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Antimicrobial Efficacy of Nanoparticles Loaded with Herbal Extracts on Polyester/Cotton Blend Fabrics in Combating Nosocomial Infection

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Abstract

A nosocomial or hospital acquired infection is an infection which a patient develops during hospitalization. Contaminated textiles in hospitals might be an important source of microbes contributing to endogenous, indirect-contact, and aerosol transmission of nosocomial-related pathogens. Textiles are an excellent substrate for bacterial and fungal growth under appropriate moisture and temperature conditions. The use of antimicrobial textiles, especially in those that are in close contact with the patients, may appreciably reduce bio burden in clinical settings and consequently reduce the risk of nosocomial infection. In view of increasing resistance to existing antimicrobial agents, herbal drugs are being looked as very important source for the discovery of new agents for treating various ailments related to bacterial infections. Nanotechnology has real commercial potential for the textile industry. This is mainly due to the fact that conventional methods used to impart different properties to fabrics often do not lead to permanent effects, and will lose their functions after laundering or wearing. Nanotechnology can provide high durability for fabrics, because nanoparticles have a large surface area-to-volume ratio and high surface energy, thus presenting better affinity for fabrics and leading to an increase in durability of the function. The study dealt with imparting antimicrobial property to polyester / cotton blend fabrics using nanoparticles loaded withcombinatorial herbal extracts and its efficacywas evaluated in a hospital environment. The results proved that the load of microorganisms was comparatively lesser than the control fabrics, this shows that these fabrics could control the transmission of nosocomial infection through fomites.

Keywords: Nosocomial Infection; Herbal Nanoparticles; Polycotton Fabrics; Antimicrobial Textiles.

Introduction

Nosocomial or hospital-acquired infection is a new infection that develops in a patient during hospitalization, nosocomial infections (NI) are estimated to occur in at least 5% of all patients hospitalized [1]. Contaminated textiles in hospitals might be an important source of microbes contributing to endogenous, indirect-contact, and aerosol transmission of nosocomial-related pathogens. Textiles are an excellent substrate for bacterial and fungal growth under appropriate moisture and temperature conditions, and it was shown that bacteria and fungi can survive for prolonged periods in hospital fabrics [2], [3], and [4]. Microbial shedding is greater in patients [5],thus bacterium, when shed into a textile fabric between the patient and the bed would readily proliferate since the moisture and temperature in the textile microenvironment would promote its proliferation [6]. Therefore, this problem could be overcome by finishing the textiles with anti-microbial agents.

The use of antimicrobial textiles, especially in those that are in close contact with the patients, may significantly reduce bio burden in clinical settings and consequently reduce the risk of NI [1]. Fortunately, antimicrobial agents on textiles may

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only reduce the density of the skin resident flora but do not completely eliminate them. But the problem with synthetic anti-microbial agents is that they would confer resistance. In view of increasing resistance to existing antimicrobial agents, herbal drugs are being looked as very important source for the discovery of new agents for treating various ailments related to bacterial infections [7]. When these plant extracts are used to finish fabrics to impart anti-microbial property, would be the need of the hour. But when the plant extracts are used as crude would get washed away easily, so coating the surface of textiles and clothing or incorporating the fibers with nanoparticles is the latest approach for the production of highly active anti-microbial textile [8].

Nanotechnology has real commercial potential for the textile industry as it can provide high durability for fabrics, because nanoparticles have a large surface area-to-volume ratio and high surface energy, thus presenting better affinity for fabrics and leading to an increase in durability of the function. In addition, a coating of nanoparticles onto the fabrics will not affect their breathability or hand feel [9].

The common hospital fabrics used are smooth 100 % cotton, 60 % cotton + 40 % polyester blend and 100 % polyester [10]. Cotton fabric which is the natural fibres contain rich source of nutrients for the multiplication of the microorganisms. On the other hand synthetic fibres are stronger than natural fibres and could replace the natural fibres, but the only disadvantage is that they do not possess much hydrophilicity. To overcome this problem, the fabrics could be pre-treated so as to remove the chemical groups that confer hydrophobicity to them. With the innovation in science and technology, surface modification of fabrics without changing the bulk properties is achievable using plasma technology.

