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Antimicrobial Treatment on Cellulosic Fibre using Agro Waste (Lemon Peel)

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ABSTRACT

Concern in environment-friendly textile processing techniques has been augmenting in recent years. Natural products are environment friendly, low toxic and less allergenic owing to the presence of large number of structurally diverse active compounds which makes natural agents as promising options for the development of antimicrobial protective textiles. The purpose of the present study was to investigate the effect of lemon peel treatment on microbial resistant property of cotton fabric. It was found that the cotton fabric treated with lemon peel extract showed 97.89, 98 and 97.16 percent reduction in the growth of *P. aeruginosa*, *E. coli* and *X. campestris* bacteria, 93.49 and 95.83 percent reduction in the growth of *B. subtilis* and *S. aureus* bacteria whereas treated fabric showed 94.99, 90.41 and 93.44 percent reduction against *A. clavatus*, *A. flavus* and *A. awamori* fungal strains, respectively. Lemon peel extract treated cotton fabric was further characterized by FESEM and FTIR spectra to study the changes occurred in the fibre structure after treatment.

1. INTRODUCTION

The natural products in textile finishing are gaining significant momentum these days [40]. Currently, functional finishes on textile fabrics are of critical importance to improve textile products with multifunctional properties. In recent years, antimicrobial finishing to textile has become extremely important in the production of technical textile products [57]. Antimicrobial textiles with improved functionality finds a variety of applications such as health and hygiene products, specially the garments worn close to the skin and several medical applications, such as infection control and barrier material [41]. The antimicrobial finish is one of the special finishes which can be applied to the textile material to protect the skin of the wearer and the textile substrate itself [24]. There is a vast resource of natural antimicrobial agents which can be used for imparting antimicrobial property to textile substrates [35], [4], [55]. The protective abilities of the textile materials are considerably influenced by many factors such as structure of molecules of antimicrobial agents, presence of absorptive groups, phytochemicals present in the plant material and other additives used for finishing of textiles [7], [62], [26]. Natural plants have their own self

defense mechanism and protect themselves from microbes due to the presence of substances known as phytochemicals [73]. The plant extract containing active substances can be used effectively to make the textiles microbial resistance. Moreover, extract from plant source with active substances are eco- friendly, non- toxic and non- allergic.

The search for new therapeutically active compounds has spurred researchers over the years to explore natural compounds [2], [1], [5], [10], [12], [13], [68], [42], [43]. In particular plant wastes express interesting sources of biologically active molecules [6], [11], [14], [15], [22], [49], [61]. Specifically, secondary metabolites from plants indicate valuable bioactive ingredients [16], [17], [20] with remarkable antibacterial properties [44], utile in the antimicrobial treatment of the textile material.

The phytochemicals are divided into primary and secondary metabolite. Primary metabolites are the compounds involved in the metabolic pathway, which are common to all living organisms [18]. On the other hand, secondary metabolites functions as defense and signal compounds that is necessary for the plant's survival and reproductive ability [72]. The effect of various plant extracts such as neem, aloevera, onion, orange, pomegranate etc. on bacteria and fungi has been reported [25].

Today, cotton textiles represent more than half of the global textile market and the demand is expected to continue [9]. This dominance of cotton fibre is mainly because of its natural comfort, appearance and excellent performance properties such as alkali resistance, hygroscopicity and moisture retention [28]. However cotton is more susceptible to microbial attack because of its hydrophilic nature [21], [27] therefore, is good carrier for various type of microorganisms and can cause health related problems to the wearer [53]. If the finishing of cotton is done using the natural products, it will provide the protection against microbes without any harmful effect on environment because natural agents are safe, non-toxic and biodegradable in nature.

With the world becoming more conscious towards ecology and environment, there is urgent need today to use the natural products for antimicrobial finishing of textiles. Although there are many natural sources that are rich in antimicrobial agents, the study on their use in textiles is very limited. The major challenges in application of natural sources for textile application are that majority of the sources are complex mixtures of several compounds and also the composition varies in different species of the same plant. Plant and fruit products are more reliable since there in bioactive compounds results in combating various microbes. Among various fruits citrus are widely used in almost all countries. Peel waste are being highly perishable and seasonal, is a serious problem to the processing industries and pollution monitoring agencies. A large number of researchers have focused on the study of peel of Citrus fruits and their antimicrobial activities [65], [19], [30], [36], [38], [51], [37]. The citrus fruit peel are rich in nutrients and phyto-chemicals. These can be resourcefully used as antimicrobial agents as they are not much costly, eco-friendly and non- toxic in nature. Moreover, remarkable climatic changes and increasing pollutants in the environment also necessitate the herbal antimicrobial treated clothes to protect the human body and keep the sound health. Hence, there is a huge demand for non- toxic and eco-friendly finish which not only improves the intrinsic functionality of the fabric but also a production process that is as environment friendly as possible. The antimicrobial property of lemon peel treated fabric was assessed and it was found that lemon peel treated cotton fabric showed excellent protection against microbial growth. Thus, use of lemon peel extract as antimicrobial finish will be beneficial for textile and also for the pollution monitoring agencies.

2. Materials and Methods

2.1 Preparation of Cotton Fabric

To ensure complete wetting of cotton fabric and uniform absorbency of the extracts during the padding, it must undergo preparatory processes. Desizing and scouring treatments were given to the woven cotton fabric to remove starch and other vegetative impurities before finishing process.

2.1.1 Desizing of the fabric

The fabric was weighed and pre wetted prior to introducing into the desizing bath. The fabric was squeezed thoroughly and was treated in a bath containing 1 percent sulphuric acid (H_2SO_4) on the weight of fabric with MLR 1:30. It was kept at room temperature for 60 minutes with occasional stirring. After required time, the fabric was taken out, washed thoroughly with water until free from acid and any residue and dried [32].

2.1.2 Scouring of the fabric

The desized fabric was initially weighed and soaked in water. It was then entered into the scouring bath containing required amounts of sodium carbonate (2.0%), sodium hydroxide (5.0%) and wetting agent (0.5%) on the weight of the fabric with MLR 1:20. The temperature of the bath was gradually raised to 90^0 C and fabric was treated for 60 minutes. The scouring was continued for 1 hour with occasional stirring. The scoured fabric was then washed with water, neutralized with weak acetic acid solution and dried at ambient temperature [64].

2.2 Preparation of lemon peel extract for application on cotton fabric

The collected lemon peels were washed, cut into small pieces and allowed to dry in shade to avoid breakdown of important compounds. After being completely dried, the material was crushed into small pieces, pulverized into coarse powder and stored in an air tight container.

2.3 Optimization of extraction parameters

The extraction process of plant materials depends on certain important parameters, i.e., concentration of extract and time. These parameters help in qualitative determination of the size, shape and yield. Here in this study, we have optimized these parameters for the maximum yield of extract.

Firstly, three concentrations of lemon peel powder i.e., 50, 100 and 150 g, keeping other variables constant were dissolved in MLR 1:20 and kept on shaking incubator for 12 hours and then sieved through muslin cloth, transferred to round bottom flask and was subjected to rotavapour. After that, the obtained dried extract was weighted and yield percentage was calculated.

Similarly, for extraction time optimization, one hundred fifty grams of lemon peel powder was mixed with 1000 ml of water and subjected to maceration method for different time periods viz., 12, 24 and 36 hours. The time at which maximum extract yield was observed, was selected as extraction time. The obtained dried extract was weighted and yield percent was calculated by using following Eq. (1):

$$\%\,Yield = \frac{Actual\ mass\ obtained}{Calculated\ mass} \times 100 \qquad (1)$$

The lemon peel powder (150 gm) was extracted with 1000 ml of distilled water using rotary evaporator for 2 to 5 hours at boil. The extract was filtered and then concentrated to dryness and kept at 4ºC before use.

