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# A versatile route based on *citrullus colocynthis* (L.) - extract and certain innocuous acids for multi functionalization of natural fabrics

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The research presented herein comprises the necessary practices concerned with development of eco – friendly highly reactive antimicrobial finishes for application to wool and cotton fabrics. Testing and analytical evolution of these finishes and fabrics treated thereof are also described. Syntheses of the finishes in question are based on processing of medicinal plant *Citrullus colocynthis* (L.) (*CC*) bitter apple fruit through grinding the whole fruit to powders which are submitted to alcoholic extraction flowed by filtration and drying. The *Citrullus colocynthis* (L.) extracts were applied to wool and cotton textiles in absence and presence of certain innocuous acids. The latter encompass ascorbic, itaconic, sorbic, benzoic, salicylic, sulphamic, citric and acetic acids. The as- finished fabrics display durable antimicrobial properties indicating that the synthesized finishes, the phenolic compound of the *Citrullus colocynthis* (L.) extract , the acid , the extract itself and the molecular structure of wool or cotton are involved in interactions with possible formation of chemical bonding among these entities . It is rather possible that the wool or cellulose molecules are dispersed in the extract containing the synthesized finish and the acid and, in so doing; they create strong bonding via interaction. Characterization and other properties of the research outcome are also performed using infrared spectroscopy and scanning electron microscope in addition to tensile strength, elongation at break, moisture regain and yellowness of the treated fabrics. The potential innovation emerged out from current work is so promising that scaling up trials are advocated.

**Keywords: Antimicrobial Finishing, *Citrullus colocynthis* (L.) *CC*, Extra Functionalization of Textiles**

## INTRODUCTION

Textile fibres of natural origin are, by and large, more susceptible to bio deterioration than those of synthetic (man-made) fibres. The porous structure of natural fibres and their hydrophilicty permit them to retain water, oxygen and nutrients; leading to perfect environments for microbial growth. Textile auxiliaries such as starch, protein derivatives, fats and oils used in textile finishing also favor microbial growth. Microorganisms may attack the entire textile substrate or may attack only one component of the substrate, such as plasticizer contained therein, or grow on dirt that has accumulated on the surface of a textile product. Plant fibres such as cotton, flax (Linen), jute and hemp are very susceptible to attack by fungi. Meanwhile, animal fibres display higher resistance to mildew growth than plant fibres. Wool decays only slowly but chemical and mechanical damage during processing can increase its masterful to bio deterioration. But when stored under adverse condition wool will eventually rot by the action of the proteolytic (protein-digesting) enzymes secreted by many micro-fungi and bacteria ( Paul, 1997).

Further, subdivisions in the bacteria family are Gram positive (*Staphylococus aureus*), Gram negative (*Escherichia coli*), Spore bearing or non-spore bearing type. Some specific types of bacteria are pathogenic and cause cross infection. Fungi, molds or mildew are complex organisms with slow growth rate. They are part of our everyday live and found everywhere in the environment and on our bodies (Boryo, 2013)

A variety of acids was investigated and classified as generally recognized as safe (GRAS) for their more effective ability to kill *Escherichia coli* O157:H7. These acids comprise lactic, malic, benzoic, sorbic, and fumaric acids (Huiying et al.,2011).

Details of a new method were reported for the grafting of cyclodextrins CDs onto cotton or wool. The novelty of this method refers to more chemical approach (the use of polycarboxylic acids as grafting intermediates) of the reaction than the process used (pad–dry–cure) (Martel et al.,2002).When the scales of woolen fabrics were cured with CA or the CA and chitosan treatment solution, no crosslinking was produced. After the woolen fabric was oxidized by preprocessing with potassium permanganate and cured with the CA and chitosan treatment solution, esterification and transamidation were produced. The modification of wool fabric with the ecofriendly application of chitosan and citric acid (CA/chitosan) greatly improved the wool physical and antimicrobial properties. Thus, strong covalent bonds were created on wool fabric because of the grafting of CA/chitosan (Hsieh et al.,2007 and Gawish et al.,2012)

Also malic acid, itaconic acid ,and citric acid were used along with chitosan with objectives of imparting multiple functionalities such as soil release, resistance to flammability and antimicrobial to cotton fabrics in order to make it suitable for the production of regular apparels, sanitary wears, kitchen and laboratory aprons, hand gloves, etc. (Sunder et al.,2014)

*Aloe vera* a naturally available herb is being increasingly used now as a functional finish on textile substrate to impart antimicrobial characteristics. *Aloe Vera* treated wool fabric displays 82.81% bacterial reduction even after 20 washing cycles (Hooda et al.,2013)

Curcumin, a common natural dye was used as an antimicrobial finish due to its bactericidal properties on dyed textiles. The antimicrobial ability of the dyed wool improves with curcumin (Hana and Yanga 2005)

Microencapsulated antimicrobial and insect-resist agents were applied to wool fabrics by a pad-dry-bake method (Hassan. and Sunderland, 2015)

Previous research has characterized plant *Hubera nitidissima* that was processed as a tinctorial raw material and antioxidant activity on the proteinaceous fibers (wool, silk) (Toussirot et al.,2014)

The wool fabric was treated with natural mordant agents extracted from cone of *Chamaecyparis Lawsoniana* plant to investigate the fastness properties, color strength (K/S) values and antimicrobial properties (Kilinca t et al., 2015).Salicylic acid and three of its derivatives were bonded using epichlorohydrin or polymer binders to provide antibacterial properties to viscose fabrics because the skin may be in direct contact with fabrics. (Kantouch, et al., 2013)

