ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Antibacterial efficacy analysis of *Punica granatum* L. leaf, rind and *Terminalia chebula* fruit extract treated cotton fabric against five most common human pathogenic bacteria

R.Rathinamoorthy[1]*, S.Udayakumar[2] and G.Thilagavathi[2]
1, Department of Fashion Technology; 2, Department of Textile Technology,
PSG College of Technology, Coimbatore, (TN) - India

Abstract

Bacterial resistance of textile material is one of the major problems faced in the field of Healthcare and hygiene textiles. Hence, in this study the water and methanol extracts of *Punica granatum* L leaf, rind and *Terminalia chebula* fruits were obtained and treated on the cotton plain woven fabric. The fabrics were tested for its antibacterial activity against bacterial strains like *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, and Salmonella typhi.* The result indicates that the treated cotton fabric shows a clear microbial resistance with 27-38 mm zone of inhibition in the agar diffusion test against all the above mentioned strains. Similarly, to measure the microbe resistance quantitatively, the AATCC Test Method 100-2004 was performed. The result indicates that, the treated samples show 99% of reduction against *Staphylococcus aureus* and 90% reduction against Escherichia coli. Further the treated cotton fabrics performance was evaluated using AATCC Test Method 61-2003 for wash durability.

Key-Words: *Punica granatum L, Terminalia chebula,* Agar diffusion method, Challenge test**,** Antimicrobial efficacy, Wash durability

Introduction

Among various functional ability the antimicrobial property of fabric being considered to be important with garment, which are in direct contact with human body[1]. Carbohydrate presents in the cellulosic fiber can act as a nutrient for the growth of micro organism. The growth of micro organism in clothing causes unpleasant odor, staining, loss of mechanical strength and etc., and also cause health related problem to the wearer[2]. Hence it is important to provide necessary protection to the wearer from the micro organism; the fabric must have the bacterial resistant properties. There are several antimicrobial agents used to improve the functional ability of the clothing material [3]. But recently there are lot of attraction towards natural based herbs as an antimicrobial agent because of its eco friendly and health hazardless ness.

*** Corresponding Author**
E.mail: r.rathinamoorthy@gmail.com

Punica granatum L. has been widely used by traditional medicine in America, Asia, Africa and Europe for the treatment of different types of diseases [4-7]. It has been highlighted in many studies as having antimicrobial activity against a range of both Gram positive and negative bacteria [8]. The fruits of *Punica granatum* (pomegranate) have been used to treat acidosis, dysentery, microbial infections, diarrhoea, helminthiasis, haemorrhage, and respiratory pathologies [9]. Melendez and Capriles [10] have also reported that extracts from *Punica granatum* fruits possess strong *in vitro* antibacterial activity against many bacterial strains tested. Many studies have shown that the pomegranate peel extract has wound healing properties [11]. Antibacterial activity [12], antifungal activity [13] and antimicrobial effect [14].

Terminalia chebula is an important medicinal plant in Indian traditional medicine and it is most frequently used herb in Ayurveda. *Terminalia chebula* is called the 'King of Medicine' in Tibet and is always listed at the top of the list in Ayurvedic Material due to its extraordinary power of healing. The dried ripe fruits of *Terminalia chebula* has traditionally been used in the treatment of asthma, sore throat, vomiting, hiccough, diarrhoea, bleeding piles,gout, heart and bladder

diseases [15]. *Terminalia chebula* is routinely used as traditional medicine by tribals of Tamil Nadu in india to cure several ailments such as fever, cough, diarrhea, gastroenteritis, skin diseases, candidiasis, urinary tract infection and wound infections [16].

Antibacterial activity of *Terminalia chebula* extracts against several bacterial strains has been reported [17]. The present investigation aims at developing an eco friendly natural antimicrobial finish from plant extracts for textile application. For this study two plants (*Punica granatum* L, *Terminalia chebula*) were selectively taken based on the literature. The methanol and water extracts of the material were treated on cotton textile and the efficacy of microbial resistance of the textile material were investigated.

