

Anti-Bacterial, Anti-Mycobacterial and Anti-Fungal Properties of *Punica granatum* as Natural Dye

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ABSTRACT

Objective: This study aimed to determine the anti-mycobacterial, anti-bacterial, and anti-fungal effects of dry/fresh pomegranate peel ethanol/methanol extracts, and the dyeing performance and antimicrobial effects of dyed fabric samples with pomegranate peel ethanol extract.

Materials and Methods: Anti-mycobacterial activity against *Mycobacterium tuberculosis* H37Ra/H37Rv and two-clinical *M. tuberculosis* strains, and anti-bacterial activity against eight bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus vulgaris*, Methicillin-resistant *Staphylococcus aureus* and *Escherichia coli*) and anti-fungal activity against five fungal pathogens (*Candida albicans*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus niger*, *Fusarium proliferatum*) were determined by microplate assay. The anti-microbial activity of dyed fabric samples (30/ 1 Rib and single jersey 100% cotton) as well as their coloring properties, were investigated using the parallel streak method (AATCC 147).

Results: Extracts showed the anti-mycobacterial efficacy between MIC 7.81-31.25 µg/ml and MBC 31.25-250 µg/ml, respectively against four strains of *M. tuberculosis*. Also, each extract showed anti-bacterial activity between MIC 0.97-62.50 µg/ml and MBC 7.81-250 µg/ml and anti-fungal activity between MIC 31.25-125 µg/ml and MBC 125-250 µg/ml. While control and mordanting of fabric samples did not show any inhibition zones, significant anti-microbial activity against *S. aureus* was obtained after dyeing using dry peel on fabric samples without mordant.

Conclusion: These findings provide valuable information for future applications of natural dyes in textiles. The anti-mycobacterial, anti-fungal, and anti-bacterial properties of pomegranate could be significant in developing a model for drug design.

Keywords: Anti-microbial activity, Coloring Properties, Natural Dye, Pomegranate, *Punica granatum*

INTRODUCTION

Pomegranate fruit, which has an important place in human history, is one of the oldest cultivated agricultural products. It is known that the homeland of the pomegranate is the Mediterranean, western Asia, and Iran, and today it is grown in the USA (California and Arizona), Argentina, China, Afghanistan, India, Arabia, Chile, and northern Mexico.^{1,2} The pomegranate is the most important plant belonging to the family Punicaceae. The name of the pomegranate is derived from *Malum granatum*, which means "granular apple" in Latin.¹ *Punica granatum* has multiple spiny branches and the leaves are elliptic; the edible fruit is a berry with seeds and pulp produced from the ovary of a white or red single flower.³ 50% of the pomegranate consist of the edible part and 50% of the peel (Fawole and Opara).⁴

Throughout history, diseases and infections have been a

great concern for humans.⁵ For this reason, natural substances such as pomegranate have been added to medicines, foods, and textiles.⁶ Pomegranate can be used in various products such as drugs, dye, pomegranate molasses and sour syrup, juice, preserves, vinegar, citric acid, and animal feed, used in the production of seed oils, and used as a refreshing additive.⁷ All the parts of the pomegranate, from the bark to the flower, have been used for treatment by many nations since ancient times.⁸ Pomegranate peel's properties are important and contain cures for and protection from cardiovascular disease, diabetes, cancers, erectile dysfunction, and dental problems.⁹ The peel of *P. granatum* is used in the treatment of genital infections, mastitis, folliculitis, acne, piles, allergic dermatitis, tympanitis, scald, dysentery, and diarrhea.¹⁰ In classical usage, the bark and rind fruits are used for tanning and as a vermifuge, especially for cold and cough.¹¹ In addition, *P. granatum* has some biological

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Submitted: 19.01.2023 • Revision Requested: 04.04.2023 • Last Revision Received: 26.05.2023 • Accepted: 27.05.2023



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properties such as anti-oxidant,^{12,13} anti-atherosclerotic,^{14,15} anti-bacterial^{16,17} and anti-viral¹⁰ activities. According to the literature, pomegranate has great therapeutic effects, due to its anti-oxidant and anti-tumor capacity, using different extracts from different parts of the plant.¹⁸ These biological activities are due to the presence of bioactive compounds called "Tanins." The main function of tannins is plant defense against microorganisms and animal attacks, due to their astringent capacity and ability to create compound with proteins and polysaccharides.^{19–21} The components of *P. granatum* contain pelargonidin, cyanidin, delphinidin, galocatechin, ellagic acid, gallic acid and sitosterol, whose therapeutical properties are known.^{22,23} In addition, in the studies, it was determined that pomegranate peel extracts showed an inhibitory effect, especially against Gram-positive bacteria, namely *Propionibacterium acnes*, *Staphylococcus aureus*,²⁴ and *Bacillus subtilis*.²⁵ In the literature, it has been determined that methanol extracts of pomegranate peel are effective on *Shigella dysenteriae* serotype 2, *Salmonella typhimurium*,²⁶ and *Escherichia coli*.²⁷ In anti-fungal activity studies, methanol extracts of pomegranate peel were tested against *Penicillium expansum*, *Penicillium digitatum* and *Botrytis cinerea* and at the end of the 20-hour incubation period, it was observed that the viability of the conidia decreased compared to the control.²⁸ In another study, it was determined that pomegranate peel extracts showed higher anti-mycobacterial activity (MIC 64–1024/ml) than potable fruit juice (MIC 256–>1024g/ml).²⁹ Furthermore, pomegranate peel has important biological properties that have anti-inflammatory (activation of white blood cells, the release of immune system chemicals) and anti-allergic effects.³⁰

Synthetic dyes are used in many industries, such as textile, rubber, paper, plastic, leather, food, pharmaceutical, petrochemical, dyestuffs, and cosmetics. The release of synthetic dyes into the environment causes environmental pollution and many health problems.³¹ Today, water pollution caused by non-biodegradable colored wastes of textile dyes is one of the leading environmental problems in the world.³² For this reason, in textile dyeing natural dyes are preferred to synthetic dyes.^{33,34} In traditional dyeing, organic photoprotective agents, such as some natural dyes, are applied to silk, cotton, and wool fabrics.^{35,36} Although synthetic fibers are preferred more in dyeing, natural fibers such as cotton are mostly used in traditional natural dyeing. Natural fibers can be dyed with natural dyes with the help of a metallizing agent.³⁷ Natural dyes do not cause an allergic reaction and are not toxic to people and the environment.^{38,39} The pomegranate peel is an anti-microbial product containing significant amounts of phenolic compounds, tannins, and pellets.²⁰ The major dyeing factor in the pomegranate peel is granatone, which is N-methyl granatone found in alkaloid form. This compound in the pomegranate provides the coloring property.⁴⁰ A color scale consisting of different shades of yellow, brown, and black is ob-

tained from fabrics dyed with pomegranate peel.⁴¹ In this study, the anti-mycobacterial, anti-bacterial, and anti-fungal activities of ethanol and methanol extracts of pomegranate (*P. granatum*) peels, which are considered natural waste, were determined. In addition, this study investigates the anti-bacterial activity of the treated and control fabric samples that were examined as per standard AATCC-147 methods (Parallel Streak Method).