There is a dearth of publications on skin health and its relationship with fabrics manufactured from either wool or other fibre types. It displayed little effect on stratum corneum (SC) hydration when dry, but an increase when wet with both wool and cotton fabrics. Effects of fibre content of socks on health of the skin on human feet have also been investigated (Laing et al., 2015). Improved barrier function, indicated by a decrease in transepidermal water loss TEWL and an elevated SC, was evident at the heel (Lambers, et al., 2006,Darlenski et al., 2009,Elkeeb et al., 2010,Laing et al., 2015,Shin et al., 2014) and Fangueiro and Rana, 2016).Grafting of cyclodextrins (b-CD) and b-chloro-triazinyal cyclodextrin onto wool was applied in presence of citric acid and some phosphorous salts like sodium hypophosphite (SHP) and sodium dihydrogenphosphate (SDP) at diversified conditions using the pad-cure technique. This led to increasing the fabric resistance to *Escherichia coli* and *Staphylococcus aureus* bacteria compared to the control fabrics and were ineffective against fungi (Gawish et al., 2009)

*Citrullus colocynthis* (L.) is an important medical plant belonging to the family *Cucurbitaceae*. It is an annual herb widely distributed in Mediterranean strip (Hediat and Salama 2012).

*Citrullus colocynthis* (L.) (*Cucurbitaceae*), ordinarily known as bitter apple, locally called Hinzel. *Citrullus colocynthis* (L.) has gained increasing attention as a natural insecticide and its activity has been evaluated against many economically important insect species. Also, it is used as a purgative and vermifuge, and for the treatment of fever, cancer, amenorrhea, jaundice, leukemia, rheumatism, and tumors and as an insect repellent. *Citrullus colocynthis* (L.) fruits were extracted by different solvents (n- hexane, methylene chloride, chloroform and ethanol). Mass analysis of the isolated and purified compound showed the molecular ion peak at m/z 719. The element analysis (C, H and N) suggesting the molecular formula to be $C_{38} H_{55} O_{13}$ (Prabuseenivasan et al., 2004,Siddiqui et al., 1955,Duke et al., 2006,Sturm et al., 2009 and kantouch et al., 1997).Synthesis of silver nanoparticles has been investigated by using leaf extracts of *Citrullus colocynthis* (L.), (Satyavani et al., 2011) .Newly silver nanoparticles colloidal solutions (AgNPs) were prepared from *Citrullus colocynthis* (L.) either a whole fruit or separate seeds or fruit without seeds) as reducing and stabilizing. Wool and viscose fabrics were treated with the prepared AgNPs colloidal solutions to accept them as antimicrobial against Gram-positive bacteria, Gram-negative bacteria, Yeast and Fungi (El-Bisi et al., 2015).

Current research work is designed to synthetize highly functionalized finishes that could act against microbes when applied to wool and cotton textiles in an ecofriendly environment. Thus work is targeted to processing of antimicrobial finishes based on *Citrullus colocynthis* (L.) extract along with certain innocuous acids. Thorough investigations into the antimicrobial properties of the as – prepared finishes before and after being applied to wool and cotton fabrics are reported. Also reported are the characterizations of the antimicrobial textiles brought about by treatment of wool and cotton fabrics by such innovated finishes using state of the art facilities.

## MATERIALS AND METHODS

### Tested materials

*Citrullus colocynthis* (L.) fruits were collected from South Sinai governorate, Egypt. The ripe fruits are marbled green with white and yellowish patches but colour of unripe fruits is green. Wool and cotton fabrics were supplied by Misr Company for Spinning and Weaving, Mahalla El–Kobra, Egypt. The fabrics were cleaned further by washing for 30 minutes in an aqueous solution containing 1 g/l sodium carbonate and 2 g/l nonionic detergent(Egyptol) at 50°C with a liquor ratio 1:50. The fabrics were rinsed several times with warm and cold water, squeezed and finally air dried at the ambient temperature. Acids used are innocuous acids including ascorbic, acetic, sorbic and benzoic acid that were purchased from El Nasr Pharmaceutical Chemicals- Abou Zaabal-Egypt . Itaconic acid was obtained from (SISCO Research Laboratories PVT. LTD-BOMBAY - 400 093, INDIA. Sulphamic acid and salicylic were purchased from Aldrich Chemicals (Germany). Extra pure anhydrous citric acid was supplied by (Loba chemise, India). Nonionic detergent (nonyl phenol ethoxylane, was supplied from Starch & Detergent Company. Alexandria, Egypt). All other chemicals and reagents were of laboratory grade.

### Preparation of *Citrullus colocynthis* (L.) extract

Three extracts solutions were prepared from dried *Citrullus colocynthis* (L.) fruits. They were obtained by grinding the whole fruit into powders and put it in Soxhlet which were extracted for three successive times by in alcohols (hexane ethanol and methanol at room temperature. The alcohol extracts were then filtered and dried at 45ºC under pressure using rotavapour device.

### Tested microorganisms

The used mould fungi, *i.e. Aspergillus flavus*, *A*. *niger* and the yeast *Saccaromyces cerevisiae, Candida album* as well as Bacterial isolates, *i.e. Bacillus subtilis*, *Pseudomonas fluorescence*, *Escherichia coli*, *Staphylococcus* sp. were kindly obtained from Plant pathology Dept., National Research Centre, Egypt.