Plasma is an ionized gas with equal density of positive and negative charges which exist over an extremely wide range of temperature and pressure. The plasma gas particles etch on the fabric surface in nano-scale so as to modify the functional properties of the fabric [11].The present study focuses on the development of antimicrobial, eco-friendly and hygienic poly cotton medical textiles to avoid the spread of nosocomial infection through fomites.

Materials and Methods

Materials

Poly cotton (40/60) blend fabric was procured from Tiruppur, India. The fabric was as such used for producing antimicrobial finished fabric. The dried leaves of *Aeglemarmelos* and flowers of *Cassia auriculata*were purchased from local herbal drug store, Coimbatore. Chitosan (95% deacetylated) was procured from India Seafood's, Kerala.

Methods

Microbial Cultures

Nine bacterial cultures *Klebsiellapneumoniae, Serratiamarcescens, Proteus vulgaris, Bacillus cereus, Pseudomonas aeruginosa Escherichia coli, Staphylococcus aureus, Acinetobacterbaumannii*and *Salmonella typhi*that were obtained from hospital were used in the study.

Antibacterial Activity

The antibacterial activity of the combinatorial plant extracts were evaluated using Agar diffusion method. The plant extracts (water, methanol and ethylacetate) were used to test its inhibitory activity against the clinical pathogens. The formation of clear zones was used to determine the antibacterial efficacy of plant extract [12]. The activities of the combinatorial extracts were compared with standard antibiotics (Tetracycline and Chloramphenicol).

Minimum Inhibitory Concentration

Minimum inhibitory concentration against nosocomial pathogens were performed to find the lowest concentration of combinatorial plant extracts that will inhibit the visible growth of a microorganism after overnight incubation. Nutrient broth was prepared, sterilized and about 100µl of it was dispensed into each well of 96 well micro titer plates. About 100µl of extract from each dilution (10^{-2} – 10^{-9}) was added to tubes containing 100µl broth and each well was inoculated with 5 µl of the test culture. And the wells were then incubated at 37°C for 18-24 hours. After incubation at 37°C for 18-24 hours, the MIC was determined in Micro plate reader ELISA - Cyber lab.

Preparation of Nanoparticles

Alginate nanoparticles were prepared by the principle involving cation induced controlled gelation of alginate [13] and [14].

Sonication was carried out to disperse the nanoparticles evenly in the liquid and to avoid the agglomeration of the nanoparticles loaded with herbal nanoparticles using Sonics vibra cell VC×500 for before characterizing.

Characterization of the nanoparticles loaded with combinatorial herbal extracts.

The nanoparticles loaded with combinatorial herbal extracts were analyzed for their physical and chemical characteristics using High Resolution Transmission ElectronMicroscopy (HR-TEM) and Fourier Transmission Infrared (FT-IR) spectroscopic analysis.

High Resolution Transmission Electron Microscopy (HR-TEM)

In the current study, nanoparticles loaded with herbal extract were analyzed for its size and morphology using HR-TEM analysis. A small amount of the liquid sample was re-dispersed by sonication and was placed on copper grid and dried. The samples were viewed at high magnification using TEM JEOL-JEM 2100.

Fourier Transmission Infrared (FT-IR) Spectroscopy

IR-spectra were obtained using a Bruker tensor 27, Germany. The crude plant extract and nanoparticle loaded with herbal extract samples were assessed by FT-IR for the presence of their chemical groups. Samples were recorded from 4000 – 400 as scanning range between wave number (cm^{1}) and % Transmittance. All samples were run in triplicate and the data presented are the average of the three measurements.