MATERIALS AND METHODS

Preparation of Extracts

Pomegranate was collected from Balıkesir (2013), and identification of the plant was made by Prof. Dr. Güldam Tümen and Fatih Satıl. The plant used in the study is kept in the herbarium of the Biology Department of Balıkesir University (Herbarium Number: FS1566). The dried and fresh pomegranate peels were cut into small pieces and weighed. For the extraction, the dry and fresh pomegranate peels (100 g) were added to ethanol (1000 ml) and methanol (1000 ml) extracts at 25 °C for two weeks. They were extracted separately. As a result of the extraction, four different extracts were obtained: dry pomegranate peel ethanol, dry pomegranate peel methanol, fresh pomegranate peel ethanol, and fresh pomegranate peel methanol. The extracts were filtered through filter paper and evaporated with a rotary evaporator. The extracts were preserved at -20 °C.^{42–44} The schematic diagram of the experimental sections is given in Figure 1.

Microorganisms

The eight strains of bacteria used were *Bacillus cereus* (BC, ATCC 10876), *Staphylococcus aureus* (SA, ATCC 538), *Salmonella typhimurium* (ST, ATCC 14028), *Pseudomonas aeruginosa* (PA, ATCC 27853), *Klebsiella pneumonia* (KP, ATCC 31488), *Proteus vulgaris* (PV, ATCC 6897), Methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 33592), and *Escherichia coli* (EC, ATCC 8739). The stock culture was maintained on a Nutrient agar (NA) medium at 4 °C in the refrigerator. Five fungal pathogens, *Candida albicans* (CA, ATCC10239), *Aspergillus flavus* Link (AF, TA41-17), *Aspergillus ochraceus* K. Wilh (AO, MUCL 39534), *Aspergillus niger* van Tiegh (AN, TA47-3), and *Fusarium proliferatum* (FP, Matsushima, Nirenberg, TA18-2), were used. The stock culture was maintained on Malt Extract Agar (MEA) medium at -20 °C. We investigated the anti-mycobacterial activities of the extracts against four tuberculosis strains (MT-H37Ra, MT-H37Rv, and two clinical isolates) by MPBA.

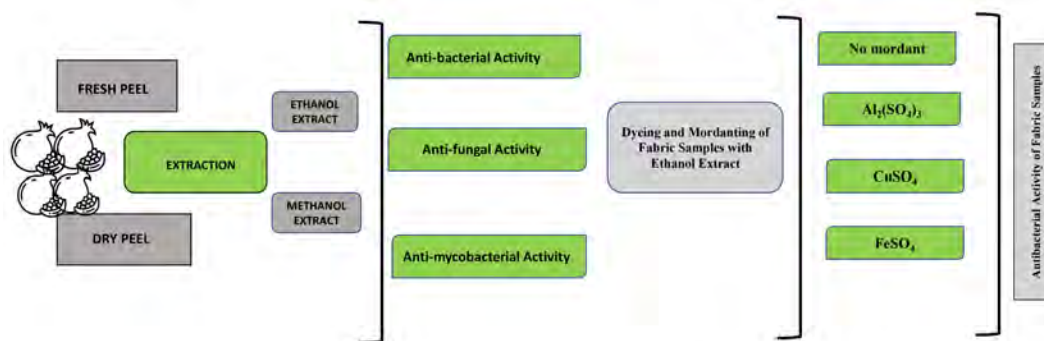


Figure 1. Schematic diagram of the experimental sections.

Preparation of Bacterial and Fungal Cultures

The samples were weighed; their weight was found to be 0.5 g. They were solubilized within 5 ml DMSO to prepare the main stock. The stock solution concentration was 50 mg/mL. All stock solutions were stored in a deep freeze at -20°C . To prepare 10 mL (500 $\mu\text{g}/\text{ml}$ concentration) of solution, 0.10 ml of stock solution was taken and 9.90 mL of DMSO was added. Therefore, the final solution concentrations were 500 $\mu\text{g}/\text{ml}$. The range of working solution concentrations was between 250 and 0.12 $\mu\text{g}/\text{ml}$. In vitro anti-bacterial activity efficiency was established by using MHA (Mueller Hinton Agar) and MHB (Mueller Hinton Broth), and anti-fungal activity assays SDA (Sabouraud Dextrose Agar) and SDB (Sabouraud Dextrose Broth). Anti-mycobacterial assays used were M7H9B (Middlebrook 7H9 Broth, Becton & Dickinson) and M7H10A (Middlebrook 7H10 Agar, Becton & Dickinson).

Anti-Bacterial and Anti-Fungal Sensitivity Assays

Minimum Inhibitory Concentration Assay (MIC)

MIC determination for anti-bacterial and anti-fungal tests was carried out according to "Methods for Dilution Antimicrobial Susceptibility Tests, Approved Standards" for fungi⁴⁵ and bacteria.⁴⁶ In this study, the negative control well contains no organisms, while the positive control well contains organisms. Final concentrations in the wells ranged from 0.12 $\mu\text{g}/\text{ml}$ to 250 $\mu\text{g}/\text{ml}$. For the incubation of all microplates, 24-48 h at 37°C for bacteria and 72 h at 28°C for fungi were selected. Anti-bacterial and anti-fungal activity tests were performed in three series. Then, Tiazolyl Blue Tetrazolium Bromide (TBTB, 20 μl , Sigma) was added to the wells and incubated at 37°C for an additional 4 h. The color change in the solution was investigated. After the indicator dye turned pink, indicating positive bacteria growth, TBTB solution was added to the other wells.

Minimum Bactericidal Concentration Assay (MBC) and Minimum Fungicidal Concentration Assay (MFC)

For MBC/MFC determination, the inoculum was taken from the MIC wells and higher concentration wells and then added to wells containing fresh and sterile SDA for fungi and MHB for bacteria. The plates were incubated at 37°C for bacteria and 28°C for fungi. Color change in positive and negative control wells was checked with a TBTB indicator. The lowest concentration without bacterial and fungal growth was accepted as MBC/MFC.