Material and Methods

Plant material extraction

Punica granatum L and *Terminalia chebula* are the plant chosen for this study is collected from the Coimbatore District, Tamilnadu, India. Fresh leaves of *Punica granatum L were* shadow dried and made into fine powder. In the same way the fine powder of *Punica granatum L rind* also obtained. The collected quantities of *Terminalia chebula* fruits were shade dried and powdered. The methanolic and water extract of the powders were obtained. 10 gm of powder of each is soaked in methanol for 24 hours to obtain 10% concentrated solution, resulting in active substances being dissolved in methanol. The extract were filtered and used for antimicrobial finishing.

Microorganisms

Bacterial cultures used in the present studies were obtained from Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh. The bacterial strains *Staphylococcus aureus (MTCC 737), Escherichia coli (MTCC 1687), Klebsiella pneumoniae (MTCC 6644), Proteus vulgaris (MTCC 742), Salmonella typhi (MTCC 733*) were used.

Application Method

Plain woven Cotton fabric with 140 PPI and 78 EPI was desized, scoured and bleached prior to the application of the antimicrobial finish.

Both the water and methanol extracts were applied to the cotton fabric by dipping in bath at material to liquor ratio of 1:10 and then Pad-dry-Cured. The fabric was then dried at 80^{o}C for ten minutes. Finally the fabric samples were tested for antimicrobial activity as per the AATCC test standards.

Agar Diffusion Method (SN 195920)

The treated and untreated fabric samples were placed in the AATCC bacteriostasis agar [18], which has been previously inoculated (Mat culture) with a test organism. After incubation, a clear area of uninterrupted growth underneath and along the side of the test material indicates the antibacterial effectiveness of the fabric. The area of the inhibition zone is a measure of antibacterial effectiveness of the material.

Hohensteins Modified Challenge Test (JIS L 1902)

Specimens of the test material were shaken in a known concentration of bacterial suspension and the reduction in bacterial activity in standard time was measured. The efficiency of the antimicrobial treatment was determined by comparing the reduction in bacterial concentration of the treated sample with that of control sample expressed as a percentage reduction of standard time [19].

The percentage of reduction of bacteria by the specimen treatments by the following formula

% of reduction = 100 (B-A)/B

Here A= the number of bacteria recovered from the inoculated treated test specimen swatches in the jar incubated over 24 hr contact time.

B= the number of bacteria recovered from the inoculated treated test sample in jar immediately after inoculation (at 0 contact time)

Durability Evaluation

To test the durability of the antimicrobial finish, treated samples were examined for antimicrobial efficacy after 5, 10 home launderings. The tests were performed as per AATCC Test Method 61-2003 [20].

Results and Conclusion

Agar Diffusion Test (SN 195920)

Various parts of *Punica granatum L have* been used for various medicinal purposes [21]. It was found that *Punica granatum L* peels contain tannins, anthocynins, flavonoids , pectins [22], three estrogen compounds luteolin, quercetin and kaempferol [23]. The result of Agar Diffusion Test for antimicrobial effectiveness of both water and Methanol extracts against standard test cultures viz., *Staphylococcus aureus (MTCC 737)* (gram positive), *Escherichia coli (MTCC 1687), Klebsiella pneumoniae (MTCC 6644), Proteus vulgaris (MTCC 742) and Salmonella typhi (MTCC 733)* (gram negative) organisms shows that the activity of treated samples is stronger for all the above mentioned strains.

The methanol and water extract of the *Punica granatum L* leaf, Peel and *Termanilla chebula* fruits were applied on the textile material by Pad-Dry-Cure method. The results indicates the presence of clear zone of inhibition of 27-38 mm diameter for both water and methanol extract treated fabrics against all the five selected micro organism namely *Staphylococcus aureus* (gram positive), *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris and Salmonella typhi* (gram negative). Table 1 (Methanol extract), Table 2

(Water extract) and Figure 1 shows the antimicrobial efficacy of treated fabric.

Hohensteins Modified Challenge Test

The Table 3 indicates that both the materials (*Punica granatum L, Terminalia chebula*) show high percentage of reduction against both *Staphylococcus aureus* and *Escherichia coli.* . As a high *Punica granatum L* leaf shows 99.9 % of reduction against *Staphylococcus aures.* And in the case of *Escherichia coli* 98.11% of reduction was achieved by *Terminalia chebula* treated fabric. The results show that, in both agar diffusion and quantitative test, the methanol extract treated samples shows high activity than the water extract against all the human pathogenic bacterial stains. This result supports the findings of Ahmed et al., 1998 [24].

