery, diarrhea, helminthiasis, acidosis, hemorrhage and respiratory infections. Four *Streptococcus mutans*, seven *Lactobacillus acidophilus* and two *Candida albicans* isolates were tested using well diffusion method in different dilutions 100%, 75%, 50%, 25% and 10% of Pomegranate juice. *Streptococcus mutans* isolates showed greatest effect while two *Lactobacillus acidophilus* isolates had no effect to all dilutions on the other hand all *Candida albicans* isolates revealed resistant to all Pomegranate juice dilutions. The results suggest that Pomegranate juice is an effective antimicrobial substance against bacteria like *Streptococcus mutans* and *Lactobacillus acidophilus*, while it has no effect on fungus like *Candida albicans* which they are major dental pathogens.

Aim of this study was to determine antimicrobial activity of Pomegranate juice against *Streptococcus mutans*, *Lactobacillus acidophilus* and *Candida albicans* isolates which they are major dental pathogens.

INTRODUCTION

Despite great improvements in the global oral health status, dental caries still remains one of the most prevalent diseases (١). Dental caries is a localized, transmissible infectious process that ends up in the destruction of hard dental tissue. It results from the accumulation of plaque on the surface of teeth and biochemical activities of complex micro-communities. *Streptococcus mutans* is one of the main opportunist pathogen of dental caries (٢), which plays a central role in fermenting carbohydrates that result in acid production, leading to the corrosion of tooth enamel. In addition, other microflora like *Lactobacillus species* are also associated with active caries lesions. *Lactobacillus acidophilus* is the most common (٣). Poor oral hygiene is one of the reasons for accumulation of these microbes and their harmful activities. The common method for maintaining good oral hygiene is brushing the teeth with dentifrices that have antimicrobial properties and can prevent the degradation of tooth enamel. Synthetic dentifrices commonly used contain chemical agents, which are known to produce harmful side effects on prolonged use (٤). *Punica granatum*, which belongs to the family of Punicaceae, is commonly known as pomegranate, grenade, granats and punica apple(٥). *Punica granatum* has been used extensively as a traditional medicine in many countries (٦), for the treatment of dysentery, diarrhea, helminthiasis, acidosis, hemorrhage and respiratory pathologies (٧). In addition, *P. granatum* is reported to have antioxidant (٨), anti-atherosclerotic (٩), antibacterial (١٠) and antiviral (١١) properties. The constituents of *P. granatum* include gallocatechins, delphinidin, cyanidin, gallic acid, ellagic acid, pelargonidin and sitosterol, which are very well known for their therapeutic properties (١٢). *P. granatum L.* gel also showed greater efficiency in inhibiting microbial adherence of different microorganisms in the oral cavity than miconazole(١٣), While one study showed that, in a test tube, extracts of the fruit can inhibit the proliferation of human breast cancer cells (١٤). Therefore, the goal of this study is to evaluate the antimicrobial activity of the *P. granatum* juice using *in vitro* model against some dental pathogens.

MATERIALS AND METHODS

Collection of samples: samples were collected from dental caries of ٢٥ students in Biology department in the College of Science. Streaked on blood agar and De Mann-Rogosa-Sharp (MRS) agar, incubated an aerobically at ٣٧ C° for ٢٤ hours and ٧٢ hours to isolate *Streptococcus mutans* and *Lactobacillus acidophilus* respectively. For *Candida albicans* isolation samples were cultured in Sabouraud dextrose agar medium. The organisms were identified by standard microbiological techniques including colony morphology, microscopic examination and biochemical tests (١٥).

Preparation of Pomegranate juice: Fresh sweet Iraqi pomegranate fruits were collected from the local market. Taxonomic identification of the plant was established. Pericarp of ripened fruit was collected and washed with sterile distilled water. Crushed into parts and squeezed to remove the crude extract. The crude extract was filtered through muslin followed by Whatman No. 1 filter paper, finally filter sterilization using a 0.2 µm filter (Millipore). Stored in sterile vials (16).

Determination of antibacterial activity: Well diffusion method was used to screen the antimicrobial activity. In-vitro antimicrobial assay was screened by using Mueller Hinton Agar (MHA) obtained from HiMedia, India. The MHA plates were prepared by pouring 10ml of molten media into sterile Petri dishes. The Plates were allowed to solidify for 10 minutes. All isolates were suspended in sterile water and diluted to 10⁸ CFU/ml. The suspension (100 µl) was spread onto the surface of MHA. Wells (6 mm) are aseptically punched on the agar using a sterile cork borer allowing at least 30 mm between adjacent wells. Serial dilutions (with sterile distilled water) were made from the concentrated Pomegranate juice v/v (70%, 50%, 20% and 10%) added in the wells. *Streptococcus mutans* plates were incubated an aerobically at 37°C for 24 hours, *Lactobacillus acidophilus* plates incubated an aerobically 37°C for 72 hours and *Candida albicans* plates incubated 37°C for 24 hours (17). The anti-microbial activity was evaluated by measuring the diameter of the inhibition zone formed around the wells.

RESULTS and DISCUSSION

In this study the antimicrobial activity of Pomegranate juice was tested against different isolates of *Streptococcus mutans*, *Lactobacillus acidophilus* and *Candida albicans* isolates, the obtained results confirm that Pomegranate juice can inhibit growth rates of the tested bacteria in different degree and all *Streptococcus mutans* isolates showed inhibition zone with variable diameters against 100%, 70%, 50% and 20% dilutions while 10% dilution had no effect to all *Streptococcus mutans* isolates as shown in table 1.