### Growth media

Potato dextrose agar (Difco Laboratories, Detroit, MI), NYDB [8 g of nutrient medium (Difco Laboratories, Detroit, MI), 5 g of yeast extract, and 10 g of dextrose in 1liter of water] and Nutrient medium (Difco Laboratories, Detroit, MI) were used for growing fungal, yeast and bacterial isolates tested in the present work. Fungal, yeast and bacterial cultures were maintained on PDA, NYD and Nutrient media agar slant media at 5±1ºC as stock cultures until use. All isolates were activated by growing at the optimum growth conditions at the beginning of the present experiments.

### Preparation of microbial growth

Fungal inoculum was grown on PDA medium at 28±1ºC until an abundant heavy growth of conidia was evident. Conidia were harvested by scraping the surface of the colonies with a spatula, transferred to sterilized distilled water and filtered through nylon mesh. All spore solutions were adjusted with sterile water to give a spore concentration of $10^4$-$10^5$ spores per milliliter. Meanwhile, bacteria and yeast inocula were grown on Nutrient broth and NYDB media and incubated in a rotary shaker at 200 rpm for 24 h at 28±1ºC. The bacterial cells were harvested by centrifugation at 6,000 rpm for 10 min, washed twice with 0.05 M phosphate buffer at pH 7.0, and re-suspended in distilled water. The concentrations of bacterial cells in the suspensions were adjusted to $10^5$-$10^6$ cells per milliliter. Concentrations of both bacterial cells and fungal spore suspensions were adjusted with the aid of a haemocyto meter slide. A few drops of the emulsifier Tween 20 (Sigma Co.) were added to the prepared tested-agents to obtain distributed separated spores/cells suspensions.

### Bio-assay tests

The inhibitory effect of *Citrullus colocynthis* (L.) extract individually or combined with certain innocuous acid solutions alone or imbedded into wool or cotton textiles was evaluated on the growth of some fungal, yeast and bacterial isolates under *in vitro* conditions. The assay method previously described by (Abdel-Kader et al., 2012 and (El-Mougy, and Abdel-Kader, 2017.) was followed. Certain volumes (10 mL) of each prepared suspension were added to melted tested sterilized media and carefully mixed by gentle swirling to ensure the equal suspension distribution before pouring into 9 cm diameter Petri dishes. After agar was solidified, a well of 5 mm using cork borer was made in the centre of each Petri dish. Volume of 1 mL of each tested solution (*Citrullus colocynthis* L.) extract individually or combined with different acids was poured into each well. In case of tested wool or cotton textiles treated with tested materials, one piece (1 cm square) was placed into the center of each Petri dish saturated with each tested suspension.

All Petri dishes were incubated for 5-7 days at 25±1°C for fungi, yeast and 30±1°C for bacteria, and then examined. This test was repeated three times and the inhibition was measured in (mm) for growth clear zone compared with the control.

### Measurements

### Infrared spectroscopy

Infrared spectra were recorded on FT-IR Nicolet 5 DX Spectrophotometer. The samples were examined as 1.5% K Br pellets.

### Scanning Electron Microscopy

The untreated as well as keratin/ECH treated fibres were mounted on aluminium stubs, and sputter coated with gold in an S150A sputter (coated Edward, UK), and examined by JEOL (JXA-840A) Electron Probe Microanalyzer (Japan).

### Tensile strength and elongation %

The tensile strength and elongation of fabric before and after treatment were evaluated using an Instron Tensile Tester (USA) according to ASTM D 76 Standard Specification for Textile Testing Machines. The average was taken for 10 samples (5 cm × 20 cm.

### Moisture regains

Measurements of moisture regain of the fabrics were performed using the standard ASTM method 2654-76 (1981). Moisture regain of the samples was calculated according to the following Equation:

Moisture regain % = $W_1$ - $W_2$ / $W_2$ ×100

Where: $W_1$ is the weight of sample (g) after saturation in the standard humidity atmosphere.

$W_2$ is the constant weight (g) of dry sample.

### Yellowness Index

Measurement of yellowness index was determined using Ultra scan Photometer (Hunter Lab) Made in USA.

### Washing durability (cycles)

Washing fastness tests were carried out according to BS1006: CO2 Test 2 with a soap solution (5 g/l, liquor ratio = 50:1) for 45 min at 48–50 ◦C **(** Achwall, 1985).

## RESULTS AND DISCUSSION

Natural extract from *Citrullus colocynthis* (L.) was screened for its antimicrobial activity against fungi (*Aspergillus flavus* and *A. niger*), gram positive bacteria (*Bacillus subtilis, Staphylococcus* sp.), gram negative bacteria (*Escherichia coli,* *Pseudomonas fluorescence*) and the yeast *Saccaromyces cerevisiae* by disc diffusion

method and the zone of growth inhibition were measured. Results of Table (1) show that the extract of *Citrullus colocynthis* has the ability to effect fungal and bacterial growth. However, *CC* extract has higher effect on fungal than bacterial growth, meanwhile no effect on the yeast was observed; therefore it is neglected from further studies. The highest inhibition zone (20mm) is observed for the growth of *Pseudomonas fluorescence*, *Escherichia coli* ($G^-$), and *Bacillus subtilis* ($G^+$). While *Staphylococcus* sp. ($G^+$) displays moderate inhibition zone which is measured as 18mm.

Inhibition zone of fungal growth measured as 17mm is recorded for both *Aspergillus flavus* and *A. niger.*

Wool and cotton fabrics treated with the finishes (*CC*) extract alone or combined with different innocuous acids were tested for their inhibition activity against the growth of bacterial and fungal isolates under *in vitro* conditions. Acids used include (As), (It), (So), (Be), (Sa), (Su), (Ci) and (Ac). Wool and cotton fabric samples treated with 1% g/l of *CC* extract alone or combined individually with the aforementioned acids and placed onto the centre of Petri plates containing the tested bacterial and fungal isolates. After incubation period, the clear zone of microbial inhibited growth was measured.

