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BIODEGRADABLE STARCH FILMS INCORPORATED WITH POMEGRANATE RIND EXTRACT AS ANTIMICROBIAL AGENT ON *Pseudomonas aeruginosa*

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ABSTRACT

Antibacterial effect of wheat starch edible film incorporating pomegranate rind extract at various concentrations was studied. Edible films were prepared from a mixture of starch, glycerol and polyethylen glycol. Pomegranate rind extract [1, 5, 10 and 20%] were incorporated in the films to act as natural antimicrobial agent, respectively. The films were characterized for antibacterial activity against *Pseudomonas aeruginosa*. The edible film exhibited antibacterial activity against tested bacteria by using agar diffusion assay method. For films tested against *P. aeruginosa* the zone of inhibition increased significantly [p<o.o5] with addition of extract at all levels.

Keywords: Edible film, wheat starch, pomegranate rind extract, antibacterial activity

INTRODUCTION

Over the last few years, considerable research has been conducted to develop and apply bio-based polymers made from a variety of agricultural commodities and lot wastes of food product industrialization. Such biopolymers include starches, cellulose derivatives, chitosan/chitin, gums, proteins and lipids. These material is present the possibility of obtaining this films and coating to corer fresh or further processed foods to extent their shelf life [1].

The use of natural plant extracts is desirable far desirable far development of new food products and nutraceuticals as well as well as active packaging systems. Antimicrobial packaging is a packaging system that is able to reduce, inhibit or

retard the growth of pathogenic microorganisms in packed foods and packaging material. Several attempts have been made in developing active packaging systems in which antimicrobial agents are incorporated into the polymeric material and slowly released on the food surface [2]. Starch based films have been particularly considered for the reason that they exhibit physical characteristics similar to synthetic polymers: transparent, semi – permeable to co_2 and resistant to o_2 passage [3].

Pomegranate (*Punica granatum* L.) is a rich source of tannins. The plant belongs to the Punicaceae, it is one of the oldest known edible fruits, that native to Persia [4] and is grown in warm climate areas of southeast Asia, the Mediterranean, the America and other parts of the world. Fruits, peels and roots of pomegranate have been commonly used in herbal remedies by local healers in many countries. Pomegranate rind is tannin–rich and its antimicrobial activity has been demonstrated against pathogenic bacteria [5, 6, 7, 8, 9, 10, 11, 12, 13, 4, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26]

Pseudomonas aeruginosa is a common Gram-negative bacterium that can cause disease in plants and animals, including humans. It is citrate, catalase, and oxidase positive. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in hypoxic atmospheres, and has, thus, colonized many natural and artificial environments [27].

Considering that antimicrobial edible packaging is a novel technology with the potential to help food preservation, the objective of the present work was to determine the antimicrobial performance of pomegranate rind extract incorporated films on *P. aeruginosa*.

MATERIALS AND METHODES:

Materials - wheat starch (*Triticum aestivum*) was obtained from Sigma – Aldriche (Germany) and the other chemicals from Merk (Germany).

Preparation of pomegranate rind extract- *Punica granatum* (Punicaceae) was obtained from Agriculture Research Institute of Yazd (Iran). Fruits were peeled and oven dried at 50^{oc} and then ground with an electric grinder in to fine powders. Dried powdered plant material was extracted with methanol 80% according to Mathabe *et al.* [10] method. 2 grams of plant sample was mixed with 50 ml of solvent. The mixture was left on a mechanical shaker at 150 rmp for 24h at room temperature and then faltered whatman No .1. The extract was

further concentrated using a rotary evaporator. The sample was then stored at 4^{oc} and further used for antibacterial test.

Organism and preparation of culture - *P. aeruginosa* (PTCC 1599) culture was obtained from Persian Type Culture Collection (Tehran, Iran). The bacterial culture was grown on nutrient agar slants and keep at 4^{oc}.