Enhancement of Hydrophilicity of the Fabric

Plasma treatment was carried out in a 12" DC plasma chamber. The samples of size (40×40 cm) were placed on the lower electrode. The chamber was initially evacuated to a base pressure of 10^{-3} mbar using a rotary pump (Hindhivac, India). Air was used as working gas and the treatment was carried out at optimized conditionssuch as an exposure time for 6mins; electrode distance of 6cm; pressure at 0.06mbar and current of 0.8mA.

Assessment of Hydrophilicity-Wicking Test (AATCC 79)

The fabric strip (2cm × 17 cm) was suspended above the distilled water surface in a glass beaker such that the horizontal bottom edge touches the surface of the water. A spontaneous wicking was observed due to capillary force. The height of the liquid rise boundary was recorded for every 5 seconds for up to 60 seconds for two trials.

Antimicrobial Finishing of the Fabrics

Padding is the most common finishing method for the application of the formulation to the textile materials in continuous process. For about 1 g of the fabric 20 ml of the nanoparticles solution / plant extracts and about 1.6 g of citric acid was used as binder, the fabric was kept immersed in the treatment solution for 20 minutes. The fabric was then passed through a padding mangle (RB Electronic and Engineering, Mumbai), at a running speed of 15m/ min with a pressure of 2 kgf/cm^2 to remove excess solution. A 100% wet pick-up was maintained for all of the treatments. Poly cotton fabrics were padded with both crude plant extracts and synthesized nanoparticles.

Antimicrobial Assessment of Finished Fabrics

- *Qualitative assessment of antimicrobial activity (AATCC 147)*

The anti-microbial efficacy of the fabric was assessed usingparallel streak test [15]. One loop full of the test culture was loaded and transferred to the surfaceof the agar plate by making five parallel inoculum streaks coveringthe central area of the petridish without refilling the loop. The testspecimen was gently pressed transversely, across the five inoculums of streaks to ensure intimate contact with the agar surface. The plates were incubated at $37^{0}C$ for 18–24h; a clear area of interrupted growth along the sides of sample indicated the anti-bacterialactivity of fabric. The average width of the zone of inhibition at thesides of the test specimen was calculated in mm using the formula:

Zone of inhibition (mm) = $\frac{T - I}{2}$

where, T is the width of clear zone with the specimen and I is thewidth of test specimen.

- *Quantitative assessment of anti-microbial activity (AATCC 100)*

The anti-microbial activity of the fabric samples was ascertained quantitatively by AATCC 100 method [15]. 1.0 ml of the test inoculawere loaded on the swatches (treated and untreated) of 4.8 ± 0.1 cm diameter. They were then transferred to the sterile AATCC bacteriostasis broth. After an incubation of 24 h, up to 10-7 serial dilutions were made for all the samples. 0.1 ml sample from each dilution were spread plated onto the sterile AATCC bacteriostasis agar plates and incubated at 37°C for 24 h. The percentage reduction of bacteria by the treatment was then calculated using the following formula:

$$(B - A) \times \frac{100}{B} = R$$

where R is the % reduction and A and B are the number of bacteria recovered from the inoculated treated and untreated swatch respectively.

Assessment of Efficacy of the Finished Fabrics in Hospitals

The end use performance of fabric samples were evaluated using the testing procedure of Edna *et al.*, 2006. The fabric samples that were collected from hospitals in sealed bags were tested for the percentage microbial load. Sterile nutrient agar plates were prepared. The fabric samples that were used in field trials were cut to size 5×5 cm and placed on the agar surface. After inoculation of the fabrics microorganisms on the agar surface for five minutes, the fabrics were removed and the plates were kept for incubation at 37° C for 24 hours. The number of colony forming units CFU was counted when growth occurred. The same procedure was repeated for treated and untreated samples. The numbers of bacterial colonies grown on the treated and untreated samples were used to calculate the percentage in reduction of bacterial load in the nanoparticles treated fabrics.