2.4 Qualitative analysis of phytochemicals

Plant sample may contain some significant phytochemicals and these can be detected by using solvent and

extraction methods. Through method described by [50], phytochemical analysis of the lemon peel extract was conducted for screening the presence of tannins, flavoniods and alkaloids. The occurrence of phenolic compounds; tannin was done by using the ferric chloride test. The presence of alkaloids in the lemon peel extract was evaluated by Mayer's test. The existence of flavoniods was assessed through the alkaline reagent test. The presence of streroid, saponins andterpenoids was demonstrated through a method described by [66], [67] in which the occurrence of steroids was analyzed by using Salkowki's test. Form test was used to test saponins. The presence of glycosides was analyzed by legal test. The presence of proteins and carbohydrates were investigated by following the standard biochemical methods of [8]. The existence of proteins was evaluated through the Ninhydrin test and iodine test was used to test carbohydrates.

2.5 Application of lemon peel extract on cotton fabric

Cotton fabric sample was treated with lemon peel extract. On the basis of weight of sample, quantity of pure extract of peel was calculated. Lemon peel extract in concentration of 5g/l was set in a bath. The material to liquor ratio was taken as 1:20. The sample was impregnated into the antimicrobial bath with pH 5.5 adjusted with acetic acid. The bath temperature was raised to 700C and it was kept at this temperature for 30 minutes. A post treatment was given with citric acid (8% on the weight of fabric) as fixing agent at room temperature (fig. 6). Finally, samples were washed with cold water and air dried [58].

2.6 Quantitative antimicrobial activity of lemon peel treated cotton fabric

Total colony forming units of controlled and treated fabric were determined to know the microbial load by using standard quantitative test method (AATCC-100 Test method). The sterilized fabric sample swatches of 2″ × 2″ were taken for determination of microbial population. The microbial resistance of the controlled and treated cotton fabric samples was quantitatively tested against Gram negative bacteria (*P. aeruginosa, E. coli* and *X. campestris*), Gram positive bacteria (*B. subtilis* and *S. aureus*) and Fungal strains (*A. clavatus, A. flavus* and *A. awamori*).50 ml broth (Nutrient broth for bacterial culture and Malt Extract broth for fungal culture) was poured in sterile 500 ml conical flasks under Laminar Air Flow. The untreated fabric samples i.e. control, the lemon peel extract treated and washed samples were aseptically transferred into separate conical flasks with media. Each flask was inoculated with the microbial concentration of 10^5CFU/ml using a pipette. These were incubated at 37^0C for 24 hours in a shaker incubator at 121 rpm. The colonies of microorganisms were counted manually and total colony forming units were calculated using following Eq. (2):

$$\text{Colony Foming Units (CFU/ml)} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Volume of culture plate}} \quad (2)$$

2.7 Characterization of lemon peel extract finished cotton fabric

2.7.1 Surface morphology

The surface morphology of cotton fabric treated with lemon peel extract, as well as the untreated control fabric, was captured using HR FESEM (High resolution field emission scanning electron microscope) SIGMA VP. at different magnifications according to test standard ISO17751:2007. It is working at an accelerating voltage of 30 kV. FESEM analysis of controlled and treated samples was got done from *Centre for Nanoscience and Nanotechnology*, JMI, Delhi.

2.7.2 FTIR attenuated total reflectance (ATR) spectroscopy

FTIR ATR spectroscopy was done to study the changes occurred on the surface of the fabric. The surface of controlled and lemon peel extract treated cotton fabric was measured by using Bruker Optikin the frequency

range of 4000- 400cm^{-1}. The resultant absorption spectrum from the bond natural vibration frequencies indicates the presence of various chemical bonds and functional groups present in the sample. FTIR analysis of controlled and treated samples was got done from CSWRI, Avikanagar, Rajasthan.

2.8 Retention of antimicrobial property of lemon peel extract treated fabric after washing

The treated sample was subjected to different washing cycles to study its effect on functional properties of the sample. It was analyzed by laundering all the samples in Laundrometer by using IS: 3361-979 test standard method, after 1, 5 and 10 washing cycles. For wash ability testing the solution was prepared using 5 g of soap and 2 g of sodium carbonate per liter. The bath was filled with water up to the overflow level (little below the axis of the rotor). Each jar was filled with necessary amount of soap solution with 1:20 M:L ratio. The jars were fitted with lids and fixed in the rotor at their respective positions and the nuts were locked tightly setting temperature at 300C for 30 minutes. The equipment was allowed to run for the set time to enable the test liquid to attain the test temperature. The jars were opened and the specimens were placed in each jar containing test liquid. After the completion of the washing cycle, all the jars were opened and specimens were removed from each jar. The specimens were rinsed twice in cold water, dried in the air and subjected to functional testing. The results were enumerated as percentage reduction in the microbial count of the treated washed fabric in comparison to the microbial count of treated fabric and were calculated by using following Eq. (3).

$$\text{Reduction in microbial count (\%)} = \frac{\frac{\text{CFU}}{\text{ml}}\text{of treated fabric} - \frac{\text{CFU}}{\text{ml}}\text{of treated washed fabric}}{\text{CFU/ml of treated fabric}} \times 100 \quad (3)$$

3. Results and Discussion

3.1 Optimization of the concentration of lemon peel powder

The obtained results of the yield percentage by maceration process of lemon peel at different concentrations showed that 1.50 g yield of extract was obtained with 50 g/l, powder, 6.0g with 100 g/l and 12.90 g with 150 g/l powder (table 1). The time period taken was 12 hours. Thus, the yield percentage of lemon peel extract was found to be highest with 8.6 % at concentration of 150 g/l. The yield percentage increased with an increase in the weight of dry powder of lemon peel i.e. 3 percent for 50 g/l, 6 percent for 100 g/l and 8.6 percent for 150 g/l. It was concluded that the optimum concentration for maximum yield of lemon peel extract was obtained with 150 g/l and therefore, selected as optimum concentration for the extraction of lemon peel extract. The reason behind increased yield percentage with the increase in concentrations may be because more phytochemical are extracted. The results are in alignment with finding of [48] who reported increase in mass yield percent of peach leaves extract with increased length of extraction period.

Table 1: Optimization of the concentration of lemon peel powder

Plant source	**Weight of the air dry powder(g)**	**Time period (hrs.)**	**Weight of the dry extract (g)**	**Yield percentage (%)**
Lemon peel	50	12	1.50	3
	100	12	6.00	6
	150	12	12.90	8.6

3.2 Optimization of extraction time

From the yield data obtained by maceration process of lemon peel at different time periods and optimized concentration i.e. 150g/l, it was found that the yield obtained for 12 hours was 12.90 g, 24 hours was 21.00g

and 36 hours was 29.40g (table 2). The yield percentage increased with an increase in the time period i.e. 8.6 percent for 12 hours, 14 percent for 24 hours and 19.6 percent for 36 hours. Thus, the yield percentage of lemon peel extract was found to be highest with 19.60 % at extraction time of 36 hours. Results are in line with the findings of [23] who reported increase in yield percent of *S. cumini* (L.) extract with increasing length of extraction period when extracted with soxhlet extraction process.

