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Research Article

Development of Naturally Coloured Cotton Fabric Based Bio-Medical Textiles for Human Wellness Behaviour Using Chitosan, Catechu and Eucalyptus Leaves as Natural Bio-Chemical Agents

Pubalina Samanta[1]*, Asis Mukhopadhyay[2] , Amiya Kumar Sinha[2], Adwaita Konar[3]

[1]Rani Birla Girls' College, 38 Shakespeare Sarani, Kolkata-700 017, WB, India
[2]Dept of Jute and Fibre Technology, University of Calcutta, 35, B C Road, Kolkata-700 019, WB, India
[3]Govt. College of Engg. & Textile Technology, Serampore,12, W C Rd, Serampore, Hooghly-712201, WB

***Corresponding author:** Pubalina Samanta
*Email-:samantapubalina@gmail.com

Abstract
The cotton cellulosic fabric was initially pre-oxidised with $K_2S_2O_8$ and then pre-treated with chitosan for its bio cationization to make it substantive to fix anionic catechu natural dye, with or without pre-mordanting with natural potash alum, to enhance colour yield, uniform dyeing and improved colour fastness to wash and finally to utilize inherent antimicrobial nature of alum (fitkari), chitosan and catechu natural dye. Further, post-dyeing bio-finishing treatment of such chitosan-based bio-cationized and catechu-dyed cotton fabrics was accomplished using eucalyptus leaf extract by pad-dry-cure technique to obtain a strong antimicrobial and UV -resistant coloured cotton fabrics as medical textiles. Detailed studies indicate that only potash alum treatment before dyeing has a good antimicrobial character. While catechu dyeing of alum pre-mordanted cotton shows that the said antibacterial effect is partly reduced probably due to the consumption of alum in fixing the catechu dye forming cellulose-alum-catechu complex. However, the lesser antibacterial criteria are shown for gram-vet bacteria than that for gram +ve bacteria in all the cases, due to the repulsion of anionic catechu dye against gram-ve bacteria (Klebsiella pneumonia). Maximum UPF value up to 25 is obtained after dyeing of chitosan-based bio-cationized pre-oxidised cotton with catechu without K-alum pre-mordanting, While chitosan based bio cationized cotton sample with K-alum pre-mordanting and catechu dyeing show somewhat higher antimicrobial criteria and UV-resistance property showing maximum 80.2 % reduction in gram -ve bacteria growth and showing maximum 85.4% reduction in gram +ve bacterial growth, showing UPF value up to 30. Further improvement of both antimicrobial and UV resistant properties are obtained after finishing the same with 10% aqua-ethanolic extract of eucalyptus leaves in acidic media (using lemon juice) showing a 90.0% reduction in bacterial growth and achieving UPF value up to 40. Thus, this work provides a newer route of eco-friendly pre-treatment, dyeing and finishing natural resource materials to cotton to produce bio-medicated coloured antimicrobial and UV-resistant cotton fabric for the development of different bio-medical textile products like surgical gowns, wound healing pads etc.

Keywords: Antimicrobial property, Alum (potash alum), Bio-Cationization, Chitosan, Catechu, Cotton, Eucalyptus Leave extract, Medical Textiles, UV Protection Factor (UPF values).

***Author for correspondence: Email:** *samantapubalina@gmail.com*

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1.0 Introduction

One of the important areas of performance-based technical textiles is medical textiles for different end uses. Hence, the development of natural fibre-based technical textiles dyed and finished with natural resources is the most targeted area of current research to produce bio-medical textiles. The role of natural colouration materials for natural dyeing and antimicrobial finishing has received immense demand amongst current researchers for use of appropriate natural dye and natural finish on natural fibre-based textile materials, which require a careful balance of colour yield and finishing performance either by using selective natural dye having antimicrobial attributes or using separately or simultaneously selective natural dye and natural finishing agents applied through natural resource-based bio -mordants (fixer) having good compatibility between such natural dye and natural finishing agents. Hence nowadays, bio-mordanting/biochemical modifications, bio-dyeing and bio-finishing (Samanta A K,2020) of natural fibre-based textiles / organic textiles have become an important and newer area of current research.

As most of the natural dyes are non-substantive, an additional step of mordanting/biochemical modification of textile substrate is essential to fix natural dyes/ natural finishes to any natural fibre-based cellulosic textile materials. Earlier used some metallic mordants like Copper sulfate, potassium dichromate, etc are not eco-safe and hence are not being used nowadays. Hence, there is a continuous search for an alternative route (for not using or minimizing the application of metallic mordants that are not eco-safe) of finding efficient natural resource-based bio-mordants/ bio-chemical modifying agents as fixing agents for non-substantive natural dyes /natural finishes to improve their wash fastness with or without attributing different functional finishing criteria like antimicrobial/Uv resistant properties in it. Selective natural bio-mordant or natural resource-based modifier/natural dye fixer used for initial biochemical modification /pre-bio-mordanting for fixing such functional performing natural dyes or natural finishing agents may provide natural fibre based cotton or other textiles better colour yield, dyeing uniformity, better wash and light fastness of colour and finally exhibiting required functional performance level for antimicrobial and /or UV resistant properties embedded in it for use of those textiles for biomedical applications.

Worldwide, consumers are looking for eco-friendly and sustainable clothing and other natural resource-based technical textile products that provide a higher degree of uniform colour yield/reproducible shades with acceptable colour fastness having required hygienic wellness (anti-microbial freshness), and odour-free characteristics (Shahid and Mohammad, 2013).

Hence it is thought appropriate in the present work that utilizing chitosan and natural potash alum as natural bio-mordants for dyeing with extracted natural colourants and associated extractable materials from catechu as tannin-based natural dye may facilitate obtaining quality antibacterial coloured cotton fabrics subsequently further finished with eucalyptus leaves extract produce uncommon sober shade and antimicrobial hygienic properties with reasonable UV resistant criteria for use as biomedical textile fabrics as efficient medical textiles for maintaining wellness behaviour.

In this endeavour, *chitosan with or without potash alum (fitkiri)* as a bio-chemical mordant, *Acacia catechu* as a natural colorabt and eucalyptus leaves (EL) extract as a natural resource based finishing agent are considered as good choices envisaging the benefits of their uniform high colour yield with excellent antimicrobial medicinal values having UV resistant criteria too to serve as excellent bio-medical textiles to meet the global needs of eco-friendly coloured sustainable medical textiles (Khan et al, 2011)

2 Materials and Methods

2.1 Fabrics:

Fabric: 100% Cotton-Cellulose fabric having plain weave structure after desized, scoured, and bleached pre-treatment with 84 ends per inch (200 ends per dm) with a warp yarn count of 9.8 tex (60Ne) and 74 picks per inch (190 Picks per dm) with weft yarn count 10.7 tex(55Ne), having fabric areal density of 145 g/m^{2}and thickness 0.25mm was used.

