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Azadirachta indica and *Punica granatum* rind treatment on linen fabric with plasma finish and exhaustion method

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In this study, neem (*Azadirachta indica*) and pomegranate (*Punica granatum*) have been used as antimicrobial agents in a fixed proportion for application on linen fabric followed by plasma treatment. The treated fabric is then assessed for antimicrobial activity. It is found that, without plasma treatment, neem and pomegranate rind extract treatments provide a semi-permanent antimicrobial finish, but after plasma treatment, the durability of the finish remains closer to the permanent finish. Some significant changes in the linen fabric properties are also observed.

Keywords: Antimicrobial activity, *Azadirachta indica*, Fabric finish, Linen fabric, Physical properties, Plasma treatment, *Punica granatum*, Zone of inhibition

1 Introduction

Synthetic antimicrobial agents, such as triclosan, metal and their salts, phenols, quaternary ammonium compounds, and organometallics, are among the active agents used in the development of antimicrobial textile materials. Although synthetic antimicrobial agents efficiently restrict bacterial growth, the majority of them are toxic and have negative health consequences along with environmental implications. According to current research, it has been found that various plant extracts may be effective against both Gram-positive and Gram-negative bacteria. As a result, research on environment-friendly antimicrobial compounds and their use in a variety of textile goods has become increasingly important across the world[1]. In textile finishing, natural antibacterial substances are obtained from plants such as neem, tea tree, azuki beans, aloe vera, tulsi leaves (*Ocimum sanctum*), clove oil, pomegranate rind, turmeric, eucalyptus oil, onion peel, and pulp extracts[2].

Azadirachta indica (neem), because of its extensive spectrum of medical benefits has gained an international reputation in recent years. Neem is widely utilized in ayurveda, unani, and homoeopathic treatments and has become a contemporary medical cynosure. Neem produces a wide range of physiologically active chemicals that are both chemically and structurally varied. The leaves, flowers, seeds, fruits, roots, and bark of the neem tree have been used to cure inflammation, infections, fever, skin ailments, and dental problems. Immunomodulatory, anti-inflammatory, anti hyperglycaemic, antiulcer, anti malarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic, and anticarcinogenic effects have been established in neem leaves and their compounds[3].

Because of its unique qualities, neem derivatives are currently used in a variety of therapies, including herbal medicine, toothpaste, cosmetics, toiletries, and pharmaceuticals. The most essential property of neem compounds is that they are less poisonous to warm-blooded creatures such as humans[4]. More than 300 active compounds have been extracted from diverse parts of the neem tree, including leaves, bark and seeds. While some of them have previously been recognized for their potential antibacterial impact, the activity of the other active compounds has yet to be determined.

It has been proven that neem extract and its primary components, such as azadirachtin, salannin, and meliantriol, are insect growth regulators and antifeedants. Therefore, it has a lot of promise as an

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antibacterial agent[5]. Antiviral and virucidal activities have also been documented for neem leaf extract[6,7]. Purified neem extract have efficacy against HIV and sexually transmitted disease pathogens, as well as contraceptive effects, according to Joshi *et al.*[7] Neem oil's antibacterial characteristics have been utilized in conjunction with other herbal oils such as clove, tulsi, and Karanga to give cotton fabrics an antimicrobial finish[8,9]. Joshi *et al.*[10] isolated an antimicrobial compound from the seeds of the neem tree (*A. indica*) and used it to impart antibacterial qualities to a polyester/cotton blend fabric, resulting in a semi-durable antibacterial finish. The ideal formula for establishing excellent cross-linking and obtaining strong antibacterial activity was found to contain around 5% w/v neem seed extract, glyoxal/glycol (4.8 %/5.2 % on the weight of bath), tartaric acid and aluminium sulphate (4 % on the weight of the resin). The antibacterial efficacy of neem leaf extract against human pathogenic bacteria such as *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *S. aureus*, and *Bacillus pumilus* has also been reported[11]. This was done by using the antibacterial capabilities of alcoholic neem leaf extracts. Extract concentrations of 200, 150, 100, 50 and 25 mg/mL were obtained using the disc diffusion method. As compared to conventional antibiotics, such as gentamycin 200 mg and gentamycin 10 mg; the methanol and ethanol extract inhibited growth of *B. pumilus*, *P. aeruginosa* and *S. aureus* in increasing order.