Table 2: Optimization of extraction time

Plant source	Time period (hrs.)	Weight of the air dry powder(g)	Weight of the dry extract (g)	Yield percentage (%)
Lemon peel	12	150	12.90	8.6
	24	150	21.00	14
	36	150	29.40	19.6

3.3 Qualitative analysis of phytochemicals

Phytochemical composition of the lemon peel revealed the presence of tannins, phenol, flavoniods, steriods, saponins, terpenoids, proteins and carbohydrates (Table 3 & fig. 1). Lemon peels contains a high concentration of tannins and represent a rich source of natural flavonoids, saponins and terpenoids. The findings of the study are found in line with the results of study carried out by [3] who found that the phytochemical analysis with the methanolic extracts of the dried fruit of *Citrus limon* plant showed the presence of saponin, sterols, steroids, terpenoids, protein and amino acid, tannins and carbohydrate. It has been reported that these compounds exert antimicrobial effects against pathogens due to the high contents of terpenoids, tannins, quinones, phenolic acids and polyphenols [47]. Phytochemical analysis of citrus peel extract has been reported with similar phytoconstitutes [45], [31].

Table 3. Phytochemical analysis of the lemon peel extract

Phytochemical Tests	Phytochemical compounds	Results
Gelatin Test	Tannins	+ + +
Ferric Chloride Test	Phenol	+
Alkaline Reagent Test	Flavoniods	+ +
Mayer's Test	Alkaloids	-
Salkowki's Test	Steriods	+
Form Test	Saponins	+ +
Terpenoids Test	Terpenoids	+ +
Legal Test	Glycosides	-
Ninhydrin Test	Proteins	+
Iodine Test	Carbohydrates	+

(+) slightly detected, (+ +) moderately detected, (+ + +) highly detected (-) not detected

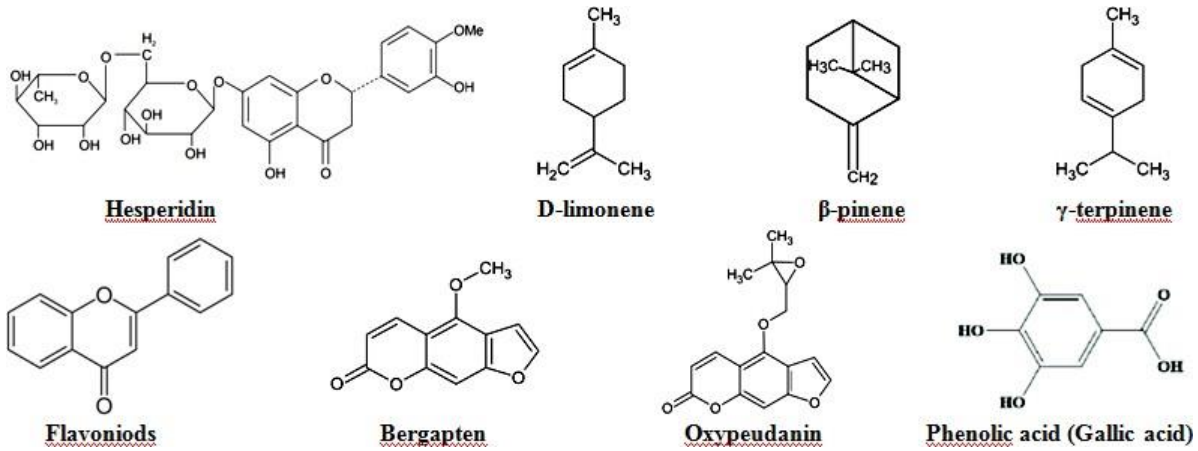


Fig. 1. Phytochemicals composition of the lemon peel

3.4 Determination of microbial resistance of cotton fabric treated with lemon peel extract

The quantitative assessment data regarding microbial resistance of the cotton fabric after treatment with lemon peel extract against the growth of the Gram negative (*P. aeruginosa, E. coli* and *X. campestris*), Gram-positive (*B. subtilis* and *S. aureus*) bacteria and Fungal strains (*A. clavatus, A. flavus* and *A. awamori*) on subsequent hours of inoculation has been presented in table 4 and fig. 2. The microbial resistance was determined by comparing the microbial colony counts and percent reduction between treated sample and controlled sample i.e. untreated desized and scoured sample. The sample having lesser microbial count (CFU) and higher percent reduction was analyzed to be more resistant towards the growth of microbial strains. Confluent lawn growth was observed on controlled sample.

After subsequent hours of inoculation there was 97.89, 98and 97.16% bacterial reduction with 5x107, $4x10^7$ and $5.8x10^7$ CFUs, respectively for *P. aeruginosa, E. coli* and *X. campestris* at 10^7 and 10^8 mean dilution factors on cotton fabric sample treated with 5g/l concentration of lemon peel extract. On the other hand, the bacterial reduction values of the same cotton fabric sample against *S. auerus* and *B. subtilis* were 93.49 and 95.83 % with $14.9x10^7$ and $8.4x10^7$ CFUs, respectively.

The percent microbial reduction of the treated cotton fabric sample against *A. clavatus, A. flavus* and *A. awamori* were 94.99, 90.41 and 93.44% with $11.5x10^7$, $22.7x10^7$ and $16x10^7$ CFUs, respectively (Fig. 2). The antimicrobial activity possessed by the extract was may be due to the reason that the extract get attached to the substrate through bond formation on the surface and also due to the presence of several phytochemicals [29]. The attached extract disturbs the cell membrane of the microbes through the physical and ionic phenomena [52].

The antimicrobial activity of treated cotton fabric may be attributed to the presence of number of secondary metabolites such as essential oils, protopine and corydaline alkaloids, lactone, polyacetylene, acyclic sesquiterpenes, hypericin and pseudohypericin compounds, flavonoid glycosides, coumarins, ß and γsitosterol, glycosides and volatile oils in the lemon peel extract [56]. The antimicrobial activity of such secondary metabolites was reported by earlier studies and revealed that these secondary metabolites inhibit the growth of microbes in many ways such as by inhibiting protein synthesis, inferring with nucleic acid synthesis, breaking the peptide bonds, acting as chelating agents, inhibiting metabolic pathway, inferring with cell wall synthesis or by preventing utilization of available nutrients by the microorganisms [63], [39], [59] revealed that the decrease in the percentage of reduction in bacterial count in the case of the fabric samples treated with extract. Also, it has been reported that by increasing the solution concentration the

reduction rates of bacteria colonies progressively increased [33].

Table 4. Microbial resistance of lemon peel extract treated cotton fabric

Cotton samples**	**Antimicrobial activities*** Microbial count (CFU/ ml) and Reduction (%)							
	Gram negative Bacteria			**Gram positive Bacteria**		**Fungal strains**		
	P. aeruginosa	***E.coli***	***X. campestris***	***S. auerus***	***B. subtilis***	***A. clavatus***	***A. flavus***	***A. awamori***
Lemon peel extract treated	$5x10^7$ (97.89%)	$4x10^7$ (98%)	$5.8x10^7$ (97.16%)	$14.9x10^7$ (93.49 %)	$8.4x10^7$ (95.83%)	$11.5x10^7$ (94.99%)	$22.7x10^7$ (90.41%)	$16x10^7$ (93.44%)
Control	Confluent growth							