2.2 General Chemicals: Commercial grade **a)** acetic acid (CH_3COOH) and b**)** sodium acetate and Sodium acetate -acetic acid buffer *for adjusting pH4-4.5 to 5* and (c) 1% NaOH Solution d) Potassium Per-sulphate ($K_2S_2O_8$) etc, were procured from E-Merck (India)**.**

2.3 Chitosan

Chemically chitosan is a linear polysaccharide (β-1-4 N-acetyl -2-amino-2-deoxy-D-glucose), which is not easily soluble in water. Hence. An alcoholic hot aqua-acidic mixture is necessary for dissolving chitosan. The presence of acid helps the protonation of the -NH_2 group of chitosan. So, in this present work, normal chitosan powder (90% de-acetylated medium molecular weight variety) was put in 10% acetic acid solution for batching 12 hrs and was then heated at 90^0 C for 30 min in acetic acid media of 50:50 water and isopropyl alcohol mixture, to obtain the required % chitosan solution for use.

The chemical formula of chitosan (90% deacetylated chitin) is given below in **Fig-1.** Which has well well-known antibacterial /antifungal effect (Inmaculada et al -2021) for its linear bio-compatible amino functional natural bio-polymer, convertible to quarternary compounds structure in the presence of acids.

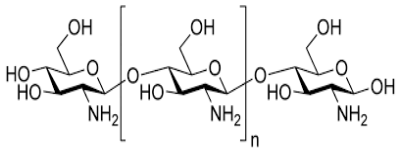


Fig-1: Chemical Structure of Chitosan

2.4 Potash alum (Fitkari), a natural resource-based metallic-mordants: Potash Alum, (K-Alium) i.e. simply as K.Al $(SO_4)_2$, $12H_2O$) called popularly Fatkiri, as an antiseptic agent having antimicrobial values) was used as one of the naturally resourced metallic mordants.

2.5 Catechu (used as a natural colorant):
Catechu (*Acacia catechu*) has its inherent antibacterial /antiseptic medicinal criteria with reasonable tannin content and it has been used from ancient history for producing Ayur-vastra. A hot aqueous solution of purified catechu extract contains catechin epi-catechin (2%-12%), and quercetin (10-12%) besides the presence of 22-30 % Flevo-tannins (catechu-flavanoids/ catechuic acid) including the low quantity of catechu red (catechol), the chemical formula of which is given in **Fig-2** as components of catechu (Samant, L.,2022 and Datta, M, 2014) In the present work, hot aqueous extraction and ethanol-water (50:50) mixture extraction after pre-soaking catechu powder /cake was used. Phytol, polyphenolic compounds saponin, etc. as antioxidants, are extractable in water-alcohol mixture-based extraction of catechu. (Samanta P, 2024)

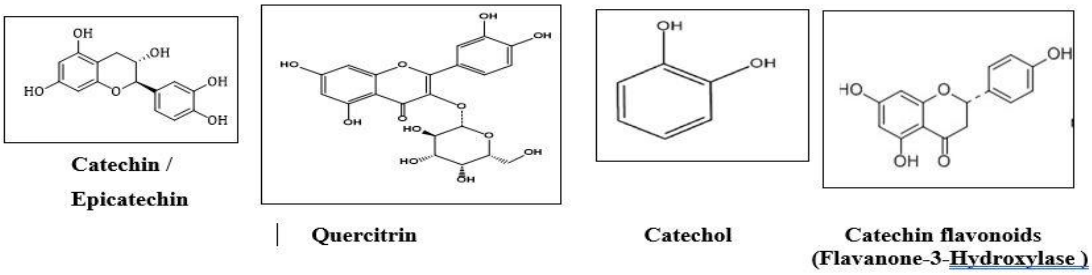


Fig-2: Chemical structure of colour components of catechu natural dye by hot water extraction.

2.6 Extraction and purification of Catechu extract:
In the case of aqueous extraction, catechu powder /cake was initially pre-soaked in alkali for 1hr. (Khan et al, 2011) and Samant, L.,2022) to make it easily soluble in hot water and was further heated to raise temperature to 60-70°C for 60 min, with Catechu: water ratio (MLR) 1:20 at alkaline pH 10, by addition of required quantity of 1% NaOH solution (Samant, L.,2022 and Datta, M, 2014) and subsequently was double filtered in 60 mesh nylon cloth followed by filtration in Whatman 40 filter paper. Finally, the resultant aqueous catechu extract was neutralized with CH_3COOH to bring the pH to 5, to obtain ready catechu-extracted aqueous dye liquor with 30 % catechu extract (based on bone-dry weight of catechu powder). For specific application purposes, catechu dye powder was also extracted in the same process by using a mixture of Ethyl alcohol and Water (50:50) at 70^0C for 60 min with MLR 1:20. To obtain an aqua-alcoholic extract of catechu.

For purification, finally, the catechu extract/solution was dried in a vacuum oven at 50^0C for 2-6 hrs until fully dried to obtain a fully dried catechu dye powder of constant weight. This dried powder catechu dye extract was further purified by subjecting it further to a distillation and extraction process for 6 hrs for 10 cycles in soxhlet apparatus using ethyl alcohol+benzene mixture (50:50). Thus, it was found that only 3% solid pure catechu components are there, after evaporation and oven dry weight to constant weight, i.e 3 gm of pure dry catechu colour components is obtained from 30% aqueous extract.

2.7 Pre-oxidation of Cotton fabrics
Control cotton fabric was initially subjected to mild pre-oxidation treatment to generate few aldehyde groups in cotton celluloses by treatment with 1% $K_2S_2O_8$ solution at room temperature (27±3°C) for a period of ½ hr batched with solid chemical: water ratio (MLR) 1:20, to obtain a very low degree of pre-oxidized cotton fabric (oxy-cotton) particularly as required for preparatory step before bio-cationization of this oxy-cotton.

2.8: Chitosan initiated biochemical modification /bio-cationization of cotton:
Biochemical pre treatment called bio-cationisation of cotton cellulose was accomplished using chitosan as a biochemical agent from a natural source (9012-76-4, extra pure, medium MW with min 90% deacetylation, HSN code 29211990), which was obtaibed from reputed chemical supplier M/S Sisco Research Laboratories (SRL) Pvt Ltd, India. For bio-captioning cotton to attract and fix catechu as an anionic natural dye under salt-free eco-friendly conditions.
Scoured and H_2O_2 oxidative bleached cotton cellulose fabric was bio-cationized by the above-mentioned by presoaking pre-oxidised /only bleached cotton cellulose fabric by treatment with acidified (adding % acetic acid) aqua-alcoholic hot solution of required % of chitosan solution applied by pad-dry-cure process, with addition

of 1/5th acidic catalyst ($MgCl_2$) aimed to enhance colour yield, uniform dyeing and improved colour fastness under salt-free eco-friendly natural dyeing and bio-finishing of chitosan modified bio cationized cotton.