Table (2) and Figure (1) demonstrate that (*CC*) extract alone signifies more inhibition activity than its combination with the acids. Also, it is observed that bacterial isolates are sensitive to either (*CC*) extract alone or in combination with the acids than fungal isolates. The acetic acid (AC) treatment for wool and cotton fabrics reveals pronounced inhibition activity against all tested fungal and bacterial isolates. Concerning wool fabrics the measured inhibition zones of *A. niger* and *A. flavus* are 38, 38, 37 and 37mm at (*CC*) extract alone or combined with acetic acid, respectively. Meanwhile, *P. fluorescence* and *B. subtilis* exhibit growth inhibition zone measured as 13, 12, 14 and 12mm, in relevant order. This observation is also true for itaconic, sorbic and sulphamic acid, in descending order. It is important to note that treatment of either wool or cotton fabrics with all the tested acids monition high inhibition activity against bacterial isolates even more than the fabrics area (10mm square), meanwhile treatment of either wool or cotton fabrics induces only protection against invasion by fungal isolates. Acetic acid has just a single carboxylic acid group. It is made out of a methyl gathering ($CH_3$) that is connected to the carbon of the carboxylic gathering at wool textures. It also gives high stability after washing especially for resistance microorganisms. On the other hand, it is certain that acid treatment gives satisfactory results against *Pseudomonas fluorescence* ($G^-$) and *Bacillus subtilis* ($G^+$). The said acids assume the part of connecting through an amidification response with $NH_2$ gatherings of wool textures and (*CC*) which its constituent's in the dry natural products (Abdel-Kader et al., 2012) .Overall, the results indicate that treated wool or cotton with *CC* extracts alone or with certain (Be), and (Ac) acids and therapy impart high solidness for washing against fungi and Bactria as shown in Table (3). Table (3) reveals that the antimicrobial activity is not stable with increasing number of washing cycles up to 10 times either for treated wool or cotton fabrics. Using acetic acid for treating wool and cotton fabrics could keep their antifungal and antibacterial activity up to the tenth washing cycle.

**Table 1: Effect of *Citrullus colocynthis* (*CC*) extract on the growth of some microorganisms *in vitro***

| Microorganism | | Growth inhibition zone (mm) |
|---|---|---|
| **Fungi** | *Aspergillus flavus* | 17 |
| | *Aspergillus niger* | 17* |
| **Yeast** | *Saccaromyces cerevisiae* | 0 |
| **Bacteria** | *Bacillus subtilis* ($G^+$) | 20 |
| | *Staphylococcus* sp.($G^+$) | 18 |
| | *Escherichia coli* ($G^-$) | 20 |
| | *Pseudomonas fluorescence* ($G^-$) | 20 |

*No sporulation formed

Benzoic acid comes after which it keeps antibacterial activity up to 10 washing cycles, meanwhile it losses this activity as antifungal at the fifth washing cycle. The lowest inhibition active stability of the acid at the fifth washing cycle is recorded with citric acid against bacterial and fungal isolates. Stability of antimicrobial activity for wool and cotton fabrics treated with different (*CC*) *extracts* and acetic acid after ten washing cycles was examined. Total MEOH extract, Hexane fraction and methanol fraction obtained from (*CC*) extract were used in this test. Table (4) and Figure (2) depict a comparison between wool and cotton treated with three *Citrullus colocynthis* (L.) extract: (1: Total MEOH extracted, 2: Hexane fraction, 3: Methanol fraction) and acetic acid.

Obviously, processed cotton and wool display high stability and resistance to tested microorganisms.

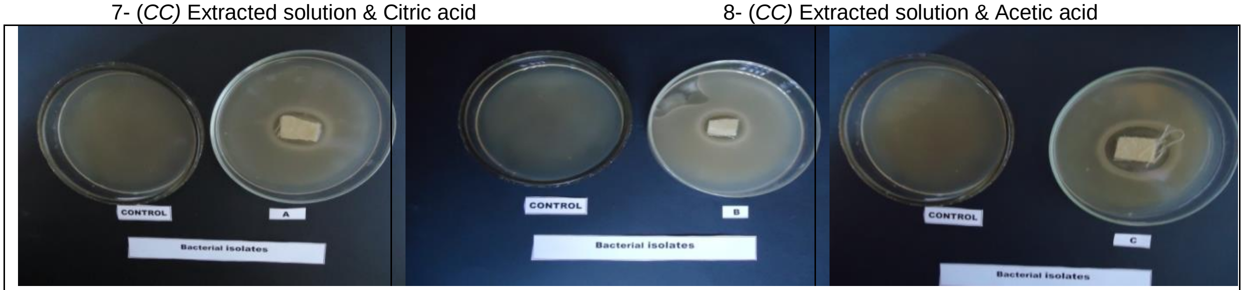
**Table 2: The growth inhibition zone of some microorganisms on wool or cotton fabrics treated with *CC* extract and different innocuous acids**