*Dilution mean of $10^7$and 10^8CFU/ml

** Concentration 5g/l

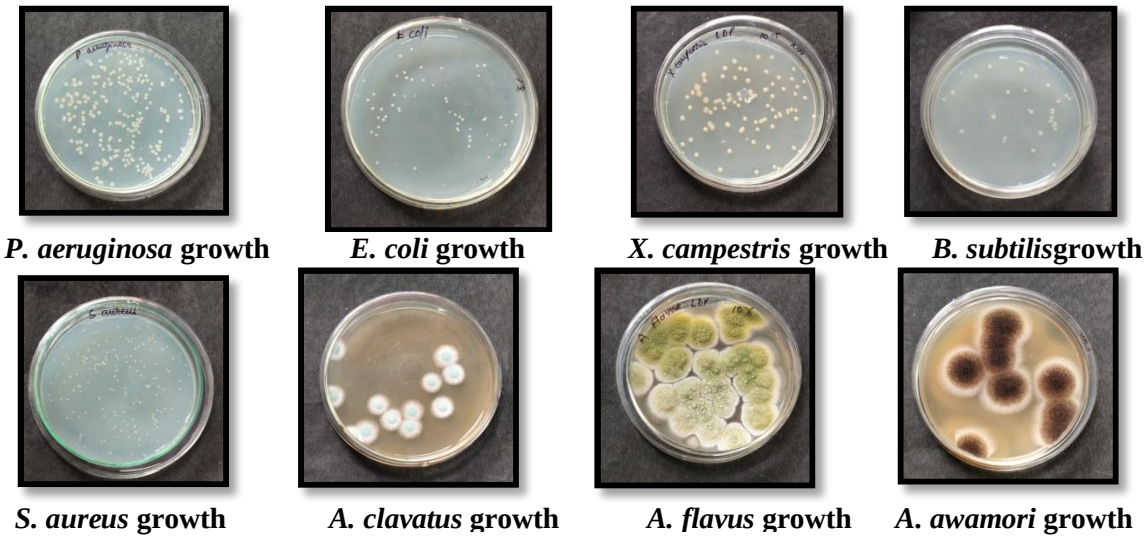


Fig. 2. Microbial growth on lemon peel extract treated fabric

3.5 Characterization of lemon peel extract treated cotton fabric

3.5.1 Surface morphology

To demonstrate that the lemon peel extract adhere to the cotton fibers and hence results in imparting antimicrobial property; the fabric samples before and after antimicrobial treatmentwere scanned under the SEM (fig. 3). The SEM photomicrographs revealed that the fibers of control cotton fabric exhibiting uniform neat plain spun structures and the cotton fibers manifested smooth surfaces. Whereas, lemon peel extract treated cotton fibers clearly became coarser and exhibited characteristic semi-granulated pattern, due to the formation of a thin layer around the fibers, composed of the lemon peel extract encapsulated in the treatment formulation. [54] studied the surface morphology using SEM showed the surface of the fibre seems to be covered with granular structure. The sizes of such granules vary from 0.2 um to 0.6 um. This could be a deposit of herbal extract on the surface of fibre. The untreated fabric depicted the smooth surface of fiber with striation while slight deposition of extracts of applied material on the surface of treated fabric [60].

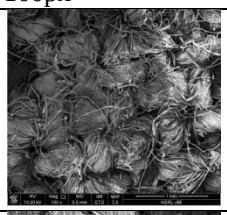
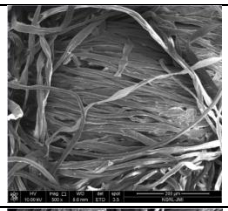
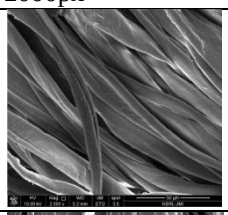
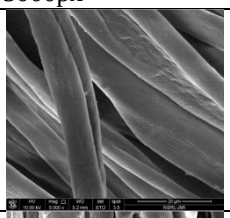
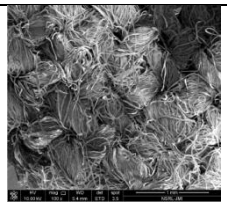
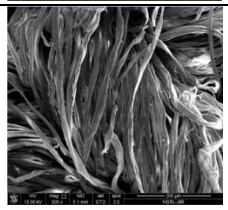
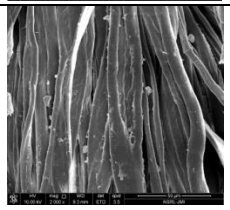
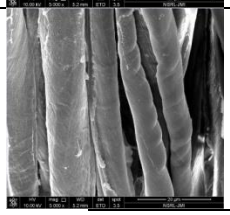
Fabric	SEM Images			
	Maginification 100px	Maginification 500px	Maginification 2000px	Maginification 5000px
Desized and scoured (Controlled)				
Lemon peel extract treated				

Fig. 3. SEM images of controlled and lemon peel extract treated cotton fabric

3.5.2 FTIR-ATR analysis of lemon peel extract treated cotton fabric

The FTIR-ATR spectra of the aforementioned untreated and treated cotton fabric with lemon peel extract by using exhuast method. It is obvious that the most characteristic peaks in Fig.4 are the main functional groups attributable to cellulosic molecular structure of cotton fabric [69]. The main characteristic peaks of cellulose molecule were appeared at 3249 cm^{-1} (O-H stretching vibration), peak at 2847 cm^{-1} that was because of methylene-C-H stretching vibration and the peak at 1371 cm^{-1} was attributed to C-H bending vibration and presence of OH-bend which showed water absorption characteristic. The peak at 1130 cm^{-1} indicting the presence of C- O-C asymmetric bridge stretching and 1079 cm^{-1} are corresponding to the C-O stretching vibration of cellulose matrix.

The FTIR-ATR spectra of the lemon peel extract treated cotton fabric had shown all the functional groups of cellulosic unit and then additionally showed the broad band (Fig.5) appearing at 3261 cm^{-1} may assigned the participation of hydroxyl group (H-bonded-O-H stretch), [46]. The peak at 2808 cm^{-1} are corresponding to the C-H aliphatic stretching and 1727 cm^{-1} is attributable to COOH groups and COOR groups (not carboxylate ions) [34]. The vibrational peaks of 1681 cm^{-1}, 1638 cm^{-1} and 1509 cm^{-1} are due to the stretching and bending vibration of carbonyl group and primary amine group of lemon peel component, respectively [70]. The peaks at1448 cm^{-1} and 1413 cm^{-1} indicated the presence of C=C stretch, quinone or conjugated ketone and organic sulphates respectively. Finally the peaks located at 1005 cm-1and 642cm-1 exhibited the presence of –C–C vibrates and aliphatic bromo compounds. The majority of the peaks correspond to the phenolic groups of the polyphenols, teriterpenoids, steroids and tannins adequately present in the lemon peel extract. These finding are inconsistent with the phytochemical analysis performed on the extract of lemon peel.