The peel of the pomegranate (*Punica granatum*) has been shown to be a potent antibacterial agent that effectively stops microbes growth. Traditional cures for diarrhea, dysentery and intestinal parasites have been observed from the peel of the fruit and the bark of the pomegranate tree. Pomegranate arils include polyphenols such as ellagitannins and flavonoids. Each 100 g serving provides 12 % of the daily value (DV) for vitamin C and 16 % of the DV for vitamin K. As a result, making an antibacterial agent out of the waste product is a novel concept[12]. It was also revealed that using the microencapsulated method stiffens the fabric and prevents it from mending creases. Resin-treated cloth's tensile strength was lowered somewhat, but its crease-healing abilities remained intact. Microencapsulation and finish, when used in conjunction with a cross-linking agent, have the potential to boost the strength of the product. Dahham *et al.*[13] investigated the antibacterial and antifungal effects of pomegranate peel extract (rind), seed extract, juice, and whole fruit on a range of bacteria and fungi. As compared to other extracts, they observed that peel extract has the greatest antibacterial activity. Antibacterial activity was found greatest against *S. aureus* among the selected bacterial and fungal cultures, whereas antifungal activity was greatest against *Aspergillus niger* among the fungi.

In textiles, finishing plays a crucial role to improve the quality and value of textiles. The human skin is constantly and continuously exposed to environmental organisms and is continuously contaminated from their secretions and excretions. The extent depends on the personal hygiene of individuals. Natural fibres are more susceptible to bacterial attacks due to their hydrophilic nature. The presence of water and oxygen in natural fibres causes rapid growth of micro-organisms[14]. Direct touch of cloth with the human body supplies warmth, humidity, and nutrients to the bacteria, providing a perfect environment for the microbial growth[9]. These micro-organisms cause an unpleasant odor, discoloration, stains, fibre damage, and a slimy feel to the textile material. Rajendran *et al.*[15] studied the ethanol, methanol, petroleum ether and water extracts of *Ocimum sanctum* leaves for their anti-microbial activities by using the agar diffusion method. The study revealed that the herb encapsulated nanoparticles could act as a biocontrol agent against bacteria in fabrics. A wide range of antibacterial textile goods is now available to the consumer with an expanding product line. By finishing with neem and pomegranate extracts, antimicrobial properties can be introduced to the fabric. The challenge is the durability of the antibacterial function of textiles[14]. The repeated washing of the finished fabric also affects the antibacterial properties of the fabric. Hence, there are processes to increase the long-term antimicrobial effect through advanced technology. The application of plasma technology with a natural antimicrobial agent increases the fabric's durability also. Further innovations are required for improving the durability and other properties of textile materials[16]. In this study, antimicrobial agents, viz. neem (*A. indica*) and pomegranate (*P. granatum*) have been used in a fixed proportion and treated with for application on linen fabric followed by plasma treatment. Then the antimicrobial activity of the treated fabric is assessed.

2 Materials and Methods

2.1 Materials

Linen fabric has been used with the specifications: weave plain, EPI 60, PPI 65 and GSM 119. The antimicrobial agents, neem and pomegranate rind (50:50) were used. Neem leaves and pomegranate rind were first dried and then fine powdered using electronic grinding and filtering. The plasma treatment is given to the linen fabric using plasma power 100–200 watt, working pressure - 0.050–0.1mbar, pumping speed- 30m^3/h and sample size rectangle - 500mm × 550mm.

2.2 Methodology

2.2.1 Agar Diffusion Test

Quantitative method AATCC-100-1998(USA) was used for the antibacterial study. Seventy-two (72) petri dishes were washed, wrapped in paper and then tied with thread. These plates were kept in an autoclave for 20 min at 121°C temperature and 15 lbs osmotic pressure for sterilization. At the same time, peptone dextrose agar media was prepared for antifungal activity, using peptone 10g, agar 20g, dextrose 40 g, and distilled water 100mL. Mild heating treatment in a conical flask was given. After boiling, the conical flask was fitted with threads and kept for autoclaving in an autoclave.