Durability Evaluation

The treated samples were evaluated for washing durability by AATCC Test Method 61. The results of wash durability of methanolic extract treated fabric are given in Table 4 and 5. There is no activity in the water extract treated fabric after subsequent washing, hence the methanolic extract samples alone subjected for wash durability test. The antimicrobial activity of the samples was assessed after 5 washes and 10 washes by using both agar diffusion and Hohensteins modified challenge test. There is a reduction in activity after subsequent washes.

Agar Diffusion Test

The agar diffusion test results shows, that there is a reduction in the inhibition zone after subsequent washes. The *Punica granatum L* rind treated samples shows inhibition zone of 22 and 26 mm respectively for *Escherichia coli* and *Klebsiella pneumonia* after 5 washes. The 10 cycles washed samples shows clear inhibition zone only against *Klebsiella pneumonia* (24mm). *Punica granatum L* Leaf treated samples show an inhibition zone of 22, 26 and 24 mm respectively for *Staphylococcus aureus, Escherichia coli* and *Proteus vulgaris* after 5 washes. The zone of inhibition was reduced after ten washes and it is around 21 mm in the case of *Escherichia coli*. At the maximum the *Terminalia chebula* treated samples shows high clear inhibition zone (above 24mm) against all the bacterial stains even after 5 and 10 wash cycles except *Salmonella typhi.*

Hohensteins Modified Challenge Test

Decreases in the percentage of reduction were obtained as the washing frequencies increased. Similarly Samples that had been washed 5 times were Shown very low percentage of bacterial reduction in the case of *Escherichia coli (Punica granatum L* leaf treated samples - 52.94% of activity, *Punica granatum L* rind treated samples -74.52% of activity and *Terminalia chebula* treated fabric - 85.62% of activity). But the sample that had been washed 10 cycles shows no activity against *Escherichia coli.* However, while considering the *Staphylococcus aureus,* these above stated samples were noted no activity for both after 5 and 10 cycles of wash. It is noted that all the treated material having poor durability after commercial laundering. The poor durability of the antimicrobial agent in the textile material may be due to the weak Vander Val's or hydrogen bonds, between antimicrobial agent and cellulose material [25].

This paper investigates the antimicrobial efficacy of *Punica granatum L* leaf, rind and *Terminalia chebula* fruit extract treated cotton fabrics. The results from agar diffusion test indicates that the extract of *Punica granatum L* leaf, rind and *Terminalia chebula* were having high potential against wide verity of human pathogenic bacteria. 27 -38 mm of inhibition zone was noticed for both water and methanol extract treated textile material. It is concluded from the challenge test results that both *Punica granatum L* leaf and rind extract treated samples have 99% of bacterial reduction against *s.areaus* (Gram positive).But in the case of *E.coli* (Gram negative) the *Punica granatum L* leaf treated sample shows 87% and the rind extract treated material showed 79% of bacterial reduction. The *Terminalia chebula* extract treated material showed higher percentage of reduction for both gram negative and gram positive (*S.areaus* -99.3% and *E.coli* 98.1%)

The wash durability result indicates that, the effectiveness of the treatment reduces with the increase in washing cycle. Though the tested samples shows poor wash durability, the unwashed samples shows high and clear zone of inhibition against wide spectrum of human pathogenic bacterial stains. The extract can be applied simply by the pad-dry-cure method. If the treated sample is for one time usage like surgical mask, bandage gauze, wound healing bandages, wound management material, dressings etc,. this method very effective and cost effective for commercialization. Since *Punica granatum L* and *Terminalia chebula* were natural resources, they were eco friendly and because of their abundant availability, the scope of implementation and commercialization of herbal extract on cotton material is much possible and this antimicrobial treatment definitely has its potential.

References

1. Sathianarayanan MP, Bhat NV, Kokate SS, Walunj VE. (2010) Antibacterial finish for cotton fabric from herbal products. Indian Journal of Fiber and Textile Research. 35: 50-8.