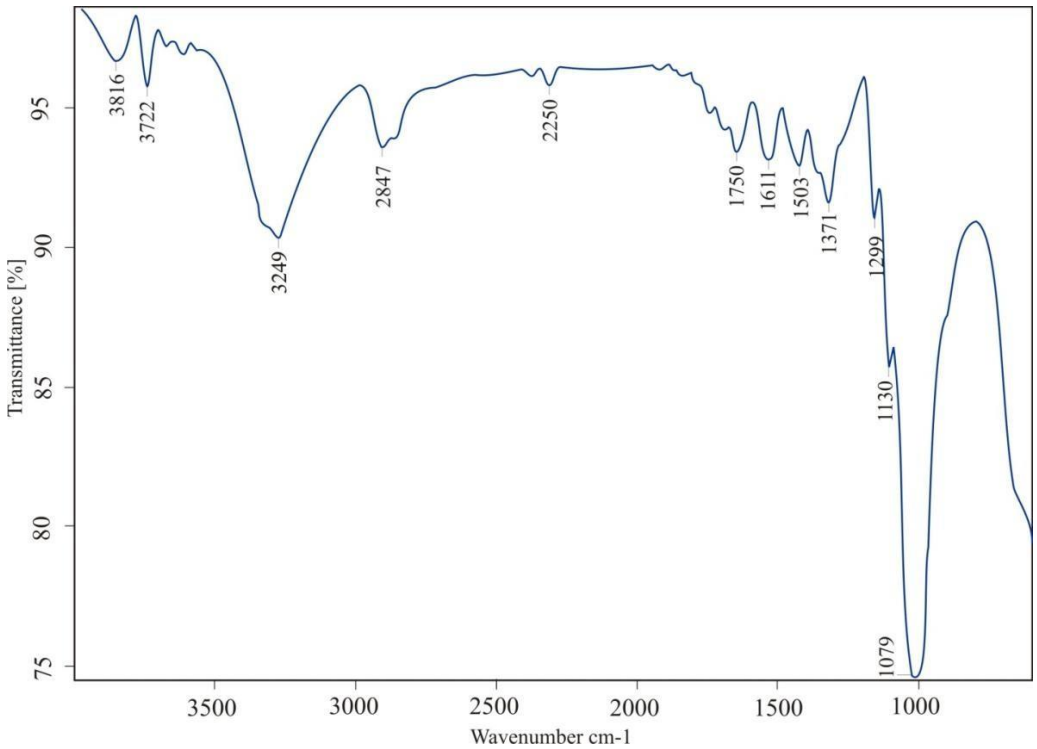


Fig.4. FTIR-ATR spectra of desized and scoured cotton fabric

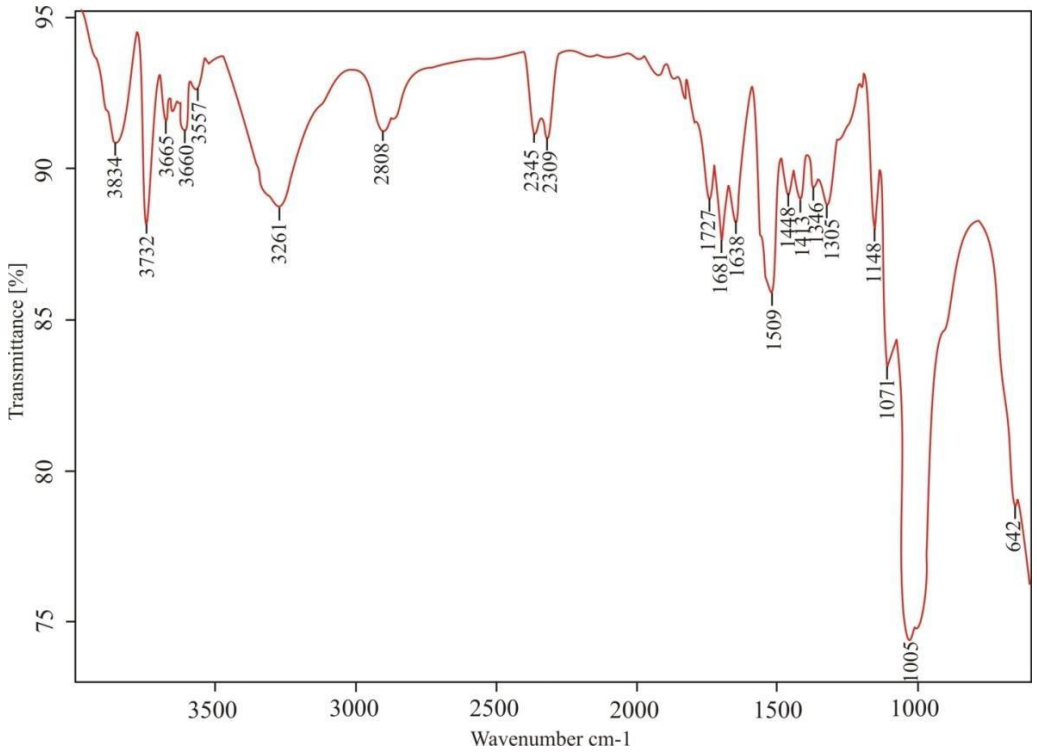


Fig.5. FTIR-ATRspectra of cotton fabric treated with lemon peel extract

3.6 Retention of antimicrobial property of lemon peel extract treated fabric after washing

It is discerned from the Table 5 that the cotton samples treated with 5g/l concentration of lemon peel extract showed decreased percent reduction in microbial growth after subsequent hours of inoculation of P. aeruginosa, *X. campestries, E. coli, S. aureus, B. subtilis, A. clavatus, A. flavus* and *A. awamori* from 97.89, 98.00, 97.16, 93.49, 95.83, 94.99, 90.41, 93.44 percent to 94.75, 96.00, 94.10, 90.50, 92.67, 91.00, 87.20,

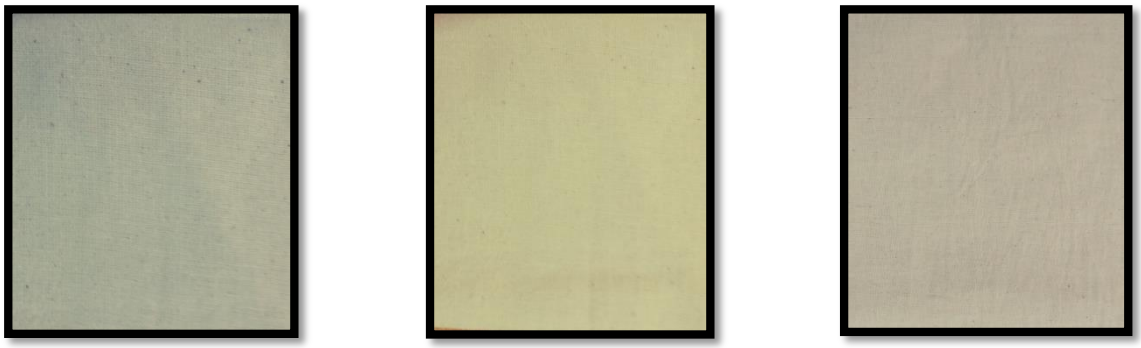
90.00 percent, respectively with progressive increase in washing cycles from 1 to 10.

Table 5. Retention of antimicrobial property of lemon peel extract treated fabric after washing

No. of washing cycles	**Microbial reduction (%)***							
	Gram negative Bacteria			**Gram positive Bacteria**		**Fungal strains**		
	P. aeruginosa	***E. coli***	***X. campestris***	***S. auerus***	***B. subtilis***	***A. clavatus***	***A. flavus***	***A. awamori***
0	97.89	98.00	97.16	93.49	95.83	94.99	90.41	93.44
1	96.20	97.80	96.89	92.98	94.70	93.56	89.88	92.10
5	95.00	96.45	95.55	91.65	93.25	92.75	88.45	91.80
10	94.75	96.00	94.10	90.50	92.67	91.00	87.20	90.00

*Dilution mean of 107and 108CFU/ml

It is thus obvious from the table that the antimicrobial efficacy of treated fabric decreased with the increase in washing cycles but still retained for some extent. The findings of the study are found in line with the results of study carried out by [71] who found that the antimicrobial activity of alum and chitosan treated dyed cotton fabric for E. coli and S. aureus bacteria diminished gradually as the number of washing frequencies increased.