2.9 Pre-mordanting with Natural potash alum (Fitkari):

Both control scoured bleached cotton and also the above said chitosan pre-treated bio-cationized cotton fabric samples were subjected optionally and separately to pre-mordanting with 10% potash alum solution at 60ºC for 30 mins using MLR 1:30. Followed by air-drying without any wash for subjecting them to dyeing with 30 % or the required % of catechu dye liquor at comparable and standardized conditions of dyeing, if not otherwise mentioned.

2.10 Dyeing of both control scoured and bleached cotton and Chitosan Bio-Cationized Cotton Fabrics with or without natural potash alum pre-mordanting

Dyeing of both scoured and bleached control cotton and chitosan pre-treated bio-cationized cotton fabric samples with or without any pre-mordanting with potash alum natural metallic mordant was carried out separately using 30% aqueous extract of catechu at pre-fixed standard dyeing conditions. The said catechu dyeing was accomplished using a lab model of IR dyeing machine (RBE, India make), by raising the required temperature of dyeing gradually from 30 to 70^{0}C for continuing dyeing for 60 min. Without the addition of any salt following pre-fixed standard dyeing conditions as studied and derived earlier for dyeing of cotton cellulose using catechu as a natural colourant using gall-nut plus K-alum dual pre-bio-mordanting published already (Samanta P, 2024) maintaining dye bath pH 5 (by using sodium acetate + acetic acid buffer), dye: liquor ratio (MLR) 1:20, time of dyeing 60 min and temperature of dyeing 70ºC without any salt.

After the dyeing was over, all such catechu chitosan modified bio-cationized cotton fabric samples were washed thrice in running tap water followed by mild soap-washing with 2 gpl neutral detergents at 50°C for 15 min for removal of loosely adhered (unfixed) catechu dyes, if any.

2.11 Aqueous and Aqua-Ethanolic Extraction of dry Eucalyptus leaves

Aqueous extract of eucalyptus leaves (EL) as a natural resource-based microbial resistant and UV resistant finishing agent, while water: ethanol (50:50) mixtyre extract of EL is supposed to have strong antimicrobial and strong UV absorption cum light fastness improver criteria. Hence, both *Aqueous* as well as *Aqua-Alcoholic (Ethanolic) Extraction*: of EL (Eucalyptus leaves) was carried out separately as follows:

Natural Materials	**Extraction method**	**λ_{max}**	**pH**	**MLR**	**Time (min)**	**Temperature (°C)**
Eucalyptus leaves (Eucalyptus globulus Labill.)	Aqueous	420 nm	9	1:20	60	80
	Water; Ethanol mixture (50:50)	418 nm	5 to 5.5	1_10	30	50

2.12 Post Dyeing finishing of chitosan + alum pre-treated catechu cotton with Eucalyptus leaves extract

Post-dyeing finishing of chitosan pre-treated and further alum pre-mordanted followed by catechu dyed cotton was carried out by applying both types of eucalyptus leaves extracts separately with the use of 2% lemon juice as natural acidic catalyst (2% lemon juice) by pad (at 100% weight pick up) - dry (at 100 ^{0}C for 5 min) and cure (at 120^{0}C for 3 min) technique.

3.0 Testing and Evaluation Methods:

3.1 Reflectance, K/S Value, Colour Difference, and related other colour interaction parameters

K/S values of the selected fabric samples [control scoured and bleached cotton, chitosan pre-treated bio-cautioned cotton with or without K-alum mordanted and subsequently dyed with 30% aqueous extract of catechu and post-dyeing finishing with 10% Aqueous and aqua-ethanolic extract of EL (eucalyptus leaves) applied on chitosan-based bio-cationised and catechu dyed cotton fabric] was assessed using UV -VIS reflectance spectrophotometer [make -Premier Colorscan, Mumbai, India] following Kubelka- Munk equation (Kubelka-P, 1948 & Kubelka, 1954).

$$K/S = \frac{(1-R_{\lambda max})^2}{2R_{\lambda max}} = \alpha \; C_D$$

Where, (K), (S), ($R_{\lambda max,)}$, (ΔH), (ΔC), (ΔL) and (ΔE) etc as important colour parameters [where K is the coefficient of absorption; S, the coefficient of scattering; $R_{\lambda max,}$ is the Reflectance value at maximum absorbance wavelength, the C_D is concentration of dye and α is the constant for a particular system, (ΔH) changes in hue, (ΔC) changes in chroma, (ΔL) changes in lightness and darkness and (ΔE) is total colour differences etc] were measured as per CIE-Lab 1976, standard formula [Shah and Gandhi, 1990 and Samanta A K, 2024] using same UV -VIS reflectance spectrophotometer.

(MI) i.e. Metamerism-Index shows the difference between a pair of samples under two different illuminants /instruments / measuring conditions etc by CIE based LABD based metamerism index formula following the given equation below [Shah and Gandhi, 1990 and Samanta A K, 2024].

$MI_{(LABD)} = [(\Delta L_1^* - \Delta L_2^*)^2 + (\Delta a_1^* - (\Delta a_2^*)^2 + (\Delta b_1^* - (\Delta b_2^*)^2]^{½}$

ΔL_1^*, Δa_1^*, and Δb_1^* are the Delta CIE Lab* colour coordinates between the standard and produced sample [Shah and Gandhi,1990 and Samanta A K, 2024].
(BI) was also assessed using ISO-2469 and 2470 equation (ISO-2469 and 2470, 1977) as follows:

$$\text{Brightness Index} = \frac{\text{Reflectance Value of the Sample at 457 nm}}{\text{Reflectance value of Standard white diffuser (white tiles) at 457 nm}} \times 100$$

3.2 Determination of Color Difference Index (CDI) value

The Colour Differences Index (CDI) between the sample and standard was also calculated using the following empirical formula available from earlier literature (Samanta A K et al, 2009).
CDI= ΔE X ΔH/ ΔC X MI

3.3 Evaluation of Color Fastness Properties:

Colour fastness to wash as per ISO-II of BIS method (BIS : IS: 3361-1979), Colour fastness to dry rubbing and wet rubbing, as per BIS standard method (BIS: IS: 766-1984) and Colour fastness to UV-light, using MBTF fade-o-meter along with 8 blue wool standard swatches (BS 1006: BOI: 1978), as per relevant BIS standard method (BIS: IS: 2454-1967) were assessed for selected dyed cotton fabric samples following said standard methods.

