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RESEARCH ARTICLE

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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properties such as anti-oxidant,[12,13] anti-atherosclerotic,[14,15] anti-bacterial[16,17] and anti-viral[10] 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, gallocatechin, 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 granatonine, which is N-methyl granatonin 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 obtained 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. Gülendam 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 ºC 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 º C. We investigated the anti-mycobacterial activities of the extracts against four tuberculosis strains (MT-H37Ra, MT-H37Rv, and two clinical isolates) by MPBA.

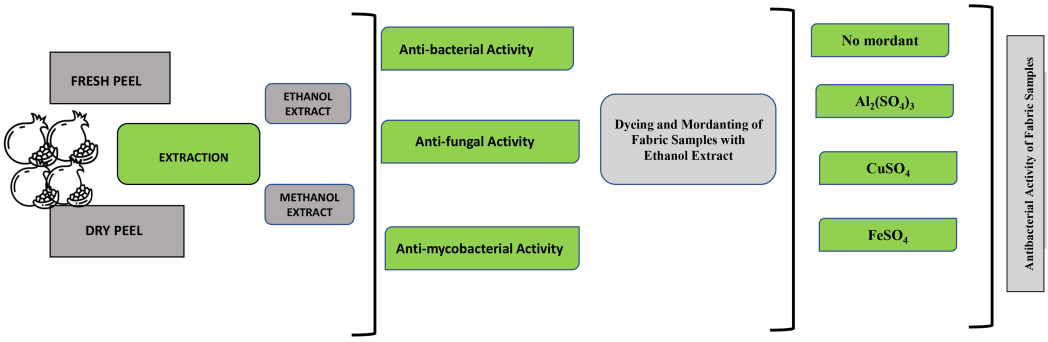

Figure 1. Schematic diagram of the experimental sections.

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 ℃. To prepare 10 mL (500 μg/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 μg/ml. The range of working solution concentrations was between 250 and 0.12 μg/ml. Invitro 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 fungi[45] 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 μg/ml to 250 μg/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 ℃ 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 μg/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 Method[49] (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 ºC 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 (μg/ml) are shown in Table 2. Each extract showed anti-mycobacterial efficacy between MIC 7.81-31.25 μg/ml and MBC 31.25-250 μg/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 μg/ml and 31.25 μg/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 μg/ml), *E. coli* (62.5 μg/ml), and *C. albicans* (31.25 μg/ml). Also, the fresh and dry peel methanol extracts showed an inhibitory effect on S. aureus (1.95 μg/ml), *E. coli* (62.5 μg/ml), and *B. cereus* (7.81 μg/ml). In addition, the inhibitory concentrations of dry

Table 1. Anti-bacterial and anti-fungal activity of pomegranate extracts (μg/mL).

	Anti-bacterial and Anti-fungal Activity (µg/mL)							
	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC
	DP-E		**DP-M**		**FP-E**		**FP-M**	
S. aureus	7.81	15.62	1.95	15.62	3.90	250	1.95	1.95
K. pneumoniae	1.95	250	0.97	250	1.95	250	1.95	1.95
E. coli	62.5	250	62.5	250	62.5	250	62.5	62.5
B. cereus	7.81	250	7.81	125	7.81	250	7.81	7.81
S. typhimurium	62.5	250	62.5	250	62.5	250	125	125
P. vulgaris	15.62	62.5	15.62	125	7.81	7.81	15.62	15.62
P. aeruginosa	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
C. albicans	31.25	125	62.5	125	31.25	125	31.25	250
A. flavus	62.5	250	125	250	62.5	250	125	250
A. niger	125	125	125	250	62.5	125	125	250
A. ochraceus	62.5	125	62.5	125	125	125	125	250
F. proliferatum	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 (μg/ml).