Desized and scoured sample **Treated sample** **Treated washed sample**

Fig. 6. Controlled, treated and washed cotton fabric

4. Conclusions

The lemon peel treated cotton fabric showed 97.89, 98 and 97.16 percent reduction in growth of *P. aeruginosa, E. coli* and *X. campestris* bacteria, 93.49 and 95.83 per cent reduction in growth of *B. subtilis* and *S. aureus* bacteria whereas showed 94.99, 90.41 and 93.44 percent reduction in *A. clavatus, A. flavus* and *A. awamori* fungal strains, respectively. Thus, it is concluded that the application of lemon peel as bio-agent on the cotton fabric is a promising approach to fulfill the requirements of the end users (consumers) for safe and eco-friendly textiles. This approach enhanced the antimicrobial property without using harmful toxic chemicals.

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Conflict of Interest Statement

All authors declare no conflict of interests.

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6. References

[1] Aiello, F., Armentano, B., Polerà, N., Carullo, G., Loizzo, M. R., Bonesi, M., Cappello, M. S., Capobianco, L., Tundis, R., 2017. From Vegetable Waste to New Agents for Potential Health Applications: Antioxidant Properties and Effects of Extracts, Fractions and Pinocembrin from Glycyrrhizaglabra L. Aerial Parts on Viability of Five Human Cancer Cell Lines. Journal of Agriculture Food Chemistry, 65, 7944–7954. doi: https://doi.org/10.1021/acs.jafc.7b0345.

[2] Aiello, F., Carullo, G., Badolato, M., Brizzi, A., 2016. TRPV1-FAAH-COX: The couples game in pain treatment. Chem Med Chem, 11, 1686–1694. doi: hppts://doi.org/10.1002/cmdc.201600111.

[3] Ali, J., Das, B., & Saikia, T., 2017. Antimicrobial activity of lemon peel (citrus limon) extract. International Journal of Current Pharmaceutical Research, 9(4), 79-82. doi: http://dx.doi.org/10.22159/ijcpr.2017v9i4.20962.

[4] Arif, D., Niazi, M. B. K., Ul-Haq, N., Anwar, M. N., Hashmi, E., 2015. Preparation of Antibacterial Cotton Fabric Using Chitosan-silver Nanoparticles. Fibers and Polymers, 16(7), 1519-1526. doi: https://doi.org/10.1007/s12221-015-5245-6.

[5] Badolato, M., Carullo, G., Cione, E., Aiello, F., Caroleo, M. C., 2017a. From the hive: Honey, a novel weapon against cancer. European Journal of Medicinal Chemistry, 142, 290- 299. doi: https://doi.org/10.1016/j.ejmech.2017.07.064.

[6] Badolato, M., Carullo, G., Perri, M., Cione, E., Manetti, F., Di Gioia, M. L., Brizzi, A., Caroleo, M. C., & Aiello, F. 2017b. Quercetin/oleic acid-based G-protein-coupled receptor 40 ligands as new insulin secretion modulators. Future Medicinal Chemistry, 9, 1873–1885. doi: https://doi.org/10.4155/fmc-2017-0113.

[7] Bajaj, P., Kothari, V. K., Ghosh, S. B., 2000. Some innovations in UV protective clothing. Indian Journal of Fibres and Textile Research, 35, 315–329. http://nopr. niscair.res.in/bitstream/123456789/24907/1/IJFTR%2025%284%29%20315-329. pdf.

[8] Bansode, D. S., Chavan, M. D., 2012. Studies on antimicrobial activity and phytochemical analysis of citrus fruit juices against selected enteric pathogens. International Research Journal of Pharmacy, 3(11), 122-126.

[9] Borland, V. S., 2010. King cotton: Here today and here tomorrow-from fiber to fashion, cotton weaves its way through the supply chain. Textile World, 160, 1–6. https://www.ellenmacarthurfoundation.org/assets/downloads/A-New-Textiles-Economy_Full-Report_Updated_1-12-17.pdf.

[10] Carullo, G., Aiello, F., Carullo, G., Cappello, A. R., Frattaruolo, L., Badolato, M., Armentano, B., Aiello, F., 2017. Quercetin and derivatives: Useful tools in inflammation and pain management. Future

Medicinal Chemistry, 9, 79–93. doi: https://doi.org/10.4155/fmc- 2016-0186.

[11] Carullo, G., Durante, M., Sciubba, F., Restuccia, D., Spizzirri, U. G., Ahmed, A., Di Cocco, M. E., Saponara, S., Aiello, F., Fusi., F., 2019. Vasoactivity of Mantonico and Pecorellograpе pomaces on rat aorta rings: An insight into nutraceutical development. Journalof Functional Foods, 57,328–334. doi: https://doi.org/10.1016/j.jff.2019.04.023.

[12] Carullo, G., Ahmed, A., Fusi, F., Sciubba, F., Di Cocco, M. E., Restuccia, D., Spizzirri, U. G., Saponara, S., Aiello, F., 2020a. Vasorelaxant Effects Induced by Red Wine and Pomace Extracts of Magliocco Dolce cv. Pharmaceuticals, 13, 87. doi: https://doi.org/10.3390/ph13050087.

[13] Carullo, G., Ahmed, A., Trezza, A., Spiga, O., Brizzi, A., Saponara, S., Fusi, F., Aiello, F., 2020b. Design, synthesis and pharmacological evaluation of ester-based quercetin derivatives as selective vascular KCa1.1 channel stimulators. Bioorganic Chemistry,105, 104404. doi: https://doi.org/10.1016/j.bioorg.2020.104404.

[14] Carullo, G., Governa, P., Spizzirri, U. G., Biagi, M., Sciubba, F., Giorgi, G., Loizzo, M. R., Di Cocco, M. E., Aiello, F., Restuccia, D., 2020c. Sangiovesecv pomace seeds extract- fortified kefir exerts anti-inflammatory activity in an in vitro model of intestinal epithelium using caco-2 cells. Antioxidants, 9, 54. doi: https://doi.org/10.3390/antiox9010054.

[15] Carullo, G., Mazzotta, S., Koch, A., Hartmann, K. M., Friedrich, O., Gilbert, D. F., Vega- Holm, M., Schneider-Stock, R., Aiello, F., 2020d. New oleoyl hybrids of natural antioxidants: Synthesis and in vitro evaluation as inducers of apoptosis in colorectal cancer cells. Antioxidants, 9, 1077. doi: https://doi.org/10.3390/antiox9111077.

[16] Carullo, G., Sciubba, F., Governa, P., Mazzotta, S., Frattaruolo, L., Grillo, G., Cappello, A.R., Cravotto, G., Di Cocco, M. E., Aiello, F., 2020e. Mantonico and pecorello grape seed extracts: Chemical characterization and evaluation of in vitro wound-healing and anti- inflammatory activities. Pharmaceuticals, 13, 97. doi: https://doi.org/10.3390/ph13050097.

[17] Carullo, G., Spizzirri, U. G., Loizzo, M. R., Leporini, M., Sicari, V., Aiello, F., Restuccia, D., 2020f.Valorization of red grape (Vitisvinifera cv. Sangiovese) pomace as functional food ingredient. Italian Journal of Food Science, 32, 367–385. doi: https://doi.org/10.14674/IJFS- 1758.

[18] Dewick, P. M., 2009. Medicinal natural products: A biosynthetic approach (3rd edn.). West Sussex: John Wiley & Sons Ltd. file:///C:/Users/rose/Downloads/ MedicinalNaturalProducts- Paul.pdf.

[19] Frassinetti, S., Caltavuturo, L., Cini, M., Della Croce, C. M., 2011. Antibacterial and antioxidant activity of essential oils from Citrus spp. Journal of Essential Oil Research, 23, 27-31. doi:10.1080/104 12905.2011.9700427.