Results and Discussion

Antimicrobial Activity of the Combinatorial Plant Extracts against Nosocomial Pathogens

The collected herbs were subjected to three different solvents such as water, methanol and ethyl acetate. The filtered plant extracts were dried and diluted with Dimethylsulfoxide (DMSO) in the concentration of one gram in ten mililiter (1g / 10 ml) and used for further studies. The extent of zone of inhibition represent the antimicrobial activity of each solvent extracts. The methanolicextracts of both the herbs used for the study showed a good bactericidal activity but was not much effective against all the test organisms. So the plants extracts were combined to show a better activity and used for the further study. The results also showed that the combinatorial extracts showed greater activity than individual extracts (Table 1). The zones of inhibition of extracts were very close and identical in magnitude and are comparable with that of standard antibiotic used for the study.

Jyothi and Rao(2010) [16] investigated the preliminary screening of antibacterial activity and the results showed that the extracts from *A. marmelos* possess good antibacterial activity. Maneemegalai and Naveen (2010) [17]concluded that the presence of phytochemicals observed in *C. auriculata*made it a potent antibacterial agent and could be used for medicinal purposes. Conferring the potentials of these plants and their uses in folk-lore medicine for treating various infections could rationalize the use of combinatorial plant extract in this study.

Synthesis and Characterization of Nanoparticles Loaded with Herbal Extracts

The combinatorial herbal extracts that showed an optimal anti-microbial activity was loaded inside the chitosan –alginate polymers using cation induced gelation. The combinatorial herbal extract loaded nanoparticles were synthesized and analyzed for their physical and chemical characteristics.

Physical Characterization

Dynamic Light Scattering (DLS)

The average size of the combinatorial herbal extract loaded nanoparticle was found to be 240.6 nm (Figure 1). The report also showed that there were particles in the range around 60nm. It is notable that the hydrodynamic diameter of the particles measured by light scattering is higher than the size estimated from microscopy particularly because of high swelling capacity of chitosan-alginate polymers that were used for nanoparticle synthesis. Hence, the actual diameter of these particles can be assumed to be significantly smaller than this.

PDI is a measure of homogeneity in dispersed systems and ranges from 0 to 1. Homogeneous dispersion has PDI value close to zero while PDI values greater than 0.3 suggest high heterogeneity [18]. The PDI of the nanoparticles loaded with combinatorial extract was 0.156 which indicated homogenous dispersion of nanoparticles in the solvent.

The significance of zeta potential is that its value can be related to the stability of colloidal dispersions. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. Zeta potential value of the synthesized nanoparticles was found to be– 23mV (milli volt) that implied that the particles were stable (Figure 2).

High Resolution- Transmission Electron Microscopy (HR-TEM)

Electron microscopy analysis confirmed the presence of nanoparticles and provided morphological information of nanoparticles loaded with herbal extracts. The HR-TEM analysis of the synthesized nanoparticles loaded with herbal extracts revealed that the particles were spherical, partially smooth and were in size range of 40-70nm and the particles were evenly dispersed (Figure 3).

Chemical Characterization of Synthesized Nanoparticle

The spectra of the combinatorial crude extracts and the nanoparticles loaded with herbal extracts are represented in the Figure 4. The FT-IR spectra of the combinatorial herbal extract and nanoparticles loaded with herbal extract reveal the presence of similar groups (Table 2).

Effect of DC air Plasma Treatment on Hydrophilicity of Polycotton Fabric

It is observed from the Figure-5, that the wicking height increased when treated under optimized conditions, which attributes to the increase in hydrophilicity of the fabric. The optimized conditions that were used in this study to enhance the hydrophilicity were pressure of 0.06 mbar at constant inter-electrode distance of 6cm, current at 0.8mA and exposure time of 6 min. This rise in hydrophilicity would have been attributed to the interaction of the energetic plasma species (ion bombardment) with the fabric surface. Moreover, the inter-electrode distance of 6 cm would have favored the extension of plasma from the cathode to the anode contributing to enhanced interaction;this in turn would have led to more etching of the fabric surface.