3.4 Assessment of Anti-microbial Properties of Textile Materials:

Microbial resistance property of selective cotton fabric samples was evaluated according to the relevant AATCC standard method (AATCC-100-2012) using (i) *Klebsiella pneumoniae*: AATCC 4352 (Gram+ve bacterial species) and also using (ii) *Staphylococcus aureus*: AATCC 6538 (Gram -ve bacterial species) mounting the samples on suitable Petri plates incubated at standard conditions at 37°C incubated for 24 h followed by counting of bacteria colonies/assessment of the per cent reduction of areas of bacterial growth separately for each sample using the following formula :

$R\ (\%) = (A - B)/A \times 100\%$

where R represents the % reduction of bacterial growth under test conditions and A and B are the area or numbers of counted bacterial colonies assessed after the test for each sample separately. These tests were done by BTRA and ICAR-CIRCOT, Mumbai.

3.5 Assessment of UV Protection Factor (UPF):

The UV protection factor (UPF) of selective samples was evaluated according to AATCC: 183: /2004 (revised in 2010/2014) method, using Lab-sphere Inc.-USA make UV-Transmittance Analyzer, was calculated as per the following equation (Gies, P.H et al, 2000) :

$$UPF = \sum_{290}^{400} E_\lambda S_\lambda \Delta\lambda \Big/ \sum_{290}^{400} E_\lambda S_\lambda T_\lambda \Delta\lambda$$

Where E_λ represent the relative erythemal spectral radiance, S_λ represents the solar ultraviolet radiance I,e UV spectral irradiance strength in $W.m^{-2}.nm^{-1}$, T_λ represents the spectral UV transmission through the fabric, Δ_λ represents the bandwidth of spectral radiance in mm, and λ is the wavelength of spectral radiance in nano-metre (nm).

3.6 Method of phytochemical analysis

The existence of alkaloids, and tannins. flavonoids and phenols, terpenoids, and saponins etc in the aqueous and aqua-ethanolic extracts of eucalyptus leaves abbreviated here as EL used as a natural resource-based bio-finishing agent were identified as per the standard procedure described by Harborne (Harbone J B 1988/1998) for qualitative phytochemical analysis as follows:

(a) *Identification of Alkaloids*:

(i) Each (2 ml) of the two types of eucalyptus extract as a bio-finishing agent was separately boiled with 2% hydrochloric acid and then it was filtered. The filtrate was afterwards treated with 2 drops of Dragendorff's reagent (potassium bismuth iodide) and the presence of alkaloids was detected by the formation of red precipitate in it.

(ii) A white or cream precipitate with the use of Mayer's reagent (mixture of potassium iodide and mercuric chloride) indicates and confirms the presence of organic alkaloids.

(iii) The presence of alkaloids was reconfirmed with Wagner's Reagent (iodine and solution of potassium iodide) test by forming a reddish-brownish precipitate in it.

(iv) Finally, the presence of alkaloids can be reconfirmed by the appearance of a Precipitate of yellow colour, if 1% picric acid is added to it.

(b) Identification of Flavonoids: 0.2 ml EL extract used here as the bio-finishing agent was boiled for 2 min in water containing ethyl-acetate and filtered to use in the following tests to determine the presence of flavonoids.

(i) Appearance of more yellowish tinge/colour in the EL extract after sequential addition of 5 ml of oil. 1% ammonia liquor and subsequently sulfuric acid (concentrated) added in sequence indicate the presence of flavonoids;

(ii) The appearance of a more yellowish tinge/colour in EL extract indicates the presence of bio-flavonoids or flavonoids after /with the addition of a few drops of 1% aluminium chloride to EL extract.

(c) Identification of Tannins:
2 ml EL extract was boiled with 45% Ethyl alcohol for 5 min and then it was cooled and filtered. The Ethanolic filtrate was then treated with 0.1% ferric chloride, where if it develops a brown-green or blue-black ink-like colour in the Ethanolic EL filtrate, it indicates the presence of tannins in it.

(d) Identification of Terpenoids:
2 ml Chloroform was separately mixed with 5 ml of EL extract. The appearance of a reddish-brown precipitate, forms on adding 2 ml of H_2SO_4 (conc) indicating the confirmatory detection of terpenoids.

(e) Identification of Saponin:
0.1 ml of EL extract after boiling for 5 min in pure distilled water (say 20 ml) was filtered. In addition to a few drops of pure olive oil the EL filtrate is vigorously shaken and it forms a good milky emulsion by this action, it indicates the presence of saponin in it.
This phytochemical analysis of selective untreated / treated samples was done at JDBI, Kolkata.

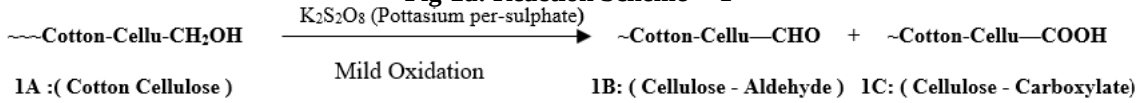
3.7 Method of LC-MS scanning and analysis
Identifiable major organic matters present in EL extract (E. globulus) were separated using Dionex ultimate 3000 ultra-HPLC instrument with Xselet HSS T3 (150 mm × 2.1 mm, 3.5 mm) column (Meng Pan, 2019). The temperature of the chromatographic column used was 30^{o}C and the sample mounting tray temperature used was 5^{o}C. In this ultra-HPLC instrument, the mobile phase used was acetonitrile phase-A and 0.1% formic acid solution was used as phase B. The flow rate used was 0.2 mL/min and the injection volume was 10 ml for each sample.
Final LC-MS peak different components of EL extract were evaluated using NIST0 Software MS-library. The presence of separated major components of EL extract was qualitatively analyzed using the reference scan/peaks available from relevant digital library/literature of MS peaks of Eaxh element/compounds in the said NIST0 Software MS-library and as per peak height etc, the relative content was quantified from observed peak lines for both aqueous and aqua-ethanolic EL extracts.

3.8 Study of FTIR spectra:
FTIR (Fourier transforms infrared spectroscopy) spectral scan of bleached and peroxidized Cotton (Oxy Cotton) and chitosan bio-cautioned cotton with catechu dyeing and Catechu dyed chitosan based bio cationized cotton after bio-finished with 10% aqua-ethanolic EL extracts for analysis of changes in functional groups content using SPECTRUM-TWO model FTIR instrument (Perkin Elmaer, Singapore make) The FTIR spectral scan was recorded at 500 to 4,000 cm^{-1} using standard KBR pellets method (F.Mizi et al, , 2012) showing each FTIR spectral scans as an average of 10 repeated scans.