Preparation of Mycobacterial Inocula and Anti-mycobacterial Activity Test

For susceptibility tests of *M. tuberculosis*, the MGIT guideline and National Committee for Clinical Laboratory Standards (NCCLS) were used.⁴⁶⁻⁴⁸ For the production of *M. tuberculosis* strains incubated at 37°C , 7H9 Broth Base (4 ml), OADC (oleic acid, albumin, dextrose, and catalase) supplement (0.5 ml), and the antibiotic mixture of PANTA (polymyxin-B, amphotericin-B, nalidixic acid, trimethoprim, and azlocillin) (0.1 ml) were used. In the mycobacterial activity of pomegranate extract in the test using Microplate Presto Blue Assay (MPBA), 198 μl of M7H9B and 2 μl of extract were added to the first column of each row then 100 μl of the medium was added to the other wells. Afterwards, 20 μl of inoculum was added to the wells and each microplate was incubated at 37°C for 5 days. The experiments also included positive and negative controls. The final extract concentration was in the range of 0.12-250 $\mu\text{g}/\text{ml}$. Anti-mycobacterial activity tests were performed in three series. After incubation, the results were evaluated with 20 μl of Presto blue (Invitrogen, Life Technologies) solution.

In the evaluation, the blue color in the well means no growth, and the pink color means positive growth. The MIC was determined as the lowest concentration at which the color did not turn pink and gave a negative result. To determine the MBC, 20 μl of the solution was taken from the non-growth wells and transferred to a new plate, and 80 μl of freshly modified

M7H9B was added. The plates were incubated at 37 °C. Color change in positive and negative control wells was checked with Presto blue indicator. The lowest concentration without bacterial growth was accepted as MBC.

Anti-Bacterial Activity of Textile Materials

The Parallel Streak Method⁴⁹ (AATCC Test Method 147-2011) is applied quickly and easily to determine the anti-bacterial activity of anti-microbial agents that can spread on treated textile materials. If a diffusible anti-microbial agent has anti-bacterial activity, a zone of inhibition is formed; this means that there is no growth of a microorganism on the surface of an agar medium near the boundaries of the sample placed in direct contact with the agar surface. Two strains of bacteria used were SA (ATCC 538) and KP (ATCC 31488). The stock culture was maintained on an NA at 4 °C in the refrigerators. Control and dyed fabric samples (non-sterile, (30/ 1 Rib and single jersey 100 % cotton) were cut by hand or with a die. Rectangular specimens cut 25 × 50 mm were recommended. The amount of bacterial inoculum was prepared using 0.5 McFarland standard (MF; 1.5×10^8 CFU/ml). The bacterial inoculum was prepared by transferring 1.0 ml of a 24 h nutrient broth culture into 9.0 ml of sterile distilled water. During the application, five strips of 6 cm in length at 10 mm intervals were drawn on the surface of the NA using a loopful of diluted inoculum. Test specimens were placed along the five inoculation lines with gentle pressure to make contact with the agar surface. All petri dishes were incubated at 37 °C for 18-24 h.⁵⁰ It was examined whether there was a growth interruption and a clear inhibition zone along the inoculum lines in the incubation plates. The mean width of the line and the inhibition zone on both sides of the test samples were measured and the calculation was made using the formula:

$$W = (T - D)/2.$$

W = width of the net inhibition zone in mm

T = total diameter of test specimen and net area in mm

D = diameter of the test specimen in mm

RESULTS

The pomegranate peel was successfully extracted using a rotary evaporator. All assays were performed using a microdilution method. Each extract showed anti-bacterial activity between 0.97-62.50 /ml as MICs and 7.81-250 g/ml as MBCs, and anti-fungal activity between 31.25-125 g/ml and 125-250 g/ml as MIC and MFC, respectively. The methanol extract of dry pomegranate peels exhibited the most significant activity, with a MIC value of 0.97 g/ml, and the ethanol extract of dry pomegranate exhibited a MIC value of 1.95 g/ml against *K. pneumonia*. The pomegranate peel ethanol extract exhibited the most significant activity against *A. flavus* and

A. ochraceous with a MIC value of 62.5 g/ml. It was also determined that the pomegranate extract was found to be effective against *C. albicans* (MIC 31.25- 62.5 g/ml) (Table1).

We also demonstrated the anti-mycobacterial activities of extracts against MT-H37Ra, MT- H37Rv, and two clinical isolates by myco-bactericidal activity test using the MPBA. The anti-mycobacterial activity of dry and fresh pomegranate extracts (ethanol and methanol) as MIC and MBC ($\mu\text{g/ml}$) are shown in Table 2. Each extract showed anti-mycobacterial efficacy between MIC 7.81-31.25 $\mu\text{g/ml}$ and MBC 31.25-250 $\mu\text{g/ml}$ against four strains of *M. tuberculosis*. The extract of peels exhibited the most significant activity against MT-H37Ra and MT-H37Rv with a MIC value of 7.81 $\mu\text{g/ml}$ and 31.25 $\mu\text{g/ml}$, respectively.

As a result of dyeing with pomegranate peel extract, a color scale ranging from dark brown to yellow was obtained. CIE color coordinates are given in Table 3.

The anti-microbial efficacy of the treated and control fabric samples was determined by the parallel streak method (AATCC 147). The extent of anti-microbial activity was measured and recorded, and the zone of inhibition of the anti-microbial activity of the dyed and control fabric samples against two pathogens is shown in Figure 1. In this study, we used two factors for measuring the inhibition zone method: the anti-microbial activity of the samples against the microorganism, and the capability of the anti-microbial agent to diffuse into the agar. In this way, fabric samples acquire anti-microbial activity by gaining bactericidal (killing bacteria) or bacteriostatic (reducing bacterial growth and development) properties (Figure 2).

The fabric samples dyed with fresh and dry pomegranate peel extract and placed in the agar surface were monitored after 24 hours. The effect of the anti-microbial agents diffused into the agar was examined from under the plate as an inhibition zone or no growth. Whereas the control fabric sample and mordanted fabric samples did not show any zone of inhibition, the fabric samples dyed with dry pomegranate peel showed better anti-bacterial activity against *S. aureus* (16 mm). The results are shown in Figure 3.

DISCUSSION

The results of the anti-microbial activities of methanol and ethanol extracts revealed that both extracts had an inhibitory effect on test microorganisms. The results of MIC and MBC indicated that the pomegranate extract had a great ability to prevent the growth of bacteria, fungi, and mycobacteria. The best inhibitory concentrations of fresh and dry peel ethanolic extracts were observed on *K. pneumoniae* (1.95 $\mu\text{g/ml}$), *E. coli* (62.5 $\mu\text{g/ml}$), and *C. albicans* (31.25 $\mu\text{g/ml}$). Also, the fresh and dry peel methanol extracts showed an inhibitory effect on *S. aureus* (1.95 $\mu\text{g/ml}$), *E. coli* (62.5 $\mu\text{g/ml}$), and *B. cereus* (7.81 $\mu\text{g/ml}$). In addition, the inhibitory concentrations of dry

Table 1. Anti-bacterial and anti-fungal activity of pomegranate extracts ($\mu\text{g/mL}$).