Testing of Hydrophilicity of the Treated Fabrics

Wicking Test

The changes in the hydrophilic properties of the fabrics treated with plasma and without plasma were studied using the standard wicking test.The hydrophilicity of the treated poly cotton fabric was around 4.8cm, whereas the untreated showed wicking of around 3.4 cm at 60 seconds. During the plasma treatment surface modification has brought about loss of certain molecules leaving the bonds free, which paved way for the addition of water molecules. Wong *et al.,*(2000) [19]has also observed that plasma pre-treatment enhanced the hydrophilicity.

Antimicrobial Finishing of the Fabrics

The plasma treated and untreated fabrics were padded with the synthesized nanoparticles loaded with combinatorial herbal extract and the crude combinatorial herbal extract.

Qualitative Assessment of Antimicrobial Activity (AATCC 147)

Antimicrobial efficacy of the fabric was found to increase when the plasma pre-treated fabrics were treated with combinatorial herbal extracts and nanoparticles loaded with herbal extracts. The antimicrobial efficiency was calculated from the zone of inhibition. The zone of inhibition obtained in the different culture plate were observed and tabulated in Table 3.

A maximum inhibition of around 37 mm against *S.aureus*was observed in the plasma pre-treated fabrics treated with synthesized nanoparticles followed by *S.marscenes* (32 mm). It was found that the fabrics pre-treated with plasma and finished with nanoparticles loaded with herbal extracts showed a very effective inhibition pattern (Figure 7). This could be attributed to the increase in hydrophilicity which in turn increases the absorbency of the nanoparticles loaded with herbal extracts; the minimum zone was obtained against *E. coli* which was around 10 mm.

Quantitative Bacterial Reduction of Antibacterial Activity (AATCC 100)

The hydrophilicity of the fabric pre-treated with plasma and finished with nanoparticles loaded with herbal extracts were found to be maximum among the other treatments. As a result of etching of the fabric the nanoparticles loaded with herbal extract uptake by the fabric would have also increased. The increase in pore radius of the same sample would have increased the retention of extract by the fabric. These might be the reasons for the increased antibacterial activity of the fabric treated with plasma. Results were similar to that obtained in qualitative assessment, 100% reduction was observed in the case of *S. aureus*and *S. marscenes*followed by *B. cereus* that showed 91% reduction (Table 3). Vukušiæ*et al,*(2011) [20] also studied the antibacterial treatment of cotton textiles modified with citric acid as efficient anti-bacterial agent for prevention of nosocomial infections their results proved to be a viable and the fabrics demonstrated excellent antibacterial activity values after the exposure to the both *S. aureus* strains and *P. aeruginosa* strains.

Wash Durability Test

The fabric samples pre-treated with plasma were finished with the combinatorial herbal extract and nanoparticles loaded with herbal extracts and subjected to laundering procedure. The fabrics were then tested for the percentage reduction of bacterial growth after every 5 wash cycles. It was seen that the fabrics pre-treated with plasma and finished with nanoparticles loaded with herbal extracts, had higher antibacterial activity against *E.coli* and *S.aureus,* when compared to control fabric (Table 4).

The increase in the wash durability of the plasma treated fabric attributed to the increase in average pore radius of the fabric which in turn increased the nanoparticlesretention capabilities of the fabric. The results obtained were correlated with Wong *et al*., (2006)[9], and Vaideki*et al.,* (2009) [21] who also discussed that plasma treatment enhanced the retention rate of the fabric.

The fabric pre-treated with plasma and finished with nanoparticle showed bactericidal activity against the test bacteria until 25 washes effectively due to the sustained release of the nanoparticles. The treated fabrics coated with crude plant extracts did not show much activity, this is due to the fact that the extracts does not possess sustained release of the antimicrobial compound like that of nanoparticles. However, the meager reduction of bacteria in the initial laundering cycles of the fabric treated with the bulk extract could be attributed to the presence of the binding agent (Citric acid) in the fabrics. Rajendran *et al.,* (2012) [22] also have reported that the higher laundering durability of the nanoparticles was due to the smaller particle size, uniform coating and controlled release of the nanoparticles.