4.0 Results and Discussion
4.1 Reaction mechanism of Bio-cationization of cotton with chitosan and subsequent dyeing with catechu.
Scoured and H_2O_2 bleached cotton fabric is when pre-oxidized by applying 1% $K_2S_2O_8$, this mild pre-oxidation reaction of cotton cellulose converts few ~~ Cellu-CH_2OH group of cotton cellulose, to generate few ~~Cellu-CHO group, making it amenable to react with chitosan containing an amino group. The possible reaction mechanisms of bio-cationization of cotton cellulose with chitosan are shown in the **Reaction schemes 1, 2, and 3** in **Fig -2a, 2b, and 2c.**

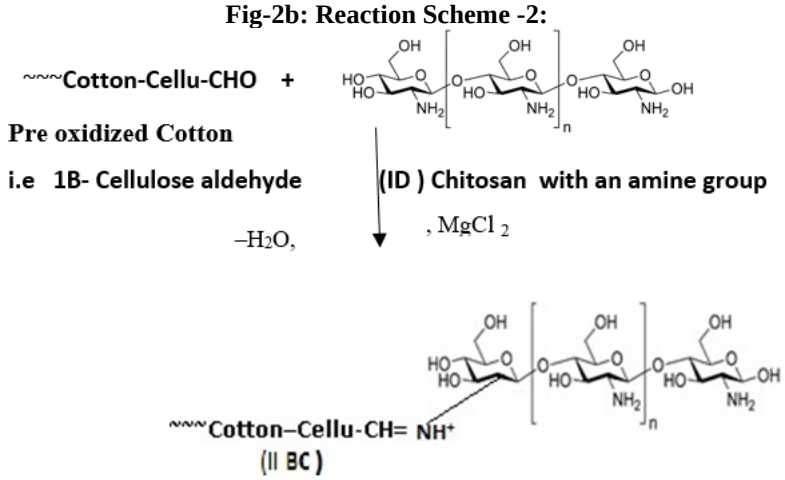
Reaction scheme-1 (**Fig-2a**), indicates the formation of the aldehyde groups in cellulose.

Fig-2a: Reaction Scheme -- 1



Already, scoured and H_2O_2 bleached cotton cellulose contain very few nos of aldehyde groups (~~~Cotton -Cellu-CHO) due to oxidative bleaching action on cotton cellulose, and 1% $K_2S_2O_8$ (Pottasium per-sulphate**)** pre-treatment at room temperature for 30 min helps to generate more nos of ~~~Cotton -Cellu-CHO groups by initial mild pre-oxidation treatment and this pre-oxidized cotton was used as control cotton fabrics for further bio-cationization treatment, with chitosan to attach with cellulose aldehyde to form an adduct via producing a cationic aldimine group functionality, as indicated in **Reaction-scheme-2 in Fig -3b.**

Fig-2b: Reaction Scheme -2:



Reaction scheme 2 thus shows that NH_2.groups of chitosan (1D) attach with the cellulose-aldehyde group to form an aldimine group when reacted with $K_2S_2O_8$ pre-oxidized cotton cellulose by reaction between the cotton -cellulose-aldehyde and the amino group of chitosan, forming an adduct (II BC) with addition of acid/ $MgCl_2$

With an acidic catalyst or by the addition of acids later, it helps to generate a protonated -$CH=NH^+$ - aldimine group as the new cationic functional group incorporated in cotton cellulose, producing chitosan-modified bio-cationized cotton, as shown in reaction-scheme-2 **in Fig-2b**.

Next, the dyeing mechanism in different ways is depicted in **Fig-3** (a, b and c) stating dyeing reaction mechanism 1, 2 and 3 as follows**:**

Fig -3: Mechanism of Dyeing: Cotton and Oxy-Cotton + Chitosan + Al (K-Alum) + Catechu (Catechin):
Fig- 3a: Dyeing Reaction Scheme -1:

Dyeing of Chitosan Bio-Cationised Cotton with anion of Catechin dye molecule of Catechu without the addition of potash alum pre-mordanting

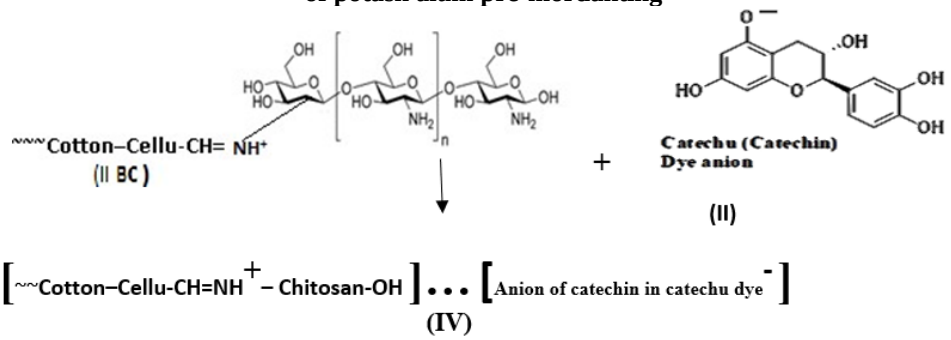


Fig 3b: Dyeing Reaction Scheme 2:
Dyeing of Chitosan Bio-Cationised Cotton with potash alum pre-mordanting and anion of Catechin dye molecule of Catechu

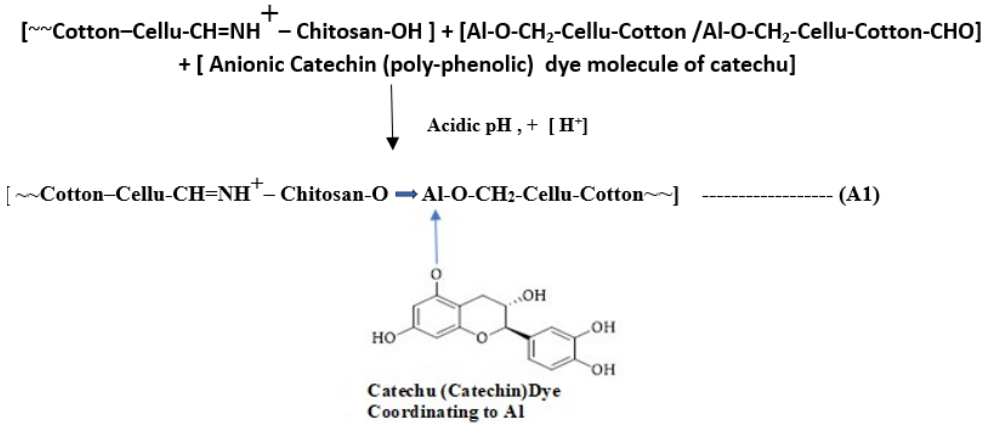
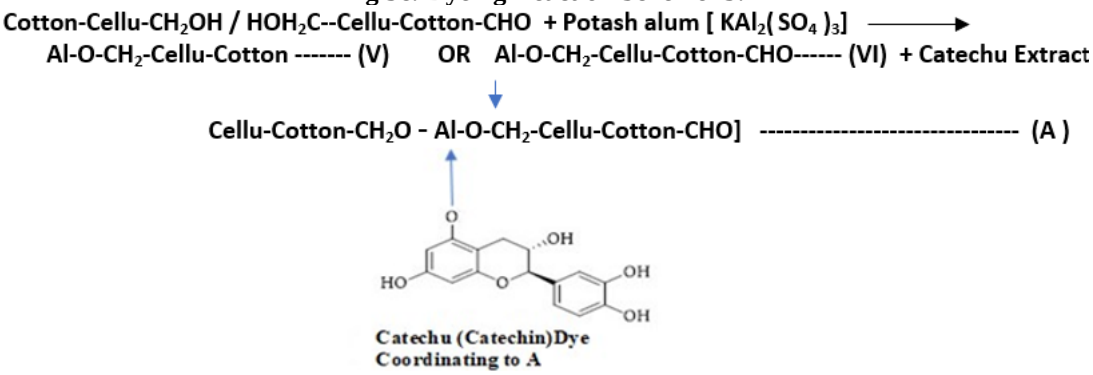