2. Raja ASM, Thilagavathi G, Kannaian T. (2010) Synthesis of spray dried polyvinyl pyrrolidone coated silver nanopowder and its application on wool and cotton for microbial resistance. Indian Journal of Fiber and Textile Research. 35: 59-64.
3. Thilagavathi G, Rajendrakumar K, Rajendran R. (2005) Development of antimicrobial textile finishes from plant species. Express Textile.
4. Gracious RR, Selvasubramanian S, Jayasundar S. (2001) Immunomodulatory activity of *Punica granatum* in rabbits: a preliminary study. J. Ethnopharmacol. 78: 85-7.
5. Kim ND, Mehta R, Yu W, Neman I, Livney T, Amichay A, Poirer D, Nicholls P, Kirby A, Jiang W, Mansel R, Ramachandran C, Rabi T, Kaplan B, Lansky E. (2002). Chemopreventive and adjuvant tharapeutic potential of pomegranate (*Punica granatum*) for human breast cancer. Breast Cancer Res. Tr. E. 71:203-17.
6. Murthy KN, Reddy VK, Veigas JM, Murthy UD. (2001). Study on wound healing activity of *Punica granatum* peel. J. Med. Food. *7*: 256-59.
7. Lamar AS, Fonseca G, Fuentes JL, Cozzi R, Cundari E, Fiore M, Ricordy R, Perticone P, Degrassi F, Salvia RD. (2008). Assessment of the genotoxic risk of *Punica granatum L* . (*Punicaceae*) whole fruit extracts. J. Ethnopharmacol. 115:416-22.
8. Navarro V, Villarreal ML, Rojas G, Lozoya X. (1996). Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. J Ethnopharmacol. 53:143-47.
9. Fuentes VR, Exposito A. (1995). Las encuestas etnobotanicas sobre plantas medicinales en Cuba. Rev. Jard. Bot. Nacion. Univ. Habana. 16: 77-144.
10. Melendez PA, Capriles VA. (2006) Antibacterial properties of tropical plants from Puerto Rico. Phytomedicine. 13: 272-76.
11. Chidambara MK, Jayaprakasha GK, Singh RP. (2002). Studies on antioxidant activity of pomegranate (punica granatum) peel extract using in vivo models. J. Agric Food Chem.;14(50): 4791.
12. Prashanth D, Asha MK, Amit A. (2001). Antibacterial activity of Punica granatum. Fitoterapia. 72:171.
13. Dutta BK, Rahman I, Das TK. (1998). Antifungal activity of Indian plant extracts. Mycoses. 41: 11-12.
14. Navarro V, Villarreal M, Rojas G, Lozoya X. (1998). Antimicrobial Evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases .J. Ethnopharma. 53(3): 143.
15. Kirtikar KR, Basu BD. (1935) Indian Medicinal Plants. no.1: 1020-1023.
16. Dash B. (1991). Materia Medica of Ayurveda. Jain Publishers, New Delhi.170-74**.**
17. Bag A, Bhattacharyya SK, Bharati P, Pal NK, Chattopadhyay RR. (2009). Evaluation of antibacterial properties of Chebulic myrobalan (fruit of Terminalia chebula Retz.) extracts against methicillin resistant Staphylococcus aureus and trimethoprim-sulphametho-xazole resistant uropathogenic Escherichia coli. Afr. J. Plant Sci.
18. Antibacterial Activity of Fabrics, Detection of: Agar Plate Method. AATCC Technical manual. 2005; 80.
19. Antibacterial finishes on textile material. AATCC Technical manual. 2005; 80: 149 -51.
20. Colour fastness to Laundering, Home and commercial: Accelerated. AATCC Technical manual. 2005; 80: 90 -94.
21. Julie Jurenka MT. (2008). Therapeutic Applications of Pomegranate *(Punica granatum L)*: A Review. Alternative Medicine Review. 13(2).
22. Nozire O, Serpil D. (1993). Valuation of the pomegranate (Punica granatumL.) peels from the stand point of pharmacy. Ankara Ecz.Fak.Derg. 22: 1-2.
23. Van-Elswijk DA, Schobel UP, Lansky EP, Irth H, Van-der- Greef G. (2004). Rapid dereplication of estrogen compounds in Punica granatum using online biochemical detection coupled to mass spectrometry. Phytochemistry. 65(2): 233.
24. Ahmad I, Mehmood Z, Mohammad F. (1998). Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol. 62(2): 183-93.
25. Thilagavathi G, Kannaian T. (2008). Application of prickly chaff (*Achyranth aspera)* Leaves as herbal antimicrobial finish for cotton fabric used in healthcare textile. Natural Product Radiance. 4(7): 330-34.