Assessment of Efficacy of the Finished Fabrics in Hospitals

Poly cotton fabrics finished with nanoparticles loaded with herbal extracts and control fabrics without any treatment were assessed for the efficacy by subjecting them to hospital environment for 5 days. The number of organisms on each plate was counted

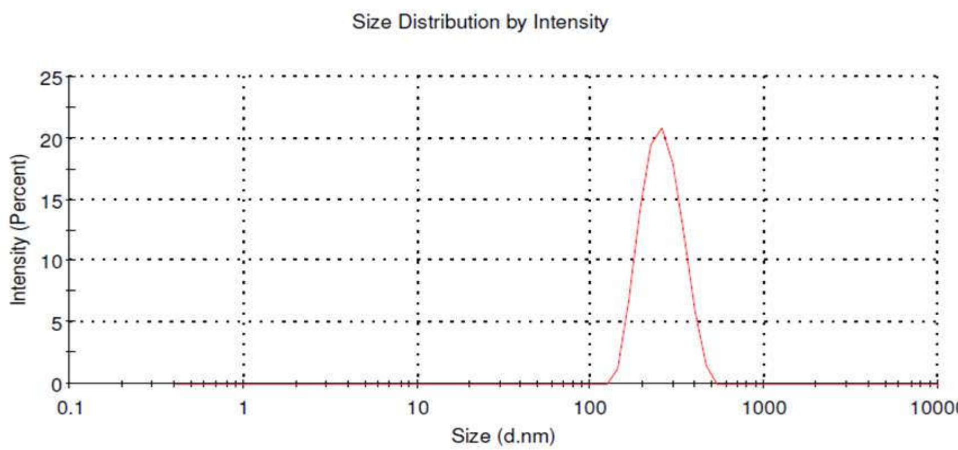

Fig. 1: Size of the nanoparticle loaded with herbal extracts

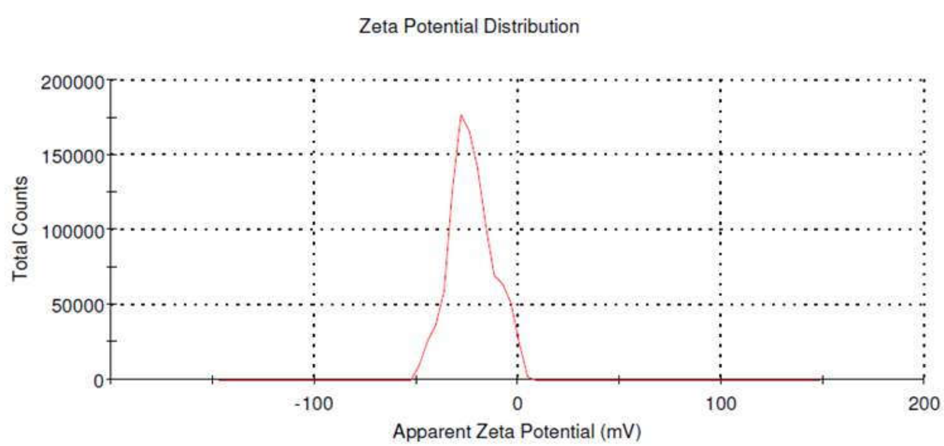

Fig. 2: Zeta potential of the nanoparticles loaded with herbal extracts

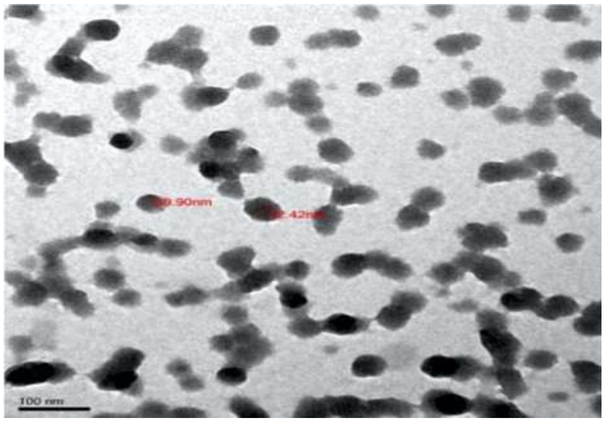

Fig. 3: HR-TEM Micrograph of Synthesized nanoparticles loaded with herbal extracts