Fig 3c: Dyeing Reaction Scheme -3:



In **Fig -3, Fig 3a**: dyeing reaction -1 scheme thus indicates the dye fixation mechanism of anionic catechin natural dye on chitosan-based bio-cationized cotton without any alum premordanting, while **Fig 3b** dyeing reaction scheme -2 indicates dyeing of chitosan-based bio-cationised cotton with additional potash alum pre-mordanting fixing anionic catechin dye molecule of catechu forming larger complex of chitosan based bio-cationized cotton -alum and catechu dye. Also, **Fig 3c** dyeing reaction mechanism-3 indicates the dyeing of normal cotton / pre-oxidized cotton with only potash alum pre-mordanting without any chitosan pre-treatment. for fixing anionic catechin dye molecule of catechu conventionally forming cotton-Al-catechu normal sized complex formation to fix catechu dye**.**

4.2 Analysis of FTIR Spectra

Fig-4 shows three FTIR spectral scans **(a)** Bleached and pre-oxidized cotton (Oxy-Cotton), (b) Chitosan bio-cationised and potash alum mordanted cotton dyed with catechu extract and (c) Catechu dyed bio-cationised oxy-cotton after potash alum mordanting and then bio-finished with Water: ethanol (50:50) mixture aqua-ethanolic extract of eucalyptus leaves(EL).

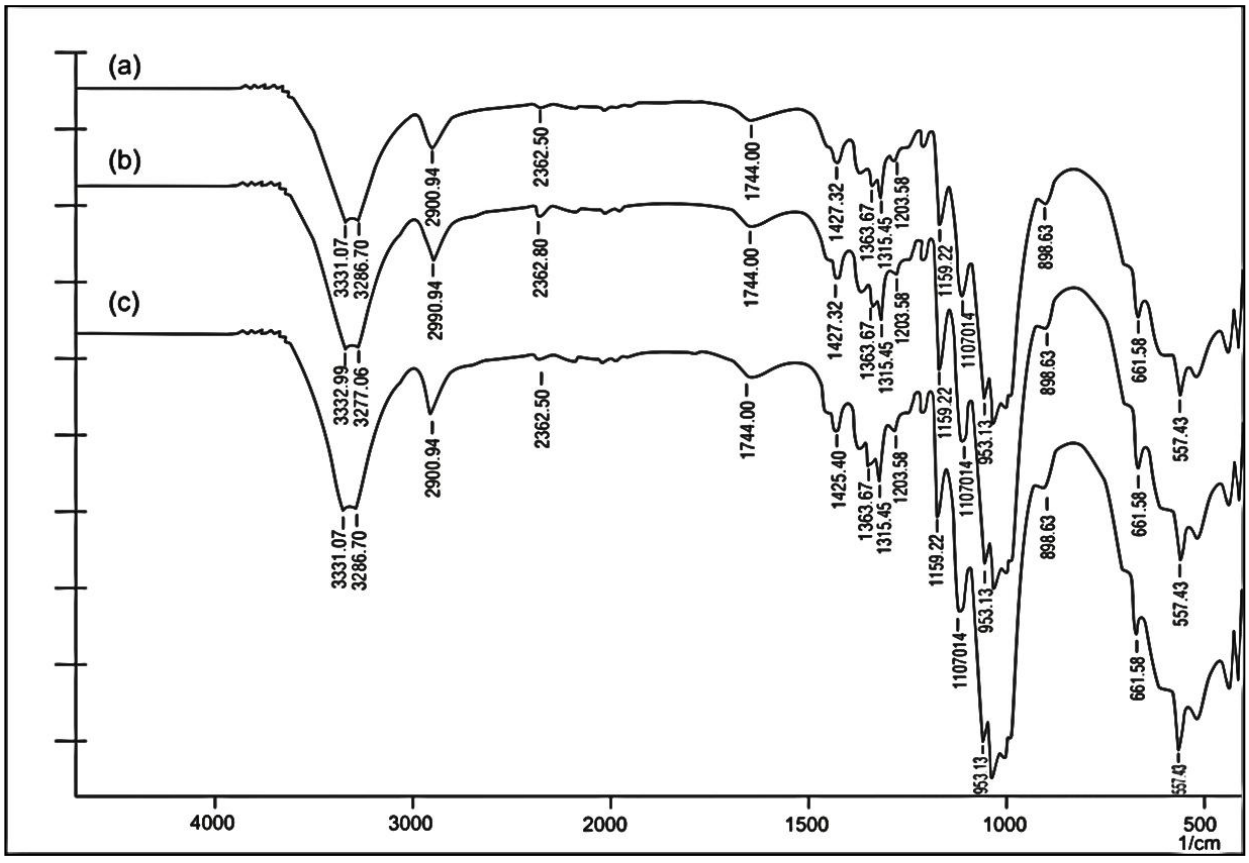


Fig-4: FTIR spectral scan of (a) Bleached and oxidized cotton (Oxy-Cotton), (b) Chitosan-based bio-cationised and potash alum mordanted cotton dyed with catechu extract and (c) Catechu dyed bio-cationised oxy-cotton after potash alum mordanting and then bo-finished with water: ethanol (50:50) mixture extracts of EL (eucalyptus leaves)

Fig -4, illustrate three different FTIR spectral scans much similar to each other, although have some finer and minute differences in peak position, and peak height/ peak area corresponding to possible differences

in functional group characteristics after chemical interactions that occur for different treatments done on control/oxy-cotton fabrics.