	Anti-bacterial and Anti-fungal Activity ($\mu\text{g/mL}$)							
	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC
	DP-E		DP-M		FP-E		FP-M	
<i>S. aureus</i>	7.81	15.62	1.95	15.62	3.90	250	1.95	1.95
<i>K. pneumoniae</i>	1.95	250	0.97	250	1.95	250	1.95	1.95
<i>E. coli</i>	62.5	250	62.5	250	62.5	250	62.5	62.5
<i>B. cereus</i>	7.81	250	7.81	125	7.81	250	7.81	7.81
<i>S. typhimurium</i>	62.5	250	62.5	250	62.5	250	125	125
<i>P. vulgaris</i>	15.62	62.5	15.62	125	7.81	7.81	15.62	15.62
<i>P. aeruginosa</i>	62.5	125	62.5	125	62.5	125	62.5	62.5
MRSA	31.25	250	62.5	250	15.62	250	7.81	7.81
<i>C. albicans</i>	31.25	125	62.5	125	31.25	125	31.25	250
<i>A. flavus</i>	62.5	250	125	250	62.5	250	125	250
<i>A. niger</i>	125	125	125	250	62.5	125	125	250
<i>A. ochraceus</i>	62.5	125	62.5	125	125	125	125	250
<i>F. proliferatum</i>	125	125	125	250	125	125	125	125

MIC: Minimum Inhibition Concentration; MBC/MFC: Minimum bactericidal/fungicidal concentration; DP-E: Dry pomegranate ethanol extract; DP-M: Dry pomegranate methanol extract; FP-E: Fresh pomegranate ethanol extract; FP-M: Fresh pomegranate methanol extract

Table 2. Anti-mycobacterial activity of pomegranate extracts ($\mu\text{g/ml}$).








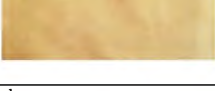
	Anti-mycobacterial activity ($\mu\text{g/mL}$)							
	H37Ra		H37Rv		Strain 1		Strain 2	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
DP-E	15.62	125	31.25	125	31.25	250	31.25	31.25
DP-M	7.81	62.5	31.25	125	31.25	250	31.25	31.25
FP-E	15.62	125	31.25	125	31.25	250	15.62	31.25
FP-M	15.62	62.5	15.62	125	31.25	250	31.25	31.25
	Standard drugs							
INH	0.13	1.04	0.52	1.04	0.52	1.04	0.52	4.16
RIF	0.64	5.12	0.32	2.56	0.64	0.64	5.12	10.24

INH: Isoniazid; RIF: Rifampicin; DP-E: Dry pomegranate ethanol extract; DP-M: Dry pomegranate methanol extract; FP-E: Fresh pomegranate ethanol extract; FP-M: Fresh pomegranate methanol extract

peel ethanolic extracts belonged to MRSA, *C. albicans* and *A. flavus* (31.25 $\mu\text{g/ml}$), and dry peel methanolic extracts (62.50 $\mu\text{g/ml}$). It can be concluded that the ethanolic solvent is more

effective than the methanolic in reacting with components and ingredients of the pomegranate extract due to an increase in the release of active substances from the plant, and enhancing con-

Table 3. Fabric samples dyed with fresh and dry pomegranate peel with and without mordants.

Sample Name	Mordant	Pom. Peel Amount (%)	Obtained Color	CIE color coordinates		
				L*	a*	b*
FPP-1	No mordant	100		55.1	7.4	9.4
DPP-1	No mordant	100		77.0	2.6	8.9
FPP-2	Al ₂ (SO ₄) ₃	100		70.4	3.5	17.9
DPP-2	Al ₂ (SO ₄) ₃	100		87.4	1.9	-2.1
FPP-3	CuSO ₄	100		52.1	14.7	52.2
DPP-3	CuSO ₄	100		76.1	5.7	2.1
FPP-4	FeSO ₄	100		58.4	8.0	11.4
DPP-4	FeSO ₄	100		67.8	3.8	14.1

DPP: Dry pomegranate peel; FPP: Fresh pomegranate peel

centrations of these substances in ethanolic extract compared to methanolic extract is more effective.⁵²

Pomegranate has a strong inhibitory effect against many microorganisms (Gram-negative and Gram-positive bacteria, filamentous fungi, and mold) and a broad spectrum of anti-microbial action. Although different parts of the pomegranate (peels, leaf seeds) showed various anti-microbial activities with different extracts, many scientists stated that the anti-microbial activity of the pomegranate peel was stronger than the other parts, and the anti-microbial activity of the peel was dependent on the total flavonoid and tannin content.⁵³ The anti-microbial activity of pomegranate peel was also shown by Ismail et al.⁵⁴ against bacteria and fungi. Ali et al.⁵⁵ showed that pomegranate peel extracts inhibited the growth of *S. aureus* (Gram-positive) and *Salmonella* (Gram-negative). In their research, they investigated the effectiveness of extracts of the different parts of pomegranate (skin, seed, juice, and whole fruit) against seven bacteria: *B. coagulans*, *B. cereus*, *B. subtilis*, *S. aureus*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. The highest effect

was seen in the pomegranate peel.⁵⁶ Other works based on the anti-fungal activity of pomegranate and some researchers⁵⁷ showed that the peel of pomegranate was the most efficient for inhibiting *C. albicans* growth. In addition, the anti-fungal activity of pomegranate against mycelial fungi has been determined in many studies.⁵⁸ Glazer et al., demonstrated the anti-fungal effects of pomegranate peel extracts against *Stemphylium botryosum*, *Alternaria alternata*, and *Fusarium species*.⁵⁹

Phenolic compounds and their derivatives are important compounds in the defense system of plants, and many pathogenic microorganisms have a deterrent effect on their development. Where plant-derived anti-microbial agents come to the fore, cessation of microorganism growth and subsequent prevention of a secondary infection are expected features.⁶⁰ It has been reported that pomegranate peel contains many important active anti-fungal compounds, such as punicalagin, castagalagin, granatin, catechin, gallo catechin, kaempferol, and quercetin.⁶¹⁻⁶⁴

In our study, the anti-mycobacterial activities of pomegranate peel extracts were tested on reference and patient strains. The