At the same time, nutrient agar was prepared in another clean conical flask. Agar powder was used from HIMEDIA-M001 (nutrient agar) with suspended 28 g in 100 mL of distilled water. After mild boiling, it is wrapped with paper, fitted with thread, and kept in an autoclave for autoclaving. The ingredients of nutrient agar are peptic digest of animal (5g/L), sodium chloride (5 g/L), beef extract (1.5 g/L), yeast extract (1.5 g/L), sodium chloride (5 g/L), agar (15 g/L) and final pH at 25°C (7.4±0.2). After completion of autoclaving, the media and sterilized petri dish were kept on the aseptic surface near the flame. Cool the media near to 60°C. Pour these media (antibacterial) under the aseptic condition in petri dish near the flame and keep finally all these petri dishes for cooling. After cooling of media in petri dish, pour fresh culture of (i) *Escherichia coli* (for antibacterial Gram-negative test) and (ii) *Staphylococcus aureus* (for the antibacterial Gram-positive test) aseptically in petri dish. Petri dish iskept as it is for 10-15 min for uniform distribution and absorption of culture on media. Then mark each petri dish with a marker pen as per the labeling for proper identification. Then take linen sample by sterilized tongue from sample bag to the flame and put it according to levelling on petri dish. After putting cotton sample on the plate, it is just pushed by the tongue, so that when the petri dish is kept in inverted condition sample may not fall. After complete transfer of the sample into the petri dish, keep all these petri dishes in an incubator at 37°C for 24 h for antibacterial activity and 48 h for antifungal activity. After completion of the incubation period, take out all petri dishes from the incubator and measure the zone of inhibition simply from the center of the sample to the clear zone on the petri dish by caliper.

2.2.2 Stiffness Test

This test is carried out using stiffness tester. Each specimen has 25 mm width and 200 mm length; 10 specimens are cut parallel to the warp and another 10 specimens are cut parallel to the weft, so that no two warp specimens contain the same warp threads and no two weft specimens contain the same weft threads. Specimen should not crease and those that tend to twist should be flattened. If a specimen is found to be twisted its mid-point should be aligned with two index lines. Readings are taken for each specimen.

2.2.3 Crease Recovery Test

This test is carried out on a crease recovery tester; a specimen is cut from the fabric with a template, (2 inches long by 1 inch wide). It is carefully created by folding it in half, placing it between two glass plates[17], and adding 2 kg weight for 5 min. They are then transferred immediately to the holder of the measuring instrument and one leg of the specimen is inserted as far as the backstop. The instrument is adjusted continuously to keep the free limb of the specimen vertical. When the limb is vertical, the crease angle is measured, after 5 min of removing the load by reading a scale.

2.2.4 Digiwash/ Washing Cycles

This test is carried outon digiwash (AATCC-61-2007. The sample and appropriate liquor are sealed inside the container and the water bath is heated to the desired temperature with required washing cycles and a soap solution is prepared by taking the materials, such as standard soap (powder form) (5 g/L) and sodium carbonate (Na_2CO_3) (2 g/L). These are mixed with water and a homogeneous soap solution is obtained by stirring the solution for a few minutes. The fabric sample and soap solution were taken

together in the steel cups of the "Digiwash" tester with different set times and temperature of 40°C. The cups were fastened inside the instrument and the lid was closed. After the completion of washing, the samples were washed and dried. The fabric samples were then subjected to microbial agar and the bacterial growth was analyzed by serial dilution, carried out after incubation.

2.2.5 Wicking Test

All samples were tested for vertical and horizontal wicking according to AATCC test method 197-2011 for vertical wicking of textiles, and AATCC test method 198-2011 for horizontal wicking of textiles.

2.2.6 Finishing Parameter

Finishing treatment was carried out using the following parameters:

M:L ratio – 1:10

Concentration – 3%, 6%, 9%

Time – 30 min

Washing cycles – 0 min, 5 min, 10 min, 15 min

2.2.7 Plasma Finish on Fabric

Cut the sample of 500mm×550mm size and clamp it on the template of same size. Then put the sample in a plasma chamber in between two aluminum plates and close the door of the chamber tightly. Then pump the oxygen gas into the closed chamber at a speed of 30m^3/h. The oxygen gas is continuously filled into the chamber till the pressure of 0.050 –0.1 mbar is obtained. Put the plasma button ON for 5 min. Expose the top side of the sample to the plasma rays and vice-versa for 5 min. After that, stop the oxygen gas filling and remove the sample from the chamber.

3 Results and Discussion

3.1 Assessment of Antimicrobial Activity

The assessment of the treated linen fabric for antimicrobial activity is done in three conditions:

- Without plasma treatment– Fabric treated with antimicrobial agent only (no plasma treatment).
- Before plasma treatment– Fabric first treated with plasma and then antimicrobial treatment.
- After plasma treatment– Fabric first treated with antimicrobial agent and then plasma treatment.

The disk diffusion agar method test shows the effectiveness of antimicrobial properties on Gram-positive and Gram-negative bacteria. Agar diffusion is spread with the Gram-positive and Gram-negative bacteria separately and then antimicrobial treated swatches are added. The bacteria are allowed to grow on the agar media. The amount of space around every antimicrobial treated swatch indicates the lethality of that antimicrobial on the bacteria.