| Treatment* | | Microorganism growth inhibition (mm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Fungi* | | | | *Bacteria* | | | |
| | | | *Aspergillus niger* | | *Aspergillus flavus* | | *Pseudomonas fluorescence* ($G^-$) | | *Bacillus subtilis* ($G^+$) | |
| Fabrics | The Acids | Control | Extract | Treated fabrics | Extract | Treated fabrics | Extract | Treated fabrics | Extract | Treated fabrics |
| Wool | 1 | 90.0 | 17 | 0 | 17 | 0 | 18 | 15 | 20 | 17 |
| | 2 | | 40 | 0 | 36 | 0 | 19 | 17 | 20 | 17 |
| | 3 | | 35 | 0 | 32 | 0 | 20 | 21 | 24 | 18 |
| | 4 | | 33 | 28 | 31 | 27 | 21 | 23 | 22 | 25 |
| | 5 | | 0 | 0 | 0 | 0 | 13 | 16 | 15 | 30 |
| | 6 | | 0 | 0 | 0 | 0 | 18 | 22 | 20 | 27 |
| | 7 | | 0 | 0 | 0 | 0 | 23 | 22 | 26 | 24 |
| | 8 | | 38 | 38 | 37 | 37 | 13 | 12 | 14 | 12 |
| Cotton | 1 | | 0 | 0 | 0 | 0 | 18 | 12 | 20 | 12 |
| | 2 | | 40 | 0 | 36 | 0 | 19 | 12 | 20 | 12 |
| | 3 | | 35 | 0 | 32 | 0 | 20 | 12 | 24 | 12 |
| | 4 | | 33 | 0 | 31 | 0 | 21 | 12 | 22 | 12 |
| | 5 | | 0 | 0 | 0 | 0 | 13 | 12 | 15 | 30 |
| | 6 | | 0 | 0 | 0 | 0 | 18 | 12 | 20 | 27 |
| | 7 | | 0 | 0 | 0 | 0 | 23 | 15 | 26 | 24 |
| | 8 | | 38 | 0 | 37 | 0 | 13 | 16 | 14 | 16 |

**Treatment conditions:** Immersed wool and cotton fabrics in 1% g /L *Cc* and 1 g/ L different polycarboxlic acids (1-8) L.R: 1:50, pickup 90%, day at room temperature, fixed at 140 ˚C for 10 min.

The Acids where:

1- (*CC)* Extracted solution & Ascorbic acid
2- (*CC)* Extracted solution & Itaconic acid
3- (*CC)* Extracted solution &Sorbic acid
4- (*CC)* Extracted solution & Benzoic acid
5- (*CC)* Extracted solution & Salicylic acid
6- (*CC)* Extracted solution & Sulphamic acid
7- (*CC)* Extracted solution & Citric acid
8- (*CC)* Extracted solution & Acetic acid




**A) *Bacillus subtilis*** **B) *Escherichia coli*** **C) *Staphylococcus* sp.**

**Figure 1: Growth inhibition (mm) of some microorganisms in response to wool and cotton textile supplemented with *Citrullus colocynthis* (L.) extract and different innocuous acids.**

**Table 3: Growth inhibition of some microorganisms on wool or cotton fabrics treated with (*CC*) extract and innocuous acids after washing for one, five and ten cycles**

| **Treatment *** | | | **Microorganism growth (mm)** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | ***Fungi*** | | | | | | ***Bacteria*** | | | | | |
| **Fabrics** | **The Acids** | **Control** | ***Aspergillus niger*** | | | ***Aspergillus flavus*** | | | ***Pseudomonas Fluorescence* (G-)** | | | ***Bacillus Subtilis* (G+)** | | |
| | | | 1** | 5 | 10 | 1 | 5 | 10 | 1 | 5 | 10 | 1 | 5 | 10 |
| **Wool** | 4 | 90 | 28 | 0 | 0 | 27 | 0 | 0 | 23 | 14 | 14 | 25 | 14 | 14 |
| | 7 | | 21 | 0 | 0 | 22 | 0 | 0 | 18 | 0 | 0 | 17 | 0 | 0 |
| | 8 | | 38 | 10 | 10 | 37 | 10 | 10 | 18 | 12 | 12 | 80 | 18 | 18 |
| **Cotton** | 4 | | 28 | 0 | 0 | 27 | 0 | 0 | 23 | 12 | 12 | 0 | 0 | 0.0 |
| | 7 | | 21 | 0 | 0 | 20 | 0 | 0 | 12 | 0 | 0 | 18 | 0 | 0 |
| | 8 | | 38.0 | 10 | 10 | 37 | 10 | 10 | 12 | 10 | 10 | 80 | 36 | 32 |

*** 4- (*CC*) extracted solution & Benzoic acid**
**7- (*CC*) Extracted solution & Citric acid**
**8- (*CC*) Extracted solution & Acetic acid**

** Fabrics wash cycles

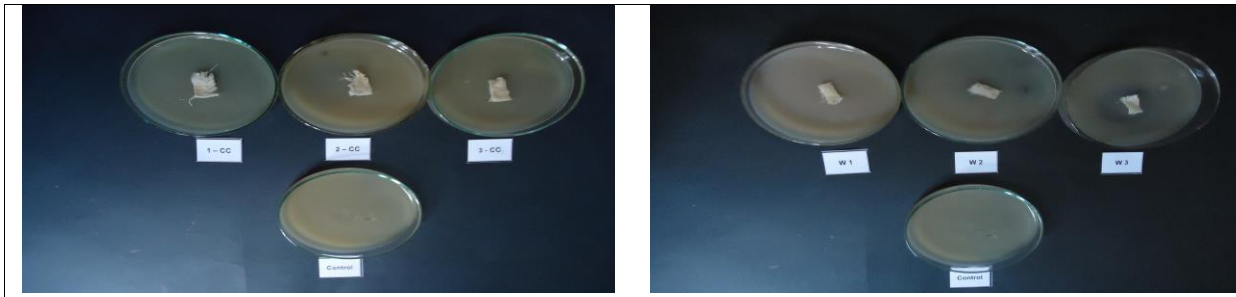
**Table 4: Growth inhibition (mm) of some microorganisms in response to wool and cotton fabrics treated with different extracts of *Citrullus colocynthis* and acetic acid after ten washing cycles.**