Mechanism Anti-bacterial Activity: Comparison of dyed and undyed fabric samples

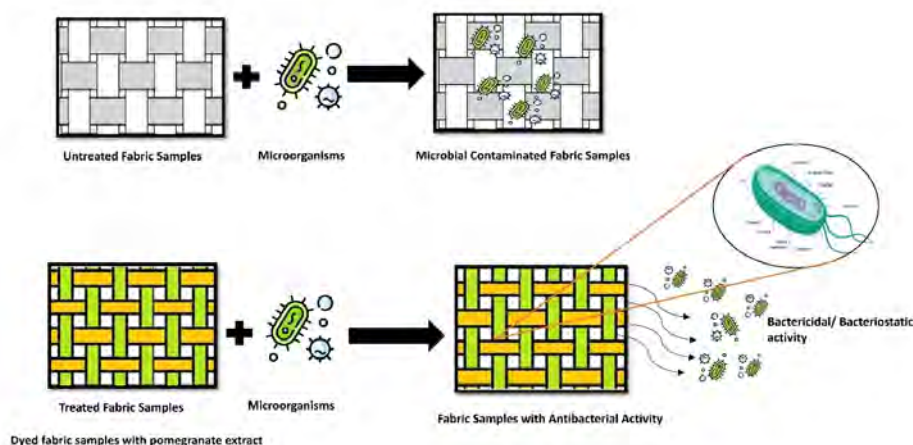


Figure 2. A mechanism between fabric samples and pomegranate extract.

extract of dried peel methanol extract exhibited the most significant activity against MT-H37Ra, with an MIC value of $7.81 \mu\text{g/ml}$, and fresh methanol peel extract exhibited the most significant activity against MT-H37Rv, with an MIC value of $15.62 \mu\text{g/ml}$. When the anti-mycobacterial activity of ethanol and methanol extracts was compared, it was found that methanol extract showed higher activity than ethanol on reference strains. This study is the first report describing the anti-mycobacterial activity of pomegranate peel in different forms (fresh/dry) and extracts (ethanol/methanol) against reference and patient strains. Another study evaluated the inhibitory effect of alcoholic extracts of *Berberis vulgaris*, *Rosa canina*, *Peganum harmala*, *P. granatum*, *Digitalis sp.*, and *Citrus lemon* on *Mycobacterium* isolates. The findings indicated that extracts of *P. harmala*, *P. granatum*, *Digitalis sp.*, and *C. lemon* exhibited inhibitory effects against non-MDR bacteria at various doses, with *P. granatum* showing the maximum inhibition zone (19.5 mm) against isoniazid and rifampin-resistant isolates.⁶⁵ The results we obtained in our study are consistent with the anti-mycobacterial activity results of these studies.

Since textile products are organic, fibrous, and absorbent materials, they offer suitable conditions for microorganisms during human contact and exposure to atmospheric dirt. For these reasons, there is increasing interest in the development of textile materials that inhibit the growth of microorganisms.⁵¹ Various mineral particles and organic compounds were evaluated as anti-microbial additives for textiles.⁶⁶ Investigations have revealed that pomegranate could be used as a natural alternative to synthetic bactericidal materials against different microbial pathogens. The pomegranate peel extract contains a higher amount of phenolic content than the pulp and thus demonstrates superior anti-bacterial activity.⁶⁷ There are studies in the literature for fabrics to gain anti-bacterial properties

by utilizing the high number of phenolic substances contained in the pomegranate peel.

Ul-Islam et. al,⁶⁸ aimed to develop low-cost bacterial cellulose-based anti-bacterial composite with pomegranate (*P. granatum L.*) peel extract for potential biomedical applications. Field-emission scanning electron microscopic (FE-SEM) observation showed a nanofibrous and microporous morphology of pristine bacterial cellulose and confirmed the development of bacterial cellulose-pomegranate peel composite. The bacterial cellulose-pomegranate peel composite exhibited better reswelling capabilities than pristine bacterial cellulose after three consecutive re-wetting cycles. The bacterial cellulose-pomegranate peel composite showed good anti-microbial activity against *S. aureus* (Gram-positive). The findings of this study indicate that bacterial cellulose-pomegranate peel composite could be a promising anti-bacterial wound dressing material.

Another study reports the simultaneous coloring, anti-oxidant activity, and anti-microbial activity of cotton fabrics dyed using silver nanoparticles (AgNPs) and pomegranate peel extract. During the reaction, hydroxyl groups present in pomegranate peel-tannins served to interact with silver ions, subsequently reducing them to AgNPs. The formation of AgNPs, and subsequently their deposition on the surface of cotton, was characterized by UV-visible spectroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy disperse X-ray (EDX) line, and ICP-MS analysis. It was determined that cotton fabrics dyed with this combination exhibited an effective anti-bacterial activity.⁶⁹

As a result of dyeing with pomegranate peel extract, a color scale was obtained ranging from dark brown to yellow. The anti-microbial efficacy of the treated and untreated fabric sample

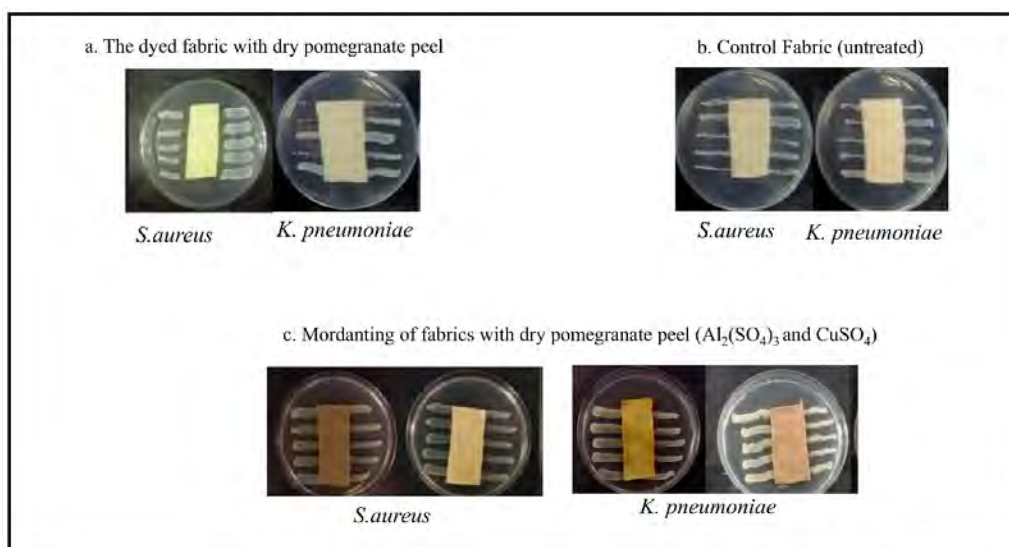


Figure 3. Assessment of anti-bacterial activity of the treated and untreated fabric samples.