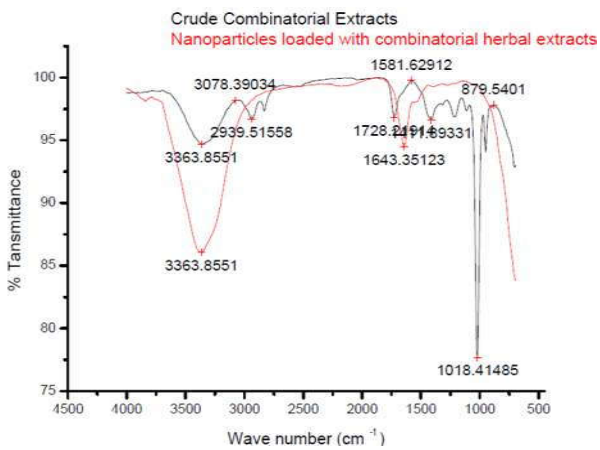

Fig. 4: FT-IR spectra of the combinatorial extract and nanoparticle loaded with herbal extracts

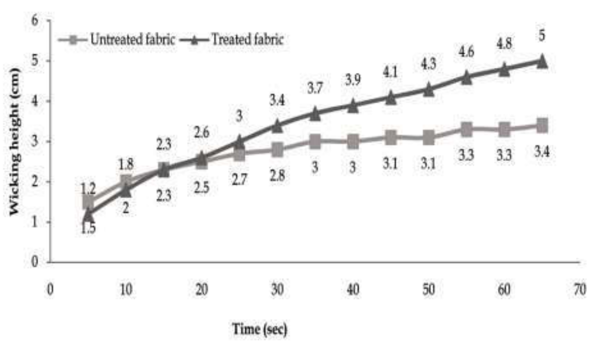

Fig. 5: Wicking height of untreated and plasma treated polycotton fabric

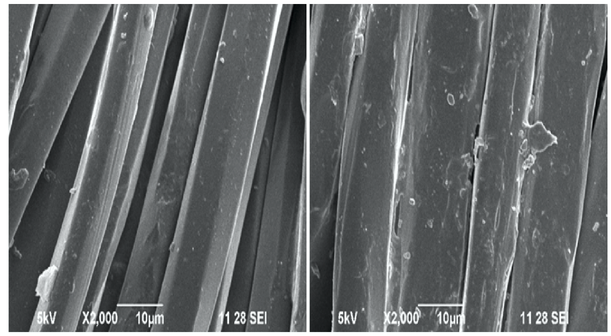

Fig. 6: SEM images of untreated and treated polycotton fabric

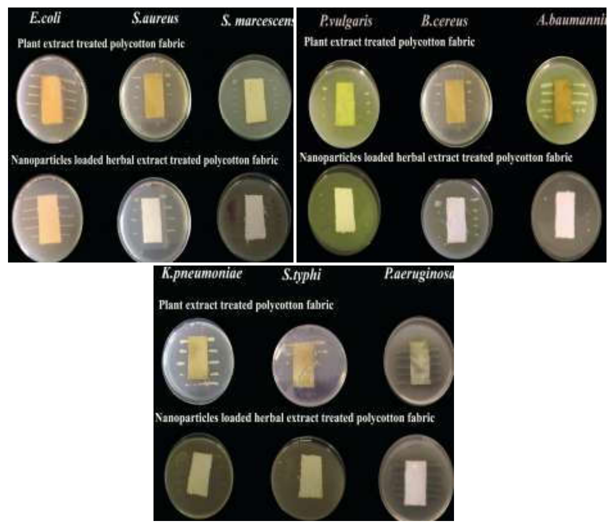

Fig. 7: Qualitative assessment of the plasma pre-treated fabrics using crude plant extracts and nanoparticles loaded herbal extracts