| **Tested materials** | | **Tested microorganisms** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Fungi** | | **Bacteria** | | | |
| | | | | **Gram positive** | | **Gram negative** | |
| | | ***Aspergillus niger*** | ***Aspergillus flavus*** | ***Bacillus subtilis*** | ***Staphylococcus* sp.** | ***Pseudomonas fluorescence*** | ***Escherichia coli*** |
| Unwashed cotton | 1* | 16** | 10 | 10 | 10 | 10 | 10 |
| | 2 | 14 | 10 | 10 | 10 | 10 | 10 |
| | 3 | 16 | 10 | 10 | 10 | 10 | 10 |
| Washed cotton | 1 | 0 | 10 | 10 | 10 | 10 | 10 |
| | 2 | 10 | 10 | 10 | 10 | 10 | 10 |
| | 3 | 14 | 12 | 10 | 10 | 10 | 10 |
| Unwashed wool | 1 | 22 | 16 | 10 | 10 | 10 | 10 |
| | 2 | 0 | 10 | 10 | 10 | 10 | 10 |
| | 3 | 0 | 10 | 10 | 10 | 10 | 10 |
| Washed wool | 1 | 10 | 10 | 10 | 10 | 10 | 10 |
| | 2 | 10 | 10 | 10 | 10 | 10 | 10 |
| | 3 | 10 | 10 | 10 | 10 | 10 | 10 |

**Treatment conditions:** Immersed cotton fabrics in 1% g /L (*CC*) & 1 g/ L different acids (1-8) L.R: 1:50, pickup 90%, day at room temperature, fixed at 140 ˚C for 10 min.

* 1 = Total MEOH extract 2= Hexane fraction 3 = Methanol fraction

**0= microorganism could not invade or overgrew on fabric pieces




Cotton fabrics Wool fabrics

*Escherichia coli*

**Figure 2: plates showing antimicrobial activity by agar diffusion method in response to wool and cotton fabrics treated with different extracts of *Citrullus colocynthis* (L.) and acetic acid as cross linking agent after ten washing cycles**

All tested extracts or fractions succeed to keep inhibition activity against fungal and bacterial isolates. Lower inhibition activity is observed when wool and cotton fabrics are exposed to 10 wash cycles as compared with unwashed fabrics. It is of interest to emphasize that cotton fabrics treated with methanol fraction have superior antifungal activity before and after washing as compared with other extract and fraction.

In order to impart highly functionalized properties onto cotton textile, specific chemical finishing treatments are one of the most important applications tested to achieve this. In the present study, (*CC*) extract alone as well as in combination with certain acids including (As), (It), (So), (Be), (Sa), (Su), (Ci) and (AC) are found to be the new range of chemicals used in imparting antimicrobial property on wool and cotton fabrics (Table, 2).

In this concern, acetic acid has just a single carboxylic acid group. It is made out of a methyl gathering ($CH_3$) that is connected to the carbon of the carboxylic gathering at wool textures. Hence, acidic is a powerless acidity, and it halfway separates in water. In fluid arrangements, acidic separates, discharging the hydrogen particle of the carboxylic gathering as a $H^{\pm}$ particle. This is the explanation behind the action of the acidic. Because of the nearness of – O-H bond in the carboxylic gathering. It also brings about high stability after washing especially for resistance to microorganism. On the other hand; all certain acid treatment gives satisfactory results against *Pseudomonas fluorescence* ($G^-$) and *Bacillus subtilis* ($G^+$).The carboxylic groups containing acids assume the part of connecting specialist through an acidification response with NH2 gatherings of wool textures. Overall, our results indicate that the treated wool and cotton fabrics with *CC* extracts alones or together with certain (Be), and (AC) acids and therapy yield high solidness for washing against fungi and Bacteria as shown in Tables (3, 4).

The work under investigation verifies the inhibition effect against the tested mould fungi and both gram positive and gram negative bacterial isolates. Results obtained make it evident that bacterial isolates are more sensitive than fungal isolates toward the ethanol extract of (*CC*). Meanwhile, no inhibition effect is observed concerning yeast isolate. Similar results are reported by other investigators. In this concern, (Bnyan et al., 2013) reported that Ethanol extract exhibits maximum inhibition effect against *Escherichia coli* (20cm) followed by *Proteus mirabilis* (16 cm) and *Staphylococcus aureus* (12cm). There is a least effect on *Streptococcus agalactia* (8 cm), while there is no inhibition effect on *Streptococcus pneumoniae* and *Klebsiella pneumonia*. Also, (Doss et al., 2011) reported that essential oil and methanol extract of *citrullus colocynthis* possess inhibition effect against bacteria $G^+$ *Staphylococcus aureus* and $G^-$ *Escherichia coli* and *Pseudomonous aeruginosa* as well as the fungus *Aspergillus niger.*(Najafi et al., 2010) stated that the preliminary qualitative phytochemical investigation showed *citrullus colocynthis* to consist of secondary metabolites such as saponins, tannins, alkaloids, glycosides and flavonoids .Also, tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them. Referring to these reports, our results also show that all tested fungal and bacterial isolates are sensitive to *citrullus colocynthis* ethanol extract. It is recorded that *Citrullus colocynthis* contains carbohydrate, protein, separated amino acid, tannins, saponins, phenolics, flavanoids, flavone glucosides, terpenoids, alkaloids, anthranol, steroids, cucurbitacins, saponarin, cardic glycoloids, trace elements and many other chemical groups. It possesses antioxidant, antimicrobial, anti-inflamatory and many other pharmacological effects against several microorganisms (Sadipo et al., 1991 and Linuma, et al.,1994).The *Citrullus colocynthis* seeds were studied *in vitro* .It is shown that antifungal and antimycotoxigenic activity of methanolic and aqueous extracts are screened against *Aspergillus ochraceus* and *Aspergillus flavus.*The results suggest that the extracts display a very good antifungal activity and antiochratoxigenic power in liquid medium. The inhibitory action could be ascribed to the presence of active compounds in the extract like glucosides and resins which inhibit enzymatic activity in cytoplasm membrane of fungal cell.