3.1.1 Without Plasma Treatment

Table 1 shows that at zero washing cycle, the zone of inhibition is more for Gram-positive and Gram-negative bacteria, indicating the high effective antimicrobial effect[18,19]. After 5 washing cycles, the zone of inhibition is decreased, i.e. the effectiveness of the antimicrobial effect is reduced. With further increases in wash cycles (up to 15 cycles), the effectiveness of the antimicrobial effect is completely diminished. Only 9% concentration shows some zone of inhibition. It is observed that the finish is durable up to 10 wash cycles only.

3.1.1 Before Plasma Treatment

Table 1 shows that at zero washing cycle, the zone of inhibition is more for Gram-positive and Gram-

Table 1 — Assessment of antimicrobial activity

Concentration %	Wash cycles	Zone of inhibition, mm					
		Without plasma treatment		Before plasma treatment		After plasma treatment	
		Gram- positive	Gram-negative	Gram-positive	Gram-negative	Gram-positive	Gram-negative
3	0	10	7	7	8	10	10
	5	6	4	7	7	9	8
	10	2	2	5	7	10	6
	15	0	0	5	7	10	3
6	0	10	6	10	8	11	10
	5	8	6	9	8	8	10
	10	6	6	8	6	10	8
	15	3	0	8	5	8	6
9	0	9	10	12	12	7	10
	5	5	6	10	10	6	8
	10	2	4	8	9	5	12
	15	0	0	8	7	4	6

negative bacteria i.e. the high effective antimicrobial effect has been produced. When it is washed up to 5 washing cycles, the zone of inhibition is decreased, i.e. the effectiveness of the antimicrobial agent is decreased. With the further increase in the wash cycles up to 15 cycles, the zone of inhibition is further decreased, i.e. the effectiveness of the antimicrobial agent persists. It means that the finish is permanent and in the case of Gram-negativebacteria, it shows a good zone of inhibition. The reason for the improvement of finish durability may be due to the reduction in surface resistivity of fabric, formation of voids and cracks on fibre surfaces, reduction in moisture regain and increased fabric water uptake[16].

3.1.2 After Plasma Treatment

Table 1 shows that at zero washing cycle, the zone of inhibition is more for Gram-positive and Gram-negative bacteria i.e. the effectiveness of antimicrobials at his best. When it is washed up to 5 washing cycles, the zone of inhibition is decreased, i.e. the effectiveness of the antimicrobial is decreased. With further increase in the washing cycles up to 15 cycles, it shows a lesser zone of inhibition, i.e. the effectiveness of the antimicrobial persists. It means that the finish was durable up to 15 washing cycles and in the case of Gram-positive bacteria, it shows a good zone of inhibition.

3.2 Physical Properties of Linen Fabric

3.2.1 Crease Recovery Testing Analysis (AATCC-61-2003)

Table 2 shows the crease recovery value of 10 samples, each with plasma treatment and without plasma treatment. The reading in warp way and weft way for crease recovery test is taken. The findings reveal that the crease recovery angle of the untreated linen fabric specimen is lower than that of the plasma-treated fabric specimen. Therefore, it is concluded that the crease recovery values of the linen specimen in warp way and weft way increase after plasma treatment.

3.2.2 Stiffness Testing Analysis (ASTM-D1388)

Bending stiffness is a very important property that affects the appearance and comfort of a garment. It is defined as the bending moment required to produce a given curvature[20]. The mean values of the bending length in warp and weft directions are reported in Table 2. It may be emphasized that the stiffness indicates handle and drape of a fibre. It is intuitively quite easy to observe that the fabric with high bending rigidity would show poor drapability. Table 2 shows that the stiffness of the linen specimen in the warp direction and weft direction has increased after plasma treatment.

3.2.3 Water Absorbency Testing Analysis

Horizontal Wicking Test Analysis (AATCC 198)

A horizontal wicking experiment is proposed to measure the wicking behavior of fabric[21]. A syringe supplying a continuous flow of distilled water remains in contact with absorbing fabric, resulting in a wicking region. The increase in the wicking area or the wicking area after a certain time is recorded. Table 2 shows that the time required to absorb one drop of water decreases. It means that the water absorbency gets increased after plasma treatment.