Table 1: antimicrobial activity of Pomegranate juice against *Streptococcus mutans*, *Lactobacillus acidophilus* and *Candida albicans* isolates

isolates	100%	70%	50%	20%	10%
	P.J.	P.J.	P.J.	P.J.	P.J.
Zone of inhibition (mm)					
<i>Streptococcus mutans</i> 1	19	16	14	10	-*
<i>Streptococcus mutans</i> 2	16	16	14	12	-
<i>Streptococcus mutans</i> 3	19	18	15	12	-
<i>Streptococcus mutans</i> 4	16	12	8	7	-

<i>Lactobacillus acidophilus</i> ١	٢٠	١٦	١٥	١٢	-
<i>Lactobacillus acidophilus</i> ٢	١٢	٦	-	-	-
<i>Lactobacillus acidophilus</i> ٣	-	-	-	-	-
<i>Lactobacillus acidophilus</i> ٤	١٢	١٠	-	-	-
<i>Lactobacillus acidophilus</i> ٥	١٦	١٣	١٣	١٢	-
<i>Lactobacillus acidophilus</i> ٦	١٧	١٦	١٤	-	-
<i>Lactobacillus acidophilus</i> ٧	-	-	-	-	-
<i>Candida albicans</i> ١	-	-	-	-	-
<i>Candida albicans</i> ٢	-	-	-	-	-

*(-) No inhibition zone

Which agree with Iraqi study in ٢٠٠٩ studied ellagic acid one constituent of pomegranate juice and found that diameter of zones of inhibition of *Streptococcus mutans* were increased as the concentration of ellagic acid increased(١٨).The inhibitory effect may due to the *In vivo* studies that demonstrated the antibacterial(١٩) effects of phytotherapeutic agents derived from *Punica granatum Linn* extract, in another study investigated *in vitro* the therapeutic potential of this agent against bacteria and yeasts either alone or pairs and groups. *In vitro* studies using biofilm models are those that come closer to a clinical situation and more precisely reflect *in vivo* conditions (٢٠). While the results indicate that two isolates of *Lactobacillus acidophilus* showed resistant to all dilutions and the effect lasted up to ٢٥% dilution in two isolates.Kakiuchi *et al.* (٢١) demonstrated the specific antimicrobial action of *Punica granatum Linn* on dental biofilm bacteria, i.e., disturbance of oliglycan synthesis, thus acting on the adherence mechanisms of these organisms to dental surface. Kirilenko *et al* (٢٢) reported that the antibacterial action of pomegranate juice varied with variety and depended on the contents of phenolic compounds, pigments and citric acid and another study found that antioxidant activities *in vitro* with pomegranate juice, punicalagin, ellagic acid, and total pomegranate tannin (polyphenol extracts from whole pomegranate juice)results identified that whole pomegranate juice are having more antioxidant activity than any of its individual constituents.The superiority of pomegranate juice compared to its individual polyphenols provides evidence of the synergy of multiple compounds in comparison to its individual polyphenols(٢٣).On the other hand all *Candida albicans* isolates showed resistant in all dilutions ,Which disagree with another study found punicalagin isolated from the fruit peel of pomegranate had

antimicrobial activity against *Candida albicans* (٢٤) and agree with other studies (٢٥) found that extract of *Punica granatum* peel did not have any effect on *C. albicans* in all concentrations. The real mechanism of the antifungal effect of tannins (the major components of *Punica granatum* extract) against *Candida albicans* is not clear. Gebara *et al.* (٢٦) reported that dental products containing natural substances have good market perspectives due to popular acceptance of phytotherapy, which represents an alternative to conventional treatments and could be introduced in the dental market as long as they are supported by scientific-based evidence. Another study founded using a pomegranate extract or fruit crust after eating or before sleeping with combination to tooth paste help to prevent dental pathogens activity (٢٧). The finding of this study support the possibility that the pomegranate juice might be used in the control of bacteria responsible for oral infections such as caries, further studies are needed to specify each active substance against each pathogen.

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دراسة الفعالية ضد ميكروبية لعصير الرمان ضد بعض الممرضات المسببة لتسوس الاسنان

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الخلاصة

تسوس الاسنان عملية تؤدي بالنهاية الى تحطيم انسجة الاسنان وبكتريا *Streptococcus mutans* واحدة من اهم الممرضات الانتهازية المسببة لتسوس الاسنان والتي تلعب دور مهم في عملية تخمير الكربوهيدرات التي تؤدي الى تكوين الحامض، مما يؤدي الى تآكل في انيميا السن بالاضافة الى انواع اخرى مثل *Lactobacillus acidophilus* التي لها دور فعال في عملية التسوس. الرمان الذي يعود الى العائلة الرمانية يستعمل في العديد من الدول لعلاج الاسهال والاصابات بالديدان والحموضة والنزف والاصابات التنفسية. تم التحقق من الفعالية ضد ميكروبية لعصير الرمان ضد اربع عزلات *Streptococcus mutans* وسبع عزلات من *Lactobacillus acidophilus* وعزلتين من *Candida albicans* وذلك باستخدام طريقة الانتشار بالاكوار وبتركيز مختلفة ١٠٠%، ٧٥%، ٥٠%، ٢٥%، ١٠%. أظهرت عزلات *Streptococcus mutans* اكبر تأثير بينما فقط عزلتين من *Lactobacillus acidophilus* كانتا مقاومتين لكل تركيز عصير الرمان، اما بالنسبة ل *Candida albicans* فكانت مقاومة لكل تركيز عصير الرمان. الدراسة تشير الى ان عصير الرمان قد يكون مادة ضد ميكروبية فعالة ضد بعض الممرضات المسببة لتسوس الاسنان.