was determined by the parallel streak method (AATCC 147). In our study, the fabric sample dyed with dry pomegranate peel showed better anti-bacterial activity against *S. aureus*. Regarding biological activities known in the literature, anti-bacterial natural dyes have gained in importance in recent years. There is the use of anti-microbial compounds, especially from natural sources^{70,71}, including pomegranate polyphenols, and they may show appropriate anti-microbial activity due to the presence of broad-spectrum antibiotic compounds in this plant.⁷² *S. aureus* (SA) is the main cause of infections in hospitals.⁷³ The use of textile products with anti-microbial activity in clinical and medical applications also has advantages, such as reducing the transmission of infection by bacteria⁷⁴ and non-toxicity in contact with human skin.⁷⁵ In recent years, measures to protect textile products from microorganisms and their toxins have increased.⁷⁶ Therefore, anti-microbial textiles have become important in terms of protection and are promising in this respect.^{77,78}

These findings provide valuable information for future applications of natural dyes in textiles. The anti-mycobacterial, anti-fungal, and anti-bacterial properties of pomegranate could be significant in developing a model for drug design. Fabrics coated with natural anti-microbial agents derived from plants can have anti-microbial properties, making them ideal for use in textiles. Furthermore, due to their environmentally friendly and non-toxic nature, these natural anti-microbial materials are still promising candidates for textile applications.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of study: T.A., P.G.; Data Acquisition: T.A., P.G.; Data Analysis/Interpretation: T.A., P.G.; Drafting Manuscript: P.G.; Critical Revision of Manuscript: T.A., P.G.; Final Approval and Accountability: T.A., P.G.; Technical or Material Support: T.A.; Supervision: T.A.. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

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REFERENCES

1. El Barnossi A, Moussaid F, Iraqi Housseini A. Tangerine, banana, and pomegranate peels valorization for a sustainable environment: A review. *Biotechnol Rep.* 2021;29.
2. Gündoğdu M, Yılmaz H, Canan İ, Nar (*Punica granatum L.*) Çeşit ve Genotiplerin Fizikokimyasal Karakterizasyonu. *IJAWS.* 2015;1(2):57-65.
3. Egharevba HO, Kunle OF. Preliminary phytochemical and proximate analysis of the leaves of *Piliostigma thionningii* (Schumach.) Milne-Redhead. *Ethnobot Leaflets.* 2010;14:570-577.
4. Fawole OA, Opara UL. Harvest discrimination of pomegranate fruit: Postharvest quality changes and relationships between instrumental and sensory attributes during shelf life. *J Food Sci.* 2013;78(8).