	Anti-mycobacterial activity (µg/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 μg/ml), and dry peel methanolic extracts (62.50 μg/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 researchers[57] 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, gallocatechin, kaempferol, and querectin.[61–64]

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

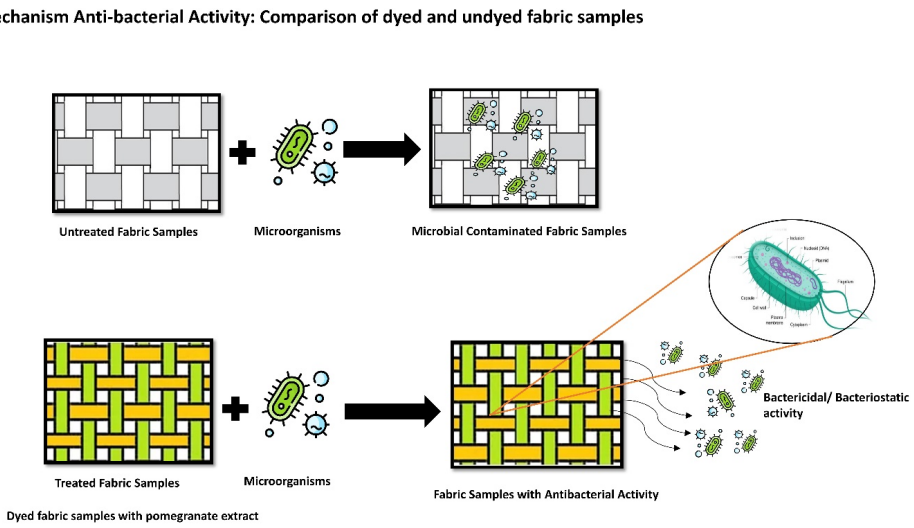

Figure 2. A mechanism between fabric samples and pomegranate extract.

extract of dried peel methanol extract exhibited the most significant activity against MT-H37Ra, with an MIC value of 7.81 μg/ml, and fresh methanol peel extract exhibited the most significant activity against MT-H37Rv, with an MIC value of 15.62 μg/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, antioxidant 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 antimicrobial efficacy of the treated and untreated fabric sample

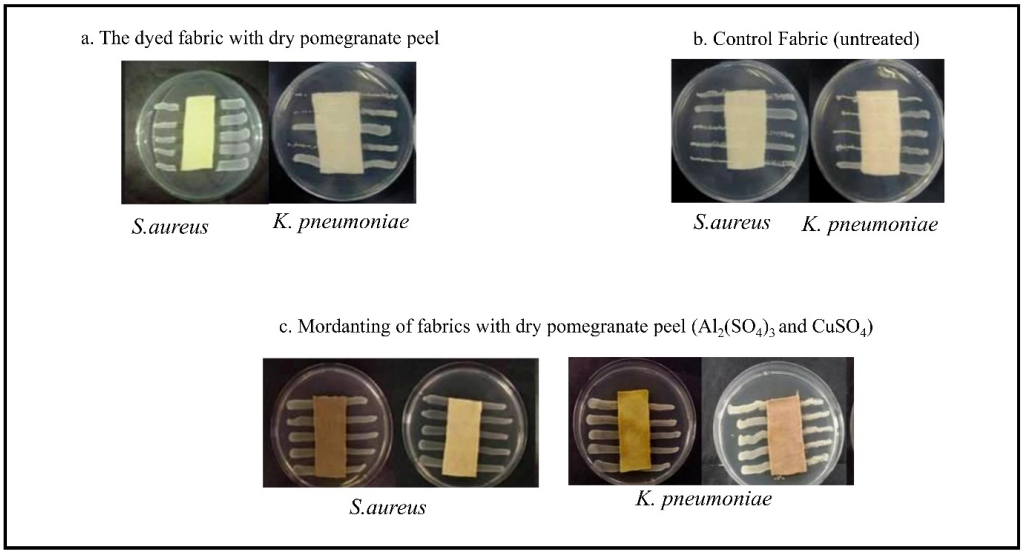

Figure 3. Assessment of anti-bacterial activity of the treated and untreated fabric samples.

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 bacteria[74] 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.

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