It is further reported that *Citrullus colocynthis* extract inhibits the growth of fungal isolates, due to the presence of the active compounds, *colocynthidin* and *colocynthin* alkaloids which may disrupt cytoplasm membrane of the microorganisms through their action on lipids and protein. Furthermore these compounds may penetrate cytoplasm membrane and compete with the active sites of certain enzymes inside the cell that are essential for multiplication of the microorganisms. It is as well to report that our results of wash durability test of both treated cotton and wool fabrics demonstrate significant antimicrobial activity up to 10 washes even after repeated wash cycles(Uma and Sekar 2014) (Jayaraman and Christina 2013, (Amine et al., 2013),(Greulach, 1973,Anthony, 1976 and Satianarayanan et al., 2010). These results are in a harmony of Satianarayanan et al., 2010, who confirmed that the wash of cotton fabrics treated with *Punica granatum* extract alone retained the antimicrobial activity only up to 5 wash cycles, which gradually decreased and became nil after 10 washes cycles.

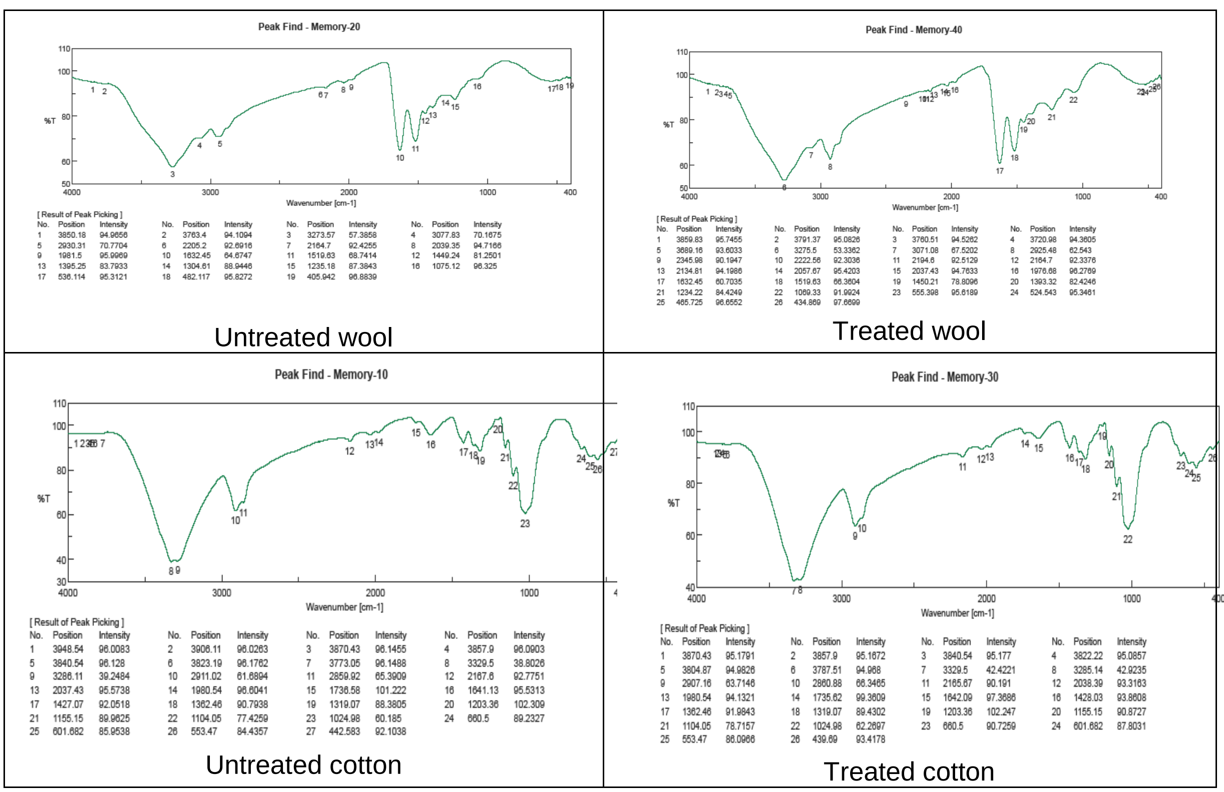
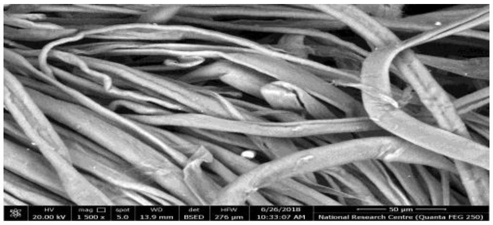
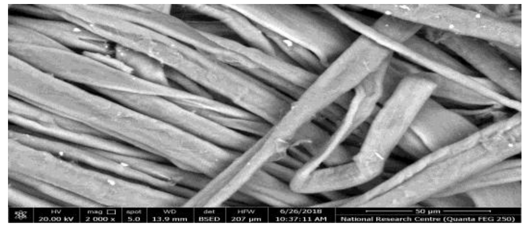
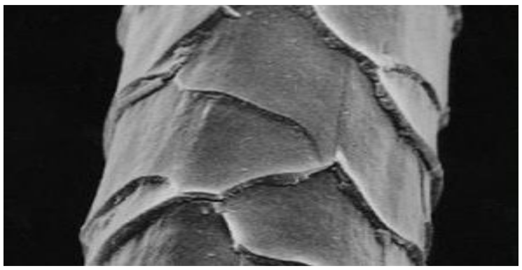