Fig. 8: Assessment of efficacy of the finished fabrics

Table 1: Anti-bacterial activity of the combinatorial herbal extracts

Test methods	Test organisms E. coli	K. pneumonia	P. aeruginosa	S. aureus	A. baumannii	S. marcescens	B. cereus	S.typhi	P. vulgaris
ZoI* (mm) MIC** (µg/ml)	8 70 µg/ml	7 60 µg/ml	7.5 80 µg/ml	10 50 µg/ml	9 60 µg/ml	10 50 µg/ml	9 80 µg/ml	6 60 µg/ml	12 70 µg/ml

*ZoI - Zone of Inhibition

**MIC – Minimal Inhibitory concentration

Table 2: Peak Characteristics of the combinatorial extracts and Nanoparticles loaded with herbal extracts

Literature cm-1	Combinatorial herbal extracts cm-1	Nanoparticles loaded with herbal nanoparticles cm-1	Peak Characteristics
900–675	879.54	-	C–H "oop"
1320–1000	1018.4	-	C–O stretch
1680–1640	-	1643.35	–C=C– stretch
3100–3000	3078.3	-	C–H stretch
3500–3200	3363.85	3363.85	O–H stretch and H–bonded

Table 3: Antimicrobial assessment of the plasma pre-treated finished fabrics

Test Methods	Fabric treatment	Test organisms and their inhibition pattern using standard methods								
		E. coli	K. pneumoniae	P. aeruginosa	S. aureus	A. baumannii	S. marcescens	B. cereus	S.typhi	P. vulgaris
AATCC 147	Crude extract	7	-	6	17	9	15	16	9	8
(mm)	Nanoparticles	11	19	14	37	18	32	21	14	14
AATCC100	Crude	63%	61%	61%	82%	62%	80%	66%	68%	56%
(%)	Nanoparticles	76%	87%	80%	100%	85%	100%	91%	78%	88%

Table 4: Wash durability analysis of the treated fabrics

S. No	Fabric treatment	No. of laundering cycles	Antibacterial activity (bacterial reduction %)			
1	Combinatorial herbal extract		*E. coli*	*P. aeruginosa*	*B. cereus*	*S. aureus*
	treated	5	54	57	52	58
	Fabrics	10	23	28	21	20
		15	12	18	-	-
		20	-	-	-	-
		25	-	-	-	-
		30	-	-	-	-
2	Nanoparticles loaded	5	72	80	87	81
	with combinatorial herbal extract	10	51	76	63	62
	treated	15	20	57	42	45
	fabrics	20	9	34	24	21
		25	-	5	7	8
		30	-	-	-	-

that revealed the number of colonies on the treated fabric were very less when compared to that of the untreated fabric. The results showed almost 95% reduction of the organisms in treated polycottonfabrics (Figure 8).

Conclusion

The reason behind the usage of semi - synthetic fabric in this study is that these fabrics would possess strength as that of synthetic fabric and hydrophilicity as that of natural fabric. To further enhance the hydrophilicity of the fabric and thereby improve its antimicrobial nature, treatment of these fabrics with plasma would play a vital role without changing the bulk properties of these fabrics. Therefore, the study concludes that when the synthetic fabric are pre-treated with plasma, could turn to be hydrophilic and these fabrics when finished with anti-microbial agents would impart anti-microbial ability to the hospital used fabric, which in turn would reduce the load of microorganisms on fabrics which could be a pose a great risk of nosocomial infection, which could be a burden to immunocompromised who are hospitalized.

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