All three FTIR spectra (a), (b) and (c) in **Fig-4,** illustrate common peaks as depicted as follows:

at 3286-3331 cm^{-1} (small duplet) for free –OH stretching;

at 2900 cm^{-1} for C–H stretching and CH_2 stretching;

small hump at 1744 cm^{-1} for –C=O i.e. ketone or carboxylate ester or aldehyde –C(H)=O group

at 1427 cm^{-1} for d(c-H) and d(C-OH) stretching);

at 1363 cm^{-1} for d(C–H) stretching and at 1315 cm^{-1} for d(C–H) stretching;

at 1203 cm^{-1} for d(CH_2) twisting and at 1164 cm^{-1} attributed to m(C–C) ring breathing (asymmetric); at 1159 cm^{-1} for (C–OH) alcohol stretching and at 1107 cm^{-1} for (C–O–C) glycosidic vibration;

at 898 cm^{-1} due to the presence of C-O-O stretching (as a result of the carboxylic acid group in cellulose after bleaching and pre-oxidation by $K_2S_2O_8$ pre-treatment);

at 661 cm^{-1} for cis-distributed alkanes like –C–H bending etc

The above common FTIR peaks of control bleached and pre-oxidized oxy-cotton (common for spectra a, b and c, in **Fig-4** support reaction schemes 2a, 2 b and 2c as shown in **Fig 2**

However, the minor but noticeable observed differences in FTIR spectra b (Chitosan pre-treated bio-cautioned cotton sample treated with potash alum pre-mordanting followed by catechu dyeing) and spectra c (Catechu dyed bio-cationised oxy-cotton after potash alum pre mordanting and then finished with water: ethanol (50:50) alcoholic EL extract**.** as compared to FT-IR Spectra a **(**in **Fig-4)** which can be summarized as follows:

The minor peak in Spectra b is observed as the additional peak for (-C-NH_2) amine group vibration (from amine group containing chitosan containing amino group anchored to cotton, after chitosan based bio-cationization, with merging of 1505 cm^{-1} of cellulose for aromatic vibration.

Additional peaks in spectra b at 1639 cm^{-1} responsible for (-C=N-) aldimine group vibration, supporting reaction scheme -2 for chitosan amino-based bio-cautioned cotton and supporting also reaction scheme -3 for chitosan modified pre-oxidized cotton

In spectra -c , - (C=O) group vibration of few -COOH group and /or (-CH=O) aldehydes in pre-oxidised cotton and showing -(-CO=O-) stretching vibration of ester group formed in chitosan-based aminated bio-cationized cotton showing an increase in height and area of FTIR paek at 1735 $cm^{-1,}$ to shift it to 1744 $cm^{-1,}$ while there is some reduction in height and area of FTIR paek at 1735 -1744 cm^{-1} due to consumption of some of the -CHO group to form aldimine adduct structure with amine group of chitosan.

There is an observed increase/shift of peak at 1059 cm^{-1} to 1072 cm^{-1} in FTIR spectra b and FTIR spectra c as compared to FTIR Spectra a, which is attributed to Al-O stretching vibration (Djebaili, K, 2015) of vibration, indicating the presence of Al metallic element for additional K-alum based pre-mordanting along with chitosan-based bio-cationization, in both spectra b and c by the formation of -Al-O-coordinated complex with phenolic –OH group of flavonoids and gallo-tannins of catechu dye as well as gallo-tannins of EL extract as depicted in the above said reaction mechanism in **Fig-3**.

Increased FTIR peak area at 2362 cm^{-1} in both spectra b and spectra c is ascribed to additional –CH=N–H– stretching vibration for reaction of Cotton-Cellu-CHO and -NH_2 group of chitosan and later addition of catechin and catechu gallo-tannin from catechu dye molecules as well as gallo-tannins of EL extract, indicating presence of both catechin and multiple phenolic gallo-tannins of ELextract in FT-IR spectra c (**Fig-4**).

FTIR peaks at 459, 595, and 656 cm−1, are also due to the Al–O stretching for its octahedral, structure and bands around 715 cm−1 and 1072 cm−1 are also related to Al–O stretching mode for its tetrahedron structural symmetric bending of Al–O–H, respectively (Djebaili, K ,2015) as observed in both spectra b and c in **Fig-4**.

The increase in peak area at 1208 cm−1 in spectra b and c is due to additional -N-H stretching of the amino group of chitosan incorporated on chitosan-based bio-cationized cotton cellulose confirms the presence of NH_2-containing chitosan in the bio cationized oxy-cotton product for both undyed and dyed with catechu.

Thus, FT-IR spectra b and c, the increase in peak height and/or area at 2362 cm^{-1} is considered for –CH=N–H– aldimine group vibration of chitosan treated oxy-cellulose with aluminium pre-mordanting of chitosan-based pre-oxidized oxy-cotton due to additional presence of –Al–O– link along with –CH=N–H– aldimine group link formation, besides the presence of sec-phenolic–OH in chitosan as well as aromatic phenols in catechol from catechu and eucalyptol of EL extract and gallotannins of both catechu and eucalyptus leaves extract too, supporting all the **reaction scheme 3a, 3b and 3c** as shown in **Fig-3,** as evident from analysis and comparison of three FTIR spectra in **Fig-4**.

4.3 Effects of chitosan-based Bio-Cationization of cotton on colour parameters for dyeing with catechu followed by bio-chemical finish EL extract

Relevant data in **Table 5A** indicate some increase in K/S values particularly after catechu dyeing after bio-cationization with 5 to 20 % Chitosan (containing amine group), with or without potash alum pre-mordanting, subsequently when dyed with 30% catechu extract as natural dye under acidic pH at 5 in a salt-free dyeing conditions.

With an increase in dosages of chitosan treatment without dyeing, the colour strength values are found to increase from 0.85 to 1.11 as compared to the K/S value of control pre-oxidized cotton (oxy-cotton) fabric as 0.22 (where K/S value of only scoured and bleached control cotton fabric is 0.02 before pre-oxidation with 1% $K_2S_2O_8$), along with the gradual minor increase of ΔE values. The data in **Table -5A** further indicate that chitosan treated oxy -cotton for the same level of 5 to 20% chitosan treatment with subsequent catechu dyeing (without ant potash alum Pre-mordanting), also shows a similar trend of increase in K/S values, ΔE and ΔC

values showing a much lesser increase in each colour parameter than that obtained by corresponding treatment with additional natural potash alum pre-mordanting of chitosan-based bio-cationized oxy-cotton.