Preparation of antimicrobial starch film - Film forming solution was prepared from a mixture of wheat starch (4gr) in 100 ml distilled water, 2% (v/v of starch solution) glycerol and 0.4% (v/v of starch solution) polyethylene glycol heated to 95^{oc} with continuous stirring for 15min before it was cooled to room temperature. Pomegranate rind extract was initially diluted to 100mg/ml concentration with dH_{20} and then incorporated into the film solution at different concentration (1, 5, 10 and 20%). The solutions (12 ml) were casted on to poly acrylic plates (10 × 10 cm) followed by oven drying at 25^{oc} for 48h.

Antibacterial activity of films - Antibacterial activity test on films was carried out using to agar diffusion method. The zone of inhibition assay on solid was used for determination of the antibacterial effects of film against *P. aeruginosa*. The edible films were cut into 10 mm diameter discs and then placed on Brain Heart Infusion Agar plates, which had been previously seeded with 0.1ml of inoculums containing approximately 1.5×10^8 $CFU/_{ml}$ of tested bacteria. The plates were then incubated at 37^{oc} for 24h. After that, the plates were examined for zone of inhibition on the film discs.

RESULTS AND DISCUSSION:

The results of the antibacterial activity of films containing pomegranate rind extract against *P.aeruginosa* are presented in Table 1. The results showed that *P. aeruginosa* was sensitive against all concentration of extract. As the concentration of extract increased, the inhibition zone increased significantly ($p< 0.05$) at all levels for films.

Table1: Antimicrobial activity of starch film incorporated with pomegranate rind extract

Content (%)	Diameter of inhibition zone (mm)
	P. aeruginosa
0	**.00^{e}**
1	**9.26^{d}**
5	**9.96^{c}**
10	**10.63^{b}**
20	**14.26^{a}**

Different lower case letters in the column indicated significant differences (P<0.05)

The agar diffusion test is a method commonly used to examine antimicrobial activity regarding the diffusion of the compound tested through water – containing agar plate. The diffusion itself is dependent on the size, shape and polarity of the diffusing material [27]. The chemical structure and the crosslinking level of the films also affect this phenomenon [28]. When antimicrobial agents are incorporated, there will be diffusing material through agar gel and further more resulting clearing zone on the bacterial growth.

Antibacterial activity of plant extract may be indicative of the presence of several metabolic toxins or broad – spectrum antibiotics. Several metabolic from herb species, including alkaloids, tannins and sterols, have previously been associated with antimicrobial activity [13].

In order to investigate components from ethanolic extract of pomegranate peel, the HPLC analysis among some other minor constituents mainly shows some major phenolic compounds, gallic acids and ellagic acids in addition to punicalagin as a major ellagitannin [13]. Gallic acid was reported to have antibacterial activity against some intestinal bacteria [29], ellagic acid has antimicrobial activity [30] and punicalagin was reported to show anti – food born pathogens [31]. This suggests that these components may also provide antibacterial activity against *P. aeruginosa* and provide a plausible explanation for the higher antibacterial activity of extract. The mechanism responsible for antibacterial activity on microorganisms was related to reaction with sulfhydryl groups of proteins and unavailability of substrates to microorganism [32].

Results obtained in this study on antibacterial activity of *P. granatum*, seen to agree with those obtained by Al-Zoreky [14] who reported that alcohol extracts of pomegranate showed antibacterial activity when tested against *P. aeruginosa*. McCarell *et al*. [12] also reported the alcoholic extract of pomegranate rind to be active against *P. aeruginosa*. Dahham *et al*. [16] showed that *P. granatum* extracts hare positive antibacterial activity against *P. aeruginosa*. In other study extract was found to be effective against *P. aeruginosa* [23]. Also it has been reported that pomegranate rind extract exhibited antibacterial activity against *P. aeruginosa* by Duman *et al*. [4]. Holetz *et al*. [7] reported a strong inhibitory effect by the direct application of pomegranate rind extract to *P. aeruginosa*. Antimicrobial activity of pomegranate peel extract against some food-born pathogen since *P.*

aeruginosa was reported by Nascimento *et al.* [33]. Another study on antimicrobial activity of pomegranate rind extract showed some inhibitory effect on *P. aeruginosa* [20]. In the current study, this inhibitory effect was incorporated and expressed in a bio–based film and pomegranate rind extract maintain its known antimicrobial activity in wheat starch based edible film.