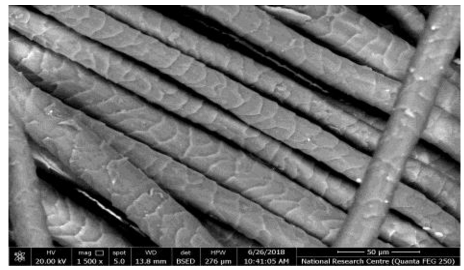

**Figure 3: The primary segments doled out from untreated and treated wool and cotton**

**Scanning electron microscopy**


Untreated cotton


Treated cotton


Untreated wool


Treated wool

## Examination of Principal Surface Groups by FTIR

Figure (3) demonstrates the doled out of the primary segments from untreated and treated wool and cotton. It is a proof of an expansion in the area band of OH for treated wool at harmony and move toward becoming agonized and moved to higher wave number at 3689.16 cm-$^{1}$.The top at 3275.5 cm-$^{1}$ relates to the O-H extending vibration.

The band at 2925.48 cm$^{-1}$ appears obviously as a groove, indicating the presence asymmetric H−O−H stretching vibration. Also the region from 2925.48 cm-$^{1}$ to 1632.45 cm-$^{1}$ is compressed and trend to H-OH bending over all clearly extending vibration at 1632.45 cm-$^{1}$ instruct to H-OH bending. The peaks increase at 1234.22 and 1069.33 $^{1}$ and stretching vibration are attributed to the region of C−O-C, indicating the presence of an ether group. Figure 4 illustrates the treated cotton peak at 3286.11 and peak at 2859.92 is shifting obviously to 2907.16 and 2165.87 respectively (H-O-H bending). Peak at 2037.43 is starching to 1980.54 (C-H (2000-1700). Peak at 1980.54 (C-H) is starching to 1735.62 (C=O (1750-1730). Peak at 1736.58 (1750- 1705 Aliphatic stretching) shifts to 1642.09 (C=O (1730-1705 Aromatic). Also peak at 1641.3 1 shifts to 1428.03(C-O-H). On the other hand, peak at 1024.98 (Alkenes) shifts to 660.5 (Alkynes). In conclusion FTIR depicts the big change and shift of groups from O-H hydroxyl group to H-OH and C-O-C. It illustrates that a connection between the fiber and the CC is finished by the gatherings of hydroxyl acids. Figures 4, 5, 6, and 7show the scanning electron micrographs of the treated and the untreated wool and cotton fabrics. As is evident, there is remarkably significant change in the scales of wool due to the treatment with *Citrullus colocynthis* (L.) extract as well as in combination with acetic acid in comparison with the untreated wool. The scales disappear completely and a smooth surface was created. In case of cotton fabric, there is just rearrangement at the surface to give ultimately a smooth surface.

From the results of table 5, it can be observed that tensile strength and the moisture regain of the treated wool and cotton fabrics are lower than those of the untreated substrates. This may be due to the existence of carboxylic groups in the finishing agent which provides a new pathway for incorporating functionality into fabrics. On the other hand, results of fabric elongation % remain intact.Meanwhile the yellowness of treated fabrics is higher after than before the treatment.

## CONCLUSION

*Citrullus colocynthis* (L.) extract alone or together with certain innocuous acids are considered eco – friendly non- toxic natural materials with promising future for multi functionalization of natural textiles, notably cotton and wool. Indeed, the function and performance of textiles which strike the attention of humun being are unequivocally the ability of textiles, particularly those made of natural fibres , to exert antimicrobial activity. Current research and the outcomes brought thereof are speaking of this concern. When wool and cotton fabrics were treated with the said extract in absence and

Presence of certain innocuous acids, the inhibition activity of thus treated fabrics was found positive against tested gram positive, gram negative and fungi, after ten wash cycles. Based on these findings, antimicrobial activity of such as – treated fabrics can be commercially utilized. These microorganisms could attack several wet or dry vegetables, onion, peanut, etc. Hence it is recommended to use wool and cotton, processed with such environmentally friendly materials in the bags used to fill these agricultural crops.The adverse effect of the present processing treatment on tensile strength and moisture regain of the treated wool and cotton fabric is a manifestation of the presence of carboxylic gatherings as completing operator gives another pathway to joining usefulness into fabrics. It is also reported that the yellowness of treated fabrics is higher after than before the treatment.

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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## AUTHOR CONTRIBUTIONS

All authors contributed in collecting and analyzing data. All authors participated in writing every part of this study. All authors read and approved the final version.



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