5. Gupta D, Khare SK, Laha A. Antimicrobial properties of natural dyes against Gram-negative bacteria. *Color Technol.* 2004;120:167–171.
6. Onar N, Aksit A, Sen Y, Mutlu M. Antimicrobial, UV-protective and self-cleaning properties of cotton fabrics coated by dip-coating and solvothermal coating methods. *Fibers Polym.* 2011;12:461–470.
7. Malviya S, Jha A, Hettiarachchy N. Antioxidant and antibacterial potential of pomegranate peel extracts. *J Food Sci Technol.* 2014; 51:4132–4137.
8. Jayaprakasha GK, Negi PS, Jena BS. Antimicrobial activities of Pomegranates. In Pomegranates, ed. CRC press. FL, USA: Boca Raton, Inc; 2006:167-168.
9. Bassiri-Jahromi S. *Punica granatum* (Pomegranate) activity in health promotion and cancer prevention. *Oncol Rev.* 2018;12(1):345.
10. Zhang J, Zhan, B, Yao X, Gao Y, Shong J. Antiviral activity of tannin from the pericarp of *Punica granatum* L. against genital Herpes virus *in vitro*. *Zhongguo Zhong yao za zhi.* 1995;20(9):556-576.
11. Vijayanand S, Hemapriya J. In vitro antibacterial efficacy of peel and seed extracts of *Punica granatum* L. against selected bacterial strains. *Int J Microbiol Res.* 2011;1(4):231-234.
12. Parmar HS, Kar A. Medicinal values of fruit pericarp from *Citrus sinensis*, *Punica granatum* and *Musa paradisiaca* with respect to alterations in tissue lipid peroxidation and serum concentration of glucose, insulin and thyroid hormones. *J Med Food.* 2008;11(2):376-381.
13. Heber D, Seeram NP, Wyatt H, Henning SM, Zhang Y. Safety and antioxidant activity of a pomegranate ellagitannin-enriched polyphenol dietary supplement in overweight individuals with increased waist size. *J Agric Food Chem.* 2007;55:10050-10054.
14. Parmar HS, Kar A. Protective role of *Citrus sinensis*, *Musa paradisiaca* and *Punica granatum* pericarp against diet-induced atherosclerosis and thyroid dysfunctions in rats. *Nutr Res.* 2007;27(11):710-718.
15. Aviram A, Rosenblat M, Gaitini Aviram M, et al. Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation. *Clin Nutr.* 2004;23:423-433.
16. Naz S, Siddiqi R, Ahmad S, Rasool SA, Sayeed SA. Antibacterial activity directed isolation of compounds from *Punica granatum*. *J Food Sci.* 2007;72(9):M341-M345.
17. Braga LC, Shupp JW, Cummings C, et al. Pomegranate extract inhibits *Staphylococcus aureus* growth and subsequent enterotoxin production. *J Ethnopharmacol.* 2005;96(1-2):335-339.
18. Zhang L, Fu Q, Zhang Y. Composition of anthocyanins in pomegranate flowers and their antioxidant activity. *Food Chem.* 2011;127:1444–1449.
19. Aguilera-Carbo A, Augur C, Prado-Barragan LA, Favela-Torres E, Aguilar CN. Microbial production of ellagic acid and biodegradation of ellagitannins. *Appl Microbiol Biotechnol.* 2008;78:189–199.
20. Prashanth D, Asha M, Amit A. Antibacterial activity of *Punica granatum*. *Fitoterapia.* 2001;72:171–173.
21. Tiwari HC, Singh P, Mishra PK, Shrivastava P. Evaluation of various techniques for extraction of natural colorants from pomegranate rind ultrasonic and enzyme assisted extraction. *IJFTR.* 2010;35:272-276.
22. Kandyli P, Kokkinomagoulos E. Food applications and potential health benefits of pomegranate and its derivatives. *Foods.* 2020;9(2):122.
23. Lansky EP, Newman RA. *Punica granatum* (Pomegranate) and its potential for prevention of treatment of inflammation and cancer. *J Ethnopharmacol.* 2007;109:177-206.
24. Panichayupakaranant P, Tewtrakul S, Yuenyongsawad S. Antibacterial, anti-inflammatory, and anti-allergic activities of standardized pomegranate rind extract. *Food Chem.* 2010;123: 400–403.
25. Turkyilmaz, M. Anthocyanin and organic acid profiles of pomegranate (*Punica granatum* L.) juices from registered varieties in Turkey. *Int J Food Sci Technol.* 2013;48:2086–2095.
26. Smaoui S, Hlima H, Ben Mtibaa AC, et al. Pomegranate peel as phenolic compounds source: Advanced analytical strategies and practical use in meat products. *Meat Sci.* 2019;158.
27. Juneja VK, Cadavez V, Gonzales-Barron U, Mukhopadhyay S, Friedman M. Effect of pomegranate powder on the heat inactivation of *Escherichia coli* O104: H4 in ground chicken. *Food Control.* 2016;70:26–34.
28. Li Destri Nicosia MG, Pangallo S, Raphael G, et al. Control of postharvest fungal rots on citrus fruit and sweet cherries using a pomegranate peel extract *Postharvest Biol Technol.* 2016;114: 54–61.
29. Diganta D, Ratnamala R, Banasri H. Antimicrobial activity of pomegranate fruit constituents against drug-resistant *Mycobacterium tuberculosis* and b-lactamase producing *Klebsiella pneumoniae*. *Pharm Biol.* 2015;53(10):1474–1480.
30. Olukunle JO, Adenubi OT, Oladele GM, Sogebi EA, Oguntoke PC. Studies on the anti-inflammatory and analgesic properties of *Jatropha curcas* leaf extract. *Acta Vet Brno.* 2011;80:259–262.
31. Crini G. Non-conventional low-cost adsorbents for dye removal: A review. *Bioresource Technol.* 2006;97: 1061-1085.
32. Forgacs E, Cserháti T, Oros G. Removal of synthetic dyes from wastewaters: A review. *Environ Int.* 2004;30(7):953-971.
33. Hou XL, Chen XZ, Cheng YX, Xu HL, Chen LF. Dyeing and UV-protection properties of water extracts from orange peel. *J Clean Prod.* 2013;52:410-419.
34. Hosseinneshad M, Gharanjig K, Razani N, et al. Green miles in dyeing technology: Metal-rich pumpkin extracts in aid of natural dyes. *Environ Sci Pollut Res.* 2022;29:50608–50616.
35. Gong K, Pan Y, Rather LJ, Wang W, Zhou Q, Li TZ. Natural pigment during flora leaf senescence and its application in dyeing and UV protection finish of silk and wool a case study of *Cinnamomum camphora*. *Dyes Pigm.* 2019;166:114–121.
36. Jose S, Pandit P, Pandey R. Chickpea husk—a potential agro waste for coloration and functional finishing of textiles, *Ind Crop Prod.* 2019;142:111833.
37. Otaviano BTH, Sannomiya M, Soares de Lima F, et al. Pomegranate peel extract and zinc oxide as a source of natural dye and functional material for textile fibers aiming for photoprotective properties. *Mater Chem Phys.* 2023;(293):126766.
38. Barhanpurkar S, Bhat P, Kumar A, Purwar R, professor A. Studies of Banana SAP used as mordant for natural dye. *Int J Text Eng Process.* 2015;1(4):2395-3578.
39. Zhou Y, Yang ZY, Tang RC. Facile and green preparation of bioactive and UV protective silk materials using the extract from red radish (*Raphanus sativus* L.) through adsorption technique. *Arab J Chem.* 2020;13:3276–3285.
40. Goodarzian H, Ekrami E. Wool dyeing with extracted dye from pomegranate (*Punica granatum*) peel. *World Appl Sci J.* 2010;8(11):1387-1389.
41. Kulkarni SS, Gokhale AV, Bodake UM, Pathade GR. Cotton dyeing with natural dye extracted from Pomegranate