CONCLUSION:

The results showed that the pomegranate rind extract incorporated in the films had antibacterial activity on *P. aeruginosa* based on the clear inhibition zone exhibited. The results obtained can serve as a guide for selection of suitable levels of pomegranate rind extract that can be in order to have an effective inhibition.

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REFERENCES

[1] Vasconez MB, Flores SV, Campos CA, Alvarado J, Gerschenson LN, Antimicrobial activity and physical properties of chitosan-tapioca starch based edible films and coatings, Food Research International, 42, 2009, 762-769.

[2] Maizura M, Fazilah A, Norziah MH, Karim AA, Antibacterial activity of modified sago starch-alginate based edible film incorporated with lemongrass oil, International Food Research Journal, 15, 2008, 233-236.

[3] Nisperos-Carriedo MO, Edible coating and films based on polysaccharides. Eds: Krochta JM, Baldwin EA, Nisperos-Carriedo MO, Edible coating and films to improve food quality, Technomic Publishing, Lancaster Pennsylvania, 1994, 305-335.

[4] Duman A D, Ozgen M, Dayisoylu K S, Erbill N, Durgac C, Antimicrobial activity of six pomegranate (*Punica granatum* L.) varieties and their relation to some of their pomological and phytonutrient characteristics, Molecules, 14, 2009, 1808-1817.

[5] Ahmad I, Beg A Z, Antimicrobial and phytochemical studies on 45 Indian medicinal plant against multi-drug and resistant human pathogens, Journal of Ethnopharmacology, 74, 2001, 113-123.

[6] Prashanth D, Asha M K, Amit A, Antibacterial activity of *Punica granatum*, Fitoterapia, 72, 2001, 171-173.

[7] Holetz F B, Pessini G L, Sanches N R, Cortez D A G, Nakamura C V, Filho B P

D, Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases, Memorias do Instituto Oswaldo Cruz, 97, 2002, 1027-1031.

[8] Machado TB, Pinto A V, Pinto M C F R, Leal I C R, Silva M G, Amaral A C F, Kuster R M, Netto-dosSantos K R, In vitro activity of Brazilian medicinal plants naturally occurring naphthoquinones and their analogues, against methicillin resistant *Staphylococcus aureus*, International Journal of Antimicrobial Agents, 21, 2003, 279-284.

[9] Braga L C, Shupp J W, Cummings C, Jett M, Takahaski J A, Carmo L S, Chartone-Souza E, Nascimento A M A, Pomegranate extract inhibits *Staphylococcus aureus* growth and subsequent enterotoxin production, Journal of Ethnopharmacology, 96, 2005, 335-339.

[10] Mathabe M C, Nikolova R V, Lall N, Nyazema N Z, Antibacterial activates of medicinal plants used for the treatment of diarreheas in Limpopo Province, South Africa, Journal of Ethnopharmacology, 105, 2006, 286-293.

[11] Shan B, Cai Y Z, Brooks J, Corke H, The in vitro antibacterial activity of dietary species and medicinal herb extracts, International Journal of Food Microbiology, 117, 2007, 112-119.

[12] Mc Carrell E, Gould S W, Fielder M D, Kelly A F, Sankary W E, Naughton D P, Antimicrobial activities of pomegranate rind extracts: enhancement by addition of metal salts and vitamin C, BMC Complementary and Alternative Medicine, 8 (64), 2008, 1-7.

[13] Choi S H, Woo J H, Lee J E, Park S J, Choo E J, Kwak Y G, Increasing incidence of quinolone resistance in human non-typhoid *Salmonella enterica* isolates in Korea and mechanisms involved in quinolone resistance, Journal of Antimicrobial Chemotherapy, 56, 2005, 1111-1114.

[14] Al-Zoreky N S, Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peel, International Journal of Food Microbiology, 134, 2009, 244-248.

[15] Panichayupakaranant P, Itsuriya A, Sirikatitham A, Preparation method and stability of ellagic acid-rich pomegranate fruit peel extracts, Pharmaceutical Biology, 48, 2010, 201-205.