ISSN: 0976-7126

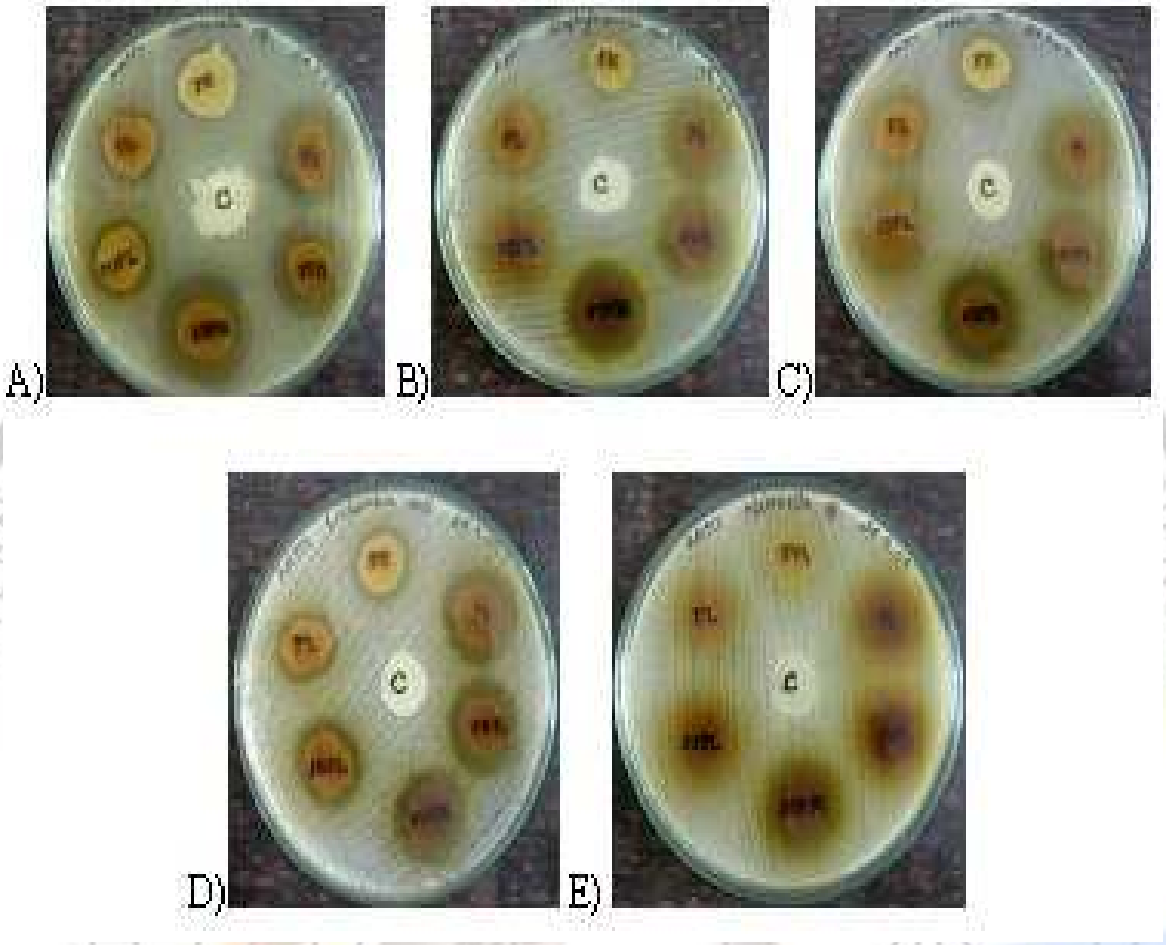

Figure 1 Agar diffusion test on *Punica granatum L* leaf and Peel treated cotton fabric: Zone of inhibition against A) *Staphylococcus aureus*, B) *Escherichia coli*, C) *Klebsiella pneumonia*, D) *Salmonella typhi*, E) *Proteus vulgaris*.