Vertical Wicking Test Analysis (AATCC 197)

It is well known that the heat and moisture transfer properties of clothing materials are critical for analyzing, as they directly affect latent heat loss from

Table 2 — Crease recovery, stiffness and horizontal wicking test analysis

S. No.	Crease recovery, degree				Stiffness testing, cm				Horizontal wicking, s	
	Without plasma		After plasma		Without plasma		After plasma		Without plasma	After plasma
	Warp	Weft	Warp	Weft	Warp	Weft	Warp	Weft		
1	68	61	61	69	4.3	3.9	4.6	4.8	2.34	1.41
2	59	65	71	72	4.4	4	3.6	4.6	2.64	1.27
3	69	64	68	68	4.2	4.1	4.7	4.6	2.26	1.17
4	59	60	71	72	3.5	3.9	4.8	4.5	2.45	1.43
5	58	62	70	63	4.5	4.2	4.6	4.3	2.39	1.21
6	70	59	73	66	4.7	3.9	4.4	4.6	2.58	1.08
7	65	63	72	71	4.5	4	4.7	4	2.21	1.36
8	61	65	69	68	4.4	3.7	4.3	4.1	2.33	1.51
9	68	64	71	64	4.1	4	4.4	4.3	2.47	1.34
10	59	66	68	69	4.8	3.6	4.1	3.9	2.24	1.11
Mean	63.4	62.9	69.4	68.2	4.34	3.93	4.42	4.37	2.39	1.31

Table 3 — Vertical wicking test analysis

Sample	Vertical wicking height, cm							
	Without plasma				After plasma			
	30 s	60 s	90 s	120 s	30 s	60 s	90 s	120 s
1	1.9	2.8	3.2	3.7	1.8	2.8	3.3	3.8
2	2.4	3.6	4	4.3	2.3	2.8	3.3	3.7
3	3.2	3.8	4.2	5	3.7	4.3	4.9	5.2
4	2.1	2.8	3.3	3.8	3.6	4.2	4.7	5
5	2.4	3.2	3.6	3.8	3.5	4.2	4.5	4.9
6	1.8	2	2.3	2.4	2.9	3.4	4.5	4.5
7	1.9	2.6	3	3.2	1.8	2.7	3.2	3.8
8	2.8	3.5	4.2	4.4	2.8	3.9	4.4	4.9
9	1.4	1.8	2.2	2.5	3.5	4.2	4.8	5
10	2.2	2.8	3.5	3.9	3.3	4	4.6	4.9
Mean (cm)	2.21	2.99	3.35	3.7	2.92	3.65	4.22	4.57

Table 4 — Weighing test analysis

Sample No.	Weight before plasma treatment, g	Weight after plasma treatment, g	Loss in weight, g	Per cent loss in weight
1	37.35	36.80	0.55	1.47
2	35.75	35.01	0.74	2.06
3	35.57	34.80	0.77	2.16
4	36.57	35.90	0.67	1.83
5	35.78	34.69	1.09	3.04
6	37.12	36.22	0.99	2.66

the human body. The objective measurement of the moisture transfer properties of clothing is therefore important to apparel product development[22]. There are two parameters most commonly used to characterize the properties of liquid moisture management performance of fabrics, which are the rate of absorbency and the capacity. Table 3 shows that after plasma treatment water uptake increases as time increases.

3.2.4 Weighting Test Analysis (ASTM D3776)

The weighing test method covers the measurement of fabric mass per unit area (weight) and applies to most of the fabrics. There are four approved options in the measurement of fabric mass per unit area, viz. Option A, Option B, Option C, and Option D. Option A may be used for the acceptance testing commercial shipments since it has been used extensively in the trade. The second option (Option B) applies to a full-width sample cut from a full piece, roll, bolt, or cut. Unless otherwise specified, these results include selveges and are based on conditioned fabric.

Option C, on the other hand, is relevant for testing a small swatch of fabric and comparing the results to the supplied sample. Measurements by this method do not include selveges and should be reported as such allowance is specified. The last Option D is intended for use with narrow fabric, as designated by trade. In this study, Option C is used for the test. Table 4 shows that after plasma treatment the weight of the fabric gets reduced.

4 Conclusion

This study has given a new approach towards finishing of linen fabric for antimicrobial activity and to study its effect on physical properties of linen after plasma treatment. It is concluded that the plasma treatment increases the durability of the antimicrobial finish on linen fabric. The stiffness of linen fabric also increases after plasma treatment. It is also found that the crease recovery of the linen fabric significantly increases after plasma treatment. Plasma treatment is also accompanied by a remarkable improvement in the hydrophilic properties of the linen fabric. The linen fabric becomes more absorbent after the plasma treatment due to improvement in wettability. The weight of the linen fabric is reduced after plasma treatment by increasing the exposure time. Without plasma treatment, neem and pomegranate rind extract are semi-permanent antimicrobial finish, but after plasma treatment, the durability of the finish is found closer to the permanent finish.

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