[16] Dahham S S, Ali M N, Tabassum H, Khan M, Studies on antibacterial and antifungal activity of pomegranate

(*Punica granatum* L.), American-Eurasian Journal of Agriculture and Environmental Science, 9 (3), 2010, 273-281.

[17] Yehia H M, Elkhadragy M F, Abdel Moneim A E, Antimicrobial activity of pomegranate rind peel extracts, African Journal of Microbiological Research, 4(22), 2011, 3664-3668.

[18] Nuamsetti T, Dechayuenyong P, Tantipaibulvut S, Antibacterial activity of pomegranate fruit peels and arils, Science Asia, 38, 2012, 319–322.

[19] Mansour E, Ben Khaled A, Lachiheb B, Abid M, Bachar Kh, Ferchichi A, Phenolic compounds, antioxidant and antibacterial activities of peel extract from Tunisian pomegranate, Journal of Agriculture Science and Technology, 15, 2013, 1393-1403.

[20] Janani J, Estherlydia D, Antimicrobial activities of *Punica granatum* extracts against oral microorganisms, International Journal of PharmTech Research, 5 (3), 2013, 973-977.

[21] Foss SR, Nakamura CV, Nakamura TU, Cortez DA, Endo EH, Dias Filho BP, Antifungal activity of pomegranate peel extract and isolated compound punicalagin against dermatophytes, Annals of Clinical Microbiology and Antimicrobials, 13, 2014, 32-38.

[22] Parashar S, Sharma H, Garg M, Antimicrobial and antioxidant activities of fruits and vegetable peels: A review, Journal of Pharmacognosy and Phytochemistry, 3 (1), 2014, 160-164.

[23] Hajoori M, Naik M, Naik K, Desai S, Evaluation of antimicrobial activity of *punica granatum* peel extracts using different solvent system, International Journal of Pharmacological Screening Methods, 4 (1), 2014, 26-31.

[24] Gaber A, Hassan MM, Dessoky ES, Attia AO, In vitro antimicrobial comparison of taif and egyptian pomegranate peels and seeds extracts, Journal of Applied Biology and Biotechnology, 3 (02), 2015, 012-017.

[25] Marchi LB, Monteiro ARG, Mikcha JMG, Santos AR, Chinellato MM, Marques DR, Dacome AS, Costa SC, Evaluation of antioxidant and antimicrobial capacity of pomegranate peel extract (*Punica granatum* L.) under different drying temperatures, Chemical Engineering Transactions, 44, 2015, 121-126.

[26] Akhtar S, Ismail T, Fraternale D, Sestili P, Pomegranate peel and peel extract: chemistry and food features, Food Chemistry, 174, 2015, 417-425.

[27] Pranoto Y, Rakshit S K, Salokhe V M, Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin, LWT, 38, 2005, 859-865.

[28] Cagri A, Ustunol Z, Ryser ET, Antimicrobial, mechanical and moisture barrier properties of low pH whey protein-based films containing p-Amminobenzoic or sorbic acid, Journal of food Science, 66(6), 2001, 865-870.

[29] Ahn YJ, Lee CO, Kweon JH, Ahn JW, Park, JH, Growth-inhibitory effects of Galla Rhois-derived tannins on intestinal bacteria, Journal of Applied Microbiology, 84, 1998, 439-443.

[30] Thiem B, Goslinska O, Antimicrobial activity of *Rubus chamaemorus* leaves, Fitoterapia, 75, 2004, 93-95.

[31] Taguri T, Tanaka T, Kouno I, Antimicrobial activity of 10 different plant polyphones against bacteria causing food-borne disease, Biological and Pharmaceutical Bulletin, 27, 2004, 1965-1969.

[32] Naz S, Siddiqi R, Ahmad S, Rasool S, Sayeed S, Antimicrobial activity directed isolation of compounds from *Punica granatum*, Journal of Food Science, 72, 2007, 341-345.

[33] Nascimento G F, Locatelli J, Freitas P, Silva G, Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria, Journal of Microbiology, 31, 2000, 247-256.