- (*Punica granatum*) peel. *Univers. J Environ Res Technol.* 2011;1(2):135-139.
42. Albu S, Joyce E, Paniwnyk L, Lorimer JP, Mason TJ. Potential for the use of ultrasound in the extraction of antioxidants from *Rosmarinus officinalis* for the food and pharmaceutical industry. *Ultrason Sonochem.* 2004;1(3),261–265.
 43. Barkhordari P, Bazargani-Gilani B. Effect of apple peel extract and zein coating enriched with ginger essential oil on the shelf life of chicken thigh meat. *J Food Meas Charact.* 2021;15(3):2727–2742.
 44. Pan Y, Wang K, Huang S, et al. Antioxidant activity of microwave-assisted extract of longan (*Dimocarpus longan Lour.*) peel. *Food Chem.* 2008;106(3):1264–1270.
 45. National Committee for Clinical Laboratory Standards (NCCLS). National Committee for Clinical Laboratory Standards. Reference Methods for broth dilution antifungal susceptibility testing of filamentous fungi. Approved Standard. Second Edition NCCLS document M38-A2. Wayne, Pennsylvania, 2008;28(16):1-29.
 46. National Committee for Clinical Laboratory Standards (NCCLS). National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Edition Aerobically; Approved Standard. Seventh Edition NCCLS Document M7-A7., Wayne, Pennsylvania, 2006;26(2):1-16.
 47. Becton Dickinson and Company Newsletter BD. Bactec MGIT 960 SIRE kit now FDA-cleared for susceptibility testing of *Mycobacterium tuberculosis*. *Microbiology News & Ideas.* 2002;13: 4-4.
 48. National Committee for Clinical Laboratory Standards (NCCLS). Susceptibility Testing of *Mycobacteria*, *Nocardiae*, and Other Aerobic Actinomycetes; Approved Standard. NCCLS document M24-A [ISBN 1-56238-500-3]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2003.
 49. AATCC Test Method 147-2004, Antibacterial activity assessment of textile materials: Parallel streak method. AATCC Technical Manual, American Association of Textile Chemists and Colorists, Research Triangle Park, NC. 2008.
 50. Bahtiyari Mİ, Benli H, Yavaş A. Printing of wool and cotton fabrics with natural dyes. *Asian J Chem.* 2008;25(6):3220-3224
 51. de Oliveira CRS, Mulinari J, Batistell MA, Augusto Ulson de Souza A, Selene Maria de Arruda Guelli Ulson de Souza. Antimicrobial effect of cotton fabric functionalized with a kaolinite-titania nano-hybrid composite. *Mater Chem Phys.* 2023;295:127078.
 52. Hajimohammadi NJR, Gharbani P, Mehrizad A. Antibacterial activity of *Punica granatum* L. and *Areca nut* (P.A) combined extracts against some food born pathogenic bacteria. *Saudi J Biol Sci.* 2022;1730-1736.
 53. Casquete R, Castro SM, Martín A, Ruiz-Moyano S, Saraiva J. Evaluation of the effect of high pressure on total phenolic content, antioxidant and antimicrobial activity of citrus peels. *Innov Food Sci Emerg Technol.* 2015;31:37–44.
 54. Ismail T, Akhtar S, Sestili P, Riaz M, Ismail A. Antioxidant, antimicrobial and urease inhibitory activities of phenolics-rich pomegranate peel hydro-alcoholic extracts. *J Food Biochem.* 2016;40(4):550–558.
 55. Ali A, Chen Y, Liu H, et al. Starch-based antimicrobial films functionalized by pomegranate peel. *Int J Biol Macromol.* 2019;129:1120–1126.
 56. Dahham, BSS, Ali MN, Tabassum H, Khan M. Studies on antibacterial and antifungal activity of pomegranate (*Punica granatum* L.). *AEJAES.* 2010;9(3):273–281.
 57. Tayel AA, El-Tras WF. Anticandidal activity of pomegranate peel extract aerosol as an applicable sanitizing method. *Mycoses.* 2010;53(2):117-122.
 58. Shuhua Q, Hongyun, Yanning Z. Inhibitory effects of *Punica granatum* peel extracts on *Botrytis cinerea*. *J Plant Dis Prot.* 2010; 36 (1):148-150.
 59. Glazer I, Masaphy S, Marciano P, et al. Partial identification of antifungal compounds from *Punica granatum* peel extracts. *J Agric Food. Chem.*2012;60 (19):4841-4848.
 60. Farag MA, Al-Mahdy DA, Salah El Dine R, Fahmy S, Yassi A. Structure activity relationships of antimicrobial gallic acid derivatives from pomegranate and acacia fruit extracts against potato bacterial wilt pathogen. *Chem Biodivers.* 2015;12 (6): 955-962.
 61. Wang R, Ding Y, Liu R, Xiang L, Du L. Pomegranate: Constituents, bioactivities and pharmacokinetics. *Fruit, Vegetable Cereal Sci Biotechn.* 2010;4 (2), 77-87.
 62. Prakash CVS, Prakash I. Bioactive chemical constituents from pomegranate (*Punica granatum*) juice, seed and peel - a review. *Int J Res in Chem Environt.* 2011;1 (1), 1-18.
 63. Nahar PP, Driscoll MV, Li L, Slitt AL, Seeram NP. Phenolic mediated antiinflammatory properties of a maple syrup extract in RAW 264.7 murine macrophages. *J Func Foods.* 2014; 6:126-136.
 64. Amyrgialaki E, Makris DP, Mauromoustakos A, Kefalas P. Optimisation of the extraction of pomegranate (*Punica granatum*) husk phenolics using water/ethanol solvent systems and response surface methodology. *Ind Crops Pro.* 2014;59:216-222.
 65. Kardan Yamchi J, Mahboubi M, Kazemian H, Hamzelou G, Feizabadi MM. The chemical composition and antimycobacterial activities of *Trachyspermum copticum* and *Pelargonium graveolens* essential oils. *Recent Pat Antiinfect Drug Discov.* 2020;15(1):68-74. doi:10.2174/1574891X14666191028113321
 66. Yang F, Wang A. Recent researches on antimicrobial nanocomposite and hybrid materials based on sepiolite and palygorskite. *Appl Clay Sci.* 2022; 219: 106454. doi: 10.1016/j.clay.2022.106454.
 67. Gozlekçi S, Saraçoğlu O, Onursal E, Ozgen M. Total phenolic distribution of juice, peel, and seed extracts of four pomegranate cultivars. *Pharmacogn Mag.* 2011;7:161. doi: 10.4103/0973-1296.80681 .
 68. Ul-Islam M, Alhajaim W, Fatima A, et al. Development of low-cost bacterial cellulose-pomegranate peel extract-based antibacterial composite for potential biomedical applications. *Int J Bio. Macromol.* 2023;231:123269.
 69. Ul-Islam S, Butola BS, Gupta A, Roy A. Multifunctional finishing of cellulosic fabric via facile, rapid in-situ green synthesis of AgNPs using pomegranate peel extract biomolecules. *Sustainable Chem Pharm.* 2019;12:100135.
 70. Ibrahim N, El-Gamal A, Gouda M, Mahrous F. A new approach for natural dyeing and functional finishing of cotton cellulose. *Carbohydr Polym.* 2010;82:1205–1211. doi: 10.1016/j.carbpol.2010.06.054
 71. Singh R, Jain A, Panwar S, Gupta D, Khare S. Antimicrobial activity of some natural dyes. *Dyes Pigm.* 2005; 66:99–102
 72. Mahmood MA, Al-Dhaher ZA, AL-Mizraqchi AS. Antimicrobial activity of aqueous extracts of pomegranate, sumac, sage, anise, hand bull tongue, thyme, cloves, lemon and mint against some food-borne pathogens. *Iraqi J Vet Med.* 2010; 34(2):85–94. doi:10.30539/iraqijvm.v34i2.635.
 73. Sanbhal N, Ma Y, Sun G, Li Y, Peerzada M, Wang L. Preparation and characterization of antibacterial polypropylene

- meshes with covalently incorporated β -cyclodextrins and captured antimicrobial agent for hernia repair. *Polymers*. 2018;10:58. doi:10.3390/polym10010058.
74. Sun G. Antibacterial textile materials for medical applications. *Mater Sci Eng*. 2011;360–375.
75. Simoncic B, Tomsic B. Structures of novel antimicrobial agents for textiles: A review. *Text Res J*. 2010;80:1721–1737.
76. Al-Zoreky N. Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. *Int J Food Microbiol*. 2009;134:244–248.
77. Ismail T, Sestili P, Akhtar S. Pomegranate peel and fruit extracts: a review of potential anti-inflammatory and anti-infective effects. *J Ethnopharmacol*. 2012;143:397–405.
78. Kim ND, Mehda R, Weiping Y, Ishak N, Talia L. Chemopreventive and adjuvant therapeutic potential of pomegranate (*Punica granatum*) for human breast cancer. *Breast Cancer Res Treat*. 2002;71:203–217.

How cite this article

Guner P, Askun T. Anti-Bacterial, Anti-Mycobacterial and Anti-Fungal Properties of *Punica granatum* as Natural Dye. *Eur J Biol* 2023;82(1): 38-48. DOI: 10.26650/Eur-JBiol.2023.1239283