[20] Fröhlich, T., Ndreshkjana, B., Muenzner, J. K., Reiter, C., Hofmeister, E., Mederer, S., Fatfat, M., El-Baba, C., Gali-Muhtasib, H., Schneider-Stock, R., et al., 2017. Synthesis of Novel Hybrids of Thymoquinone and Artemisinin with High Activity and Selectivity Against Colon Cancer. ChemMedChem, 12, 226–234. doi: https://doi.org/10.1002/cmdc.201600594.

[21] Gao, Y., Cranston, R., 2008. Recent advances in antimicrobial treatments of textiles. Textile Research Journal, 78(1), 60-72. doi: https://doi.org/10.1177/0040517507082332.

[22] Governa, P., Carullo, G., Biagi, M., Rago, V., Aiello, F., 2019. Evaluation of the in vitro wound-healing activity of calabrian honeys. Antioxidants, 8, 36. doi: https://doi.org/10.3390/antiox8020036.

[23] Gupta, V., Deepika, C., Saloni, G., Nirmal, Y., 2017. Environment friendly anti-bacterial and UV protective finish on cotton using syzygium cumini (L.) leaves extract. International Journal of Textile and Fashion Technology. 7(1):53-62.

[24] Hooda, S., Khambra, K., Yadav, N., Sikka, V. K., 2013a. Antimicrobial activity of herbal treated wool fabric, American International Journal of Research in Formal, Applied & Natural Sciences, 66-69.

[25] Hooda, S., Khambra, K., Yadav, N., Sikka, V. K., 2013b. Eco-friendly antibacterial finish for wool fabric. Journal of Life Sciences, 5, 11–16. http://www.krepublishers.com/0 2- Journals/JLS/JLS-05-0-000-13-Web/JLS-05-1-000-13-Abst-PDF/JLS-0 5-1-011-13-147-Hooda-S/JLS-05-1-011-13-147-Hooda-S-Ab.pdf.

[26] Hustvedt, D., Crews, P. C., 2005. The ultraviolet protection factor of naturally pigmented cotton. The Journal of Cotton Science, 9, 47–55. https://digitalcommons. unl.edu/cgi/viewcontent.cgi? article=1000&context=textiles_facpub.

[27] Ibrahim, N. A., Gouda, M., Husseiny, S. M., El-Gamal, A. R., Mahrous, F., 2009. UV- protecting and antibacterial finishing of cotton knits. Journal of Appllied Polymer Science, 112(6), 3589-3596. doi: https://doi.org/10.1002/app.29669.

[28] Ibrahim, N. A., Khalifa, T. F., El-Hossamy, M. B., Tawfik, T. M., 2010. Effect of knit structure and finishing treatments on functional and comfort properties of cotton knitted fabrics. Journal of Industrial Textile, 40(1), 49-64. doi: https://doi.org/10.1177/1528083709357975.

[29] Jaswal, P., Preet, A., Simran, & Goel, G., 2017. Antimicrobial activity of herbal treated cotton fabric. International Research Journal of Engineering and Technology. 4(8).

[30] Javed, S., Javaid, A., Mahmood, Z., Javaid, A., Nasim, F., 2011. Biocidal activity of citrus peel essential oils against some food spoilage bacteria. Research Journal of Medicinal Plant, 5(16), 3697-3701.

[31] John, S., Monica, S. J., Priyadarshini, S., Arumugam, P., 2017. Investigation on phytochemical profile of Citrus limonumpeel extract. International Journal of Food Science and Nutrition, 2(1),65- 67. https://www.researchgate.net/publication/330675184_Investigation_on_phytochemical_profile_of_Citrus_limonum_peel_extract.

[32] Jose, S., Prabu, H. G., Ammayappan, L., 2016. Eco-friendly dyeing of silk and cotton textiles using combination of three natural colorants. Journal of Natural Fibers, 456(18): 1- 10.doi: 10.1080/15440478.2015.1137530.

[33] Jothi, D., 2009. Experimental study on antimicrobial activity of cotton fabric treated with aloe gel extract from Aloe vera plant for controlling the Staphylococcus aureus (bacterium). African Journal of

Microbiology Research, 3(5), 228-232. https://academicjournals.org/article/article1380185091_Jothi.pdf

[34] Kanmani, P., Dhivya, E., Aravind, J., Kumaresan, K., 2014. Extraction and Analysis of Pectin from Citrus Peels: Augmenting the Yield from Citrus limon Using Statistical Experimental Design. Iranica Journal of Energy and Environment, 5(3), 303-312.

[35] Kavitha, S., Annapoorani, S. G., 2013. Aloe Vera Finish on Cotton and Organic Cotton Fabrics. Global Research Analysis, 2, 104-105. doi: https://doi.org/10.15373/2249555X.

[36] Khushwaha, A., Singh, R. P., Gupta, V., Singh, M., 2012. Antimicrobial properties of peel of Citrus fruits. International Journal of Universal Pharmacy and Life Sciences, 2(2), 24-38.

[37] Kupwade, S. R., Manglani, N. S., Wadkar, S. S., Inamdar, F. R., Shete, C. C., Ghosh, J. S., 2017. Antimicrobial Activity of Lemon Peel Extract against the Bacterium Belonging to the Genus Xanthomonas. International Journal of Pharmaceutical Sciences Review and Research, 45(2), 251-254.

[38] Lawal, D., Bala, J. A., Aliyu, S.Y., Huguma, M. A., 2013. Phytochemical screening and in vitro anti-bacterial studies of the ethanolic extract of Citrus senensis(Linn.) peel against some clinical bacterial isolates. International Journal of Innovation and Applied Studies, 2(2), 138- 145.

[39] Lazić, V., Šaponjić, Z., Vodnik, V., Dimitrijević, S., Jovančić, P., Nedeljković, J., Radetić, M., 2012. The study of antibacterial activity and stability of dyed cotton fabrics modified with different forms of silver. Journal of the Serbian Chemical Society, 77(2), 225-234.

[40] Lee, Y. H., Hwang, E. H., Kim, H. D., 2009. Colorimetric assay and antimicrobial activity of cotton, silk and fabrics dyed with peony, clove, Coptischinensis and gallnut. Materials, 2, 10–21. doi: https://dx.doi.org/10.3390%2Fma2010010.

[41] Malpani, S., 2013. Antibacterial Treatment on Cotton Fabric from Neem Oil, Aloe Vera &Tulsi. International Journal of Advance Research in Science and Engineering, 2, 35-43.

[42] Mazzotta, S., Carullo, G., Moriello, A. S., Amodeo, P., Di Marzo, V., Vega-Holm, M., Vitale, R. M., Aiello, F., Brizzi, A., De Petrocellis, Design, L., 2020a. Synthesis and In Vitro Experimental Validation of Novel TRPV4 Antagonists Inspired by LabdaneDiterpenes. Marine Drugs, 18, 519. doi: https://doi.org/10.3390/md18100519.

[43] Mazzotta, S., Cebrero-Cangueiro, T., Frattaruolo, L., Vega-Holm, M., Carretero-Ledesma, M., Sánchez-Céspedes, J., Cappello, A. R., Aiello, F., Pachón, J., Vega-Pérez, J. M., et al., 2020b. Exploration of piperazine-derived thioureas as antibacterial and anti-inflammatory agents. In vitro evaluation against clinical isolates of colistin-resistant Acinetobacterbaumannii. Bioorganics and Medicinal Chemistry Letters, 30, 127411. doi: https://doi.org/10.1016/j.bmd.2020.127411.

[44] Mazzotta, S., Frattaruolo, L., Brindisi, M., Ulivieri, C., Vanni, F., Brizzi, A., Carullo, G., Cappello, A. R., Aiello, F., 2020c. 3-Amino-alkylated indoles: Unexplored green products acting as anti-inflammatory agents. Future Medicinal Chemistry, 12, 5-17. doi: https://doi.org/10.4155/fmc-2019-0234.