[PL = *Punica granatum L* leaf – Water Extract, PR = *Punica granatum L* rind – Water Extract, K = *Terminalia chebula* fruit –Water Extract, MPL = P *Punica granatum L* leaf – Methanol Extract, MPR = *Punica granatum L* rind – Methanol Extract, MK = *Terminalia chebula* fruit – Methanol Extract]

Table 1: Zone of inhibition of treated textile material for different strains (MethanolExtract)

S.No	Bacteria	Zone of inhibition (in mm)			
		Control	*Punica granatum L* Leaf extract	*Punica granatum L* Rind Extract	*Terminalia chebula* fruit extract
1	*Staphylococcus aures*	-	30	28	38
2	*Escherichia coli*	-	28	26	33
3	*Klebsiella pneumonia*	-	26	25	36
4	*Proteus vulgaris*	-	32	32	34
5	*Salmonella typhi*	-	30	28	32

ISSN: 0976-7126

S.No	Bacteria	Zone of inhibition (in mm)			
		Control	*Punica granatum L* Leaf extract	*Punica granatum L* Rind Extract	*Terminalia chebula* fruit extract
1	*Staphylococcus aures*	-	32	35	34
2	*Escherichia coli*	-	31	34	32
3	*Klebsiella pneumonia*	-	27	29	34
4	*Proteus vulgaris*	-	38	34	34
5	*Salmonella typhi*	-	30	33	32

Table 2: Zone of inhibition of treated textile material for different strains (Water Extract)

Table 3: Percentage of bacterial reduction by Hohensteins modified challenge test (Quantitative measurement) for Methanol extract

S.No	Bacterial Stains	Sample	At "0" hour contact	At "24" hour contact	% of Reduction
1		*Punica granatum L* leaf	$244X10^{-5}$	$122x10^{-2}$	99.95 %
2	*S.Areaus*	*Punica granatum L* rind	$220X10^{-4}$	$110X10^{-2}$	99.5%
3		*Terminalia chebula*	$224X10^{-3}$	$142X10^{-1}$	99.36%
4		*Punica granatum L* leaf	136X10-4	124x10-3	90.88%
5	*E.coli*	*Punica granatum L* rind	140X10-3	21X10-3	85%
6		*Terminalia chebula*	104X10-3	196x10-2	98.11%

ISSN: 0976-7126

Table 4: Durability evaluation of Treated samples by agar diffusion method

S.No	Sample	Bacterial Stain	After 5 wash Inhibition zone (in mm)	After 10 wash Inhibition zone (in mm)
1	*Punica granatum L* rind	*Staphylococcus aures*	-	-
		Escherichia coli	22	-
		Klebsiella pneumonia	26	24
		Proteus vulgaris	-	-
		Salmonella typhi	-	-
2	*Punica granatum L* Leaf	*Staphylococcus aures*	22	-
		Escherichia coli	26	21
		Klebsiella pneumonia	-	-
		Proteus vulgaris	24	-
		Salmonella typhi	-	-
3	*Terminalia chebula*	*Staphylococcus aures*	28	25
		Escherichia coli	24	24
		Klebsiella pneumonia	36	26
		Proteus vulgaris	26	24
		Salmonella typhi	-	-

- No zone formation

Table 5: Durability test by quantitative measurement

-

S.No	Bacterial Stains	Sample		At "0" hour contact	At "24" hour contact	% of Reduction
1	*S.Areaus*	*Punica granatum L* leaf	Control fabric	-	-	No reduction
			After 5 Wash	-	-	No reduction
			After 10 wash	-	-	No reduction
2		*Punica granatum L* rind	Control fabric	-	-	No reduction
			After 5 Wash	-	-	No reduction
			After 10 wash	-	-	No reduction
3		*Terminalia chebula*	Control fabric	-	-	No reduction
			After 5 Wash	-	-	No reduction
			After 10 wash	-	-	No reduction
4	*E.coli*	*Punica granatum L* leaf	Control fabric	-	-	No reduction
			After 5 Wash	$272X10^{-5}$	$128X10^{-5}$	52.94%
			After 10 wash	-	-	No reduction
5		*Punica granatum L* rind	Control fabric	-	-	No reduction
			After 5 Wash	$212X10^{-4}$	$540X10^{-3}$	74.52%
			After 10 wash	-	-	No reduction
6		*Terminalia chebula*	Control fabric	-	-	No reduction
			After 5 Wash	$160X10^{-3}$	$230X10^{-2}$	85.62%
			After 10 wash	-	-	No reduction