[45] Mishra, R. P., Yadav, S., Anjali, 2012. Study of Antimicrobial Activities of Citrus limetta. Journal

of Pharmaceutical and Biomedical Science, 19(15), 1-4.

[46] Mohanraj,S., Dhurai, B., 2017. Comparison of natural agents to develop antibacterial kitchen textiles. International Research Journal of Pharmacy, 8(7), 93-99.

[47] Racowski, I., Piotto, J., Procópio, V., Freire, V. T., 2017. Evaluation of Antimicrobial Activity and Phytochemical Analysis of Thaiti Lemon Peels (Citrus latifolia Tanaka). Journal of Microbiology Research, 7(2), 39-44.

[48] Rani, S., Punia, P., 2018. Herbal Antibacterial Treatment on Cotton Fabric. Contemporary Social Sciences, 27(1), 127-134.

[49] Restuccia, D., Giorgi, G., Gianfranco Spizzirri, U., Sciubba, F., Capuani, G., Rago, V., Carullo, G., Aiello, F., 2019. Autochthonous white grape pomaces as bioactive source for functional jams. International Journal of Food Science Technology, 54, 1313–1320.

[50] Rosida A., Sharifah, S. S., Asdren, Z., Nizam, 2018. Antimicrobial and wash durability of cotton fabric treated with Punicagranatum and Cirtuslimen peel extracts against skin pathogens. Malaysain Applied Biology, 47(3), 129-135.

[51] Saeb, S., Amin, M., Gooybari, R. S., Aghel, N., 2016. Evaluation of Antibacterial Activities of Citrus limon, Citrus reticulata, and Citrus grandis against Pathogenic Bacteria. International Journal of Enteric Pathogens, 4(4), 37103. doi: https://doi.org/10.15171/ijep.2016.13.

[52] Sarkar, R., Munishi, 2003. Effect of soap and detergents on strength of cotton fabrics using natural herbal extracts. The Indian Textile and Trade Journal, 38, 71-75.

[53] Salama, A. A., Kotb, R. M., Shaker, R. N., 2015. Effect of treatment durability and coloration of coated cotton fabrics on antibacterial, UV-blocking, healing and anti- inflammatory properties. Journal of Chemical and Pharmaceutical Research, 7, 181–193.

[54] Sathianarayanan, M. P., Wazed, A. S., Bhat, N. V., Kokate, S. S., Walunj, V. E., 2010. Antibacterial finish for cotton fabric from herbal products. Indian Journal of Fibre and Textile Research, 35(1), 50-58.

[55] Sewani, S., Qureshi, M., 2016. Antimicrobial activity of Neem, Clove, Curry leaves, Cardamom, Tulsi stem and Tulsi leaves, International Research Journal of Biological Sciences, 5(1), 42-46.

[56] Shahnah, S. M., Ali, S., Ansari, H., Bagri. P., 2007. New sequiterpene derivative from fruit peel of citrus limon (Linn) Burn. International Journal of Pharma and Bio Sciences, 5(1), 165-170.

[57] Simoncie, B., & Tomsic, B., 2010. Structures of novel antimicrobial agents for textiles – A review. Textile Research Journal, 17, 17–21. https://www.tib.eu/en/search/id/tema%3ATEMA20101100651/Structures-of-novel-antimicrobial-agents-for-textiles/

[58] Singh, N., Punia, P., Singh, V., 2015. Antibacterial efficacy of cotton and silk fabric finished with plants extracts. International Journal of Textile and Fashion Technology, 5(5), 1-4.

[59] Singh, N. , 2017. Microbial resistance of cotton and silk finished with plant extracts. Ph.D. Thesis, CCS Haryana Agricultural University, Hisar, Haryana.

[60] Singh, N., Singh, V., Punia, P., 2019. Antibacterial finish on silk fabric with pomegranate and onion peel extracts.Journal of Pharmacognosy and Phytochemistry, 5, 129-131.

[61] Spizzirri, U. G., Carullo, G., De Cicco, L., Crispini, A., Scarpelli, F., Restuccia, D., Aiello, F., 2019. Synthesis and characterization of a (+)-catechin and L-(+)-ascorbic acid cocrystal as a new functional ingredient for tea drinks. Heliyon, 5, 02291. doi: https://doi.org/10.1016/j.heliyon.2019.e02291.

[62] Srinivasan, M., Gatewood, B. M., 2000. Relationship of dye characteristics to UV protection provided by cotton fabric. TCC and ADR, 32, 36–43.

[63] Sumitra, C., Kaneria, M., 2011. Indian nutraceutical plant leaves as a potential source of natural anti-microbial agents. Science against microbial pathogens: communicating current research and technological advances. A. Méndez-Vilas (Ed.): 1251-1259.

[64] Sushila, Arya, N., Singh, V., Devi, S., Reena, 2021. Effect of pretreatment on physical properties of cotton fabric. International Journal of Ecology and Environmental Sciences, 3(1), 472-476.

[65] Tao, N., Gao, Y., Liu, Y., Ge, F., 2010. Carotenoids from the Peel of ShatainPummelo (Citrus grandisOsbeck) and its antimicrobial activity. American-Eurasian Journal of Agricultural Environmental Sciences, 7(1), 110-115.

[66] Teli, M. D., Pandit, P., 2017. Development of thermally stable and hygienic colored cotton fabric made by treatment with natural coconut shell extract. Journal of Industrial Textiles, 45(3), 119-125.

[67] Teli, M. D., Pandit, P., Basak, S., 2017. Cocunut shell extract imparting multifunction properties to lingo-cellulosic material. Journal of Industrial Textiles, 45(32), 345-353.

[68] Tundis, R., Frattaruolo, L., Carullo, G., Armentano, B., Badolato, M., Loizzo, M. R., Aiello, F., Cappello, A. R., 2018. An ancient remedial repurposing: Synthesis of new pinocembrin fatty acid acyl derivatives as potential antimicrobial/anti-inflammatory agents. Natural Products Research, 6419, 1–7. doi: https://doi.org/10.1080/14786419.2018.1440224.

[69] Usha, S., Ramappa, K. T., Hiragoudar, S., Vasanthkumar, G. D., Aswathanarayana, D. S., 2017. Biosynthesis and characterization of copper nanoparticles from tulasi (OcimumsantumL.) leaves. International Journal of Current Microbiology and Applied Sciences, 6(11), 2219-2228. doi: 10.20546/ijcms.2017.611.263.

[70] Vankar, P. S., Shukla, D., 2012. Biosynthesis of silver nanoparticles using lemon leaves extract and its application for antimicrobial finish on fabric. Applied Nanoscience, 2, 163– 168. doi: https://doi.org/10.1007/s13204-011-0051-y.

[71] Verma, M., Gahlot, N., Singh, S. S. J., Rose, N. M., 2021. UV protection and antibacterial treatment of cellulosic fibre (cotton) using chitosan and onion skin dye. Carbohydrate Polymers, 257, 117612.

[72] Wink, M., 2003. Evolution of secondary metabolites from an ecological and molecular phytogenetic perspective. Phytochemistry, 64, 3–19. doi: https://doi.org/10.1016/S0031- 9422(03)00300-5.

[73] Yalavarthi, C., Thiruvengadarajan, V. S., 2013. A review on identification strategy of phyto constituents present in herbal plants. International Journal of Research and Pharmaceutical Sciences, 4, 123–140. https://pharmascope.org/index.php/ijrps/ article/view/1127.