Table -5A: Effects of chitosan-based Bio-Cationization of cotton on colour parameters for dyeing of chitosan-based bio-cationized oxy-cotton with catechu dye with or without potash alum pre-mordanting followed by the bio-chemical finish with eucalyptus leaves

Sample/ Treatments.	**K/S**	**ΔE***	**ΔL***	**Δa***	**Δb***	**ΔC**	**ΔH**	**BI**	**MI (LABD)**	**CDI**
Scoured and bleached cotton	0.05							46.12	2.83	-
Scoured and bleached cotton peroxidized with 1% $K_2S_2O_8$ (Oxy-Cotton)	0.22							**20.4**	**2.73**	-
CHITOSAN -5% only on Oxy Cotton (-No Dye)	0.85	2.09	-1.15	1.56	1.57	11.57	-12.02	10.23	2.1	1.03
CHITOSAN -10%, only on Oxy Cotton (-No Dye)	0.95	2.18	-1.49	1.66	1.75	10.59	-12.36	10.27	2.4	1.06
CHITOSAN -15%, only on Oxy Cotton (-No Dye)	1.01	2.47	-1.53	1.51	1.60	10.36	-12.24	9.76	2.8	1.04
CHITOSAN -20% only on Oxy Cotton (-No Dye)	1.11	2.32	-1.83	1.87	1.86	10.01	-12.74	10.24	2.9	1.01
CHITOSAN -5%, on Oxy Cotton + Catechu-30%	2.50	3.09	-1.87	1.70	1.79	11.14	-12.06	8.50	3.10	1.07
CHITOSAN-10%, on Oxy Cotton **+ Catechu-30%**	**3.11**	**3.34**	**-2.09**	**1.86**	**1.88**	**11.75**	**-12.41**	**7.40**	**3.40**	**1.03**
CHITOSAN -15%, on Oxy Cotton + Cat-30%	3.14	3.48	-2.22	1.83	1.96	11.91	-12.38	7.18	3.60	1.00
CHITOSAN -20%, on Oxy Cotton + Catechu-30%	3.28	3.53	-2.25	1.88	1.97	11.53	-12.83	8.02	3.70	1.06
CHITOSAN -5%, on Oxy Cotton + K-Alum-10% _+ Catechu -30%	3.50	3.69	-2.59	1.74	1.97	11.65	-12.73	8.60	3.23	1.24
CHITOSAN -10%, on Oxy Cotton **+ K-Alum-10% _+ Catechu -30%**	**4.28**	**3.82**	**-2.36**	**1.96**	**2.12**	**11.70**	**-12.28**	**7.34**	**3.75**	**1.06**
CHITOSAN -15% on Oxy Cotton, + K-Alum-10% _+ Catechu -30%	4.20	3.79	-2.19	2.20	2.17	10.17	-11.28	7.56	3.90	1.07
CHITOSAN -20%, on Oxy Cotton + K-Alum-10% ++ Catechu -30%	4.21	3.75	-2.68	1.84	2.07	10.05	-12.98	7.62	3.45	1.33
CHITOSAN -10%, on Oxy Cotton **+ K-Alum-10% _+ Catechu -30% + Eucalyptus Leaves extract-1* -10% & 2% lemon juice by pad-dry-curing**	4.4	4.01	-2.62	2.01	2.11	10.7	-13.4	7.70	4.40	1.14
CHITOSAN -10%, on Oxy Cotton **+ K-Alum-10% _+ Catechu -30% + Eucalyptus Leaves extract-2** -10% & 2% lemon juice by pad-dry-curing**	4.7	4.21	-3.12	2.31	2.42	11.7	-14.1	7.91	4.90	1.03
***Eucalyptus leaves extract-1: Aqueous extract of Eucalyptus leaves** ****Eucalyptus leaves extract-2: Aqua-Ethanolic (50:50) extract of Eucalyptus leaves**										

On subsequent salt-free dyeing at acidic pH of dye bath at 5 , using 30% aqueous extract of catechu natural dye applied on the said 5-20% varying chitosan pre-treated oxy-cotton followed by potash alum pre-mordanting, the K/S values are found to further enhance from 3.50 to 4.28 with some increase in ΔE and ΔC values having

small increasing trend also in CDI values, with a noticeable and reasonable decrease in BI values **(Table-5A),** where 10% chitosan treatment shows maximum K/S value as 4.28 and ΔE value as 3.28 and ΔC values as 11.75 and further enhancing chitosan concentrations do not increase K/S values, ΔE and ΔC values probably due to the surface masking effect of chitosan film formation allowing not more catechu dye molecules to absorb/diffuse any more.

While, from relevant data in **Table -5A**, at the same 10% level of comparable chitosan based bio cationization pre-treatment with 10% potash alum Pre-mordanting with 30% catechu dye extract application in acid bath salt-free dyeing is when further bio-finished with 10% Eucalyptus extract by pad -dry -cure process, there is a minor further enhancement in K/S values, ΔE and ΔC values, due to addition of light yellow colour of EL extract [for using both aqueous and water: ethanol (50:50) mixture extract of EL based bio-finish].

Colour fastness results as depicted in **Table 5B**, show moderate to good overall colour fastness results for washing for salt-free catechu dyeing at acidic pH, after bio-cationizing with chitosan pre-treatment indicating the higher amount and better colourant binding of catechu dye molecules due to formation of ionic bonding between chitosan aminated bio-cationized oxy-cotton and anionic catechu dye, with higher attraction of anionic dye molecules of catechu for fibre-cationic agent (chitosan)- anionic catechu dye complex formation. The addition of 10% potash alum pre-mordanting agent after chitosan based bio cationization of oxy cotton, on subsequent 30% catechu dyeing, shows improvement of the all-around colour fastness to soap washing, UV-light fading and dry and wet rubbing to ½ degree higher in each case indicating +ve role of alum pre-mordanting along with bio cationization as beneficial forming stronger Fibre- Chitosan cationic agent -Alum-Catechu Dye bigger sized insoluble complex formation as shown in dyeing reaction mechanism 3c in **Fig-3**.

Table -5B: Colour Fastness for combined action of Chitosan-based bio-cationization and K-alum pre-mordanting and salt-free dyeing with 30% catechu dye followed by bio-finishing with eucalyptus leaves extract

Pre-treatment /pre-mordanting and Natural Dye /Finish Used	Color Fastness rating to				
	Soap Wash		UV-Light	Rubbing	
	LOD	ST		Dry	Wet
CHITOSAN-10%- + CATECHU -30% (No Alum)	**3**	**3**	**3**	**3**	**2**
CHITOSAN-5 % + K-ALUM-10% (1: 2)- + CATECHU 30%	**3**	**3**	**3**	**3**	**3**
CHITOSAN-10 % + K-ALUM-10% (1:1)- + CATECHU 30%	**3-4**	**3-4**	**3-4**	**3-4**	**3**
CHITOSAN-15 % + K-ALUM-10% (1.5:1)- + CATECHU 30%	**3-4**	**3-4**	**3-4**	**3-4**	**3**
CHITOSAN-20 % + K-ALUM-10% (2:1)- + CATECHU 30%	**3-4**	**3-4**	**3-4**	**3**	**3**
CHITOSAN -10%, on Oxy Cotton + K-Alum-10% _+ Catechu -30% + Eucalyptus Leaves extract-1* -10% & 2% lemon juice by pad-dry-curing	**4**	**4**	**4**	**4**	**3-4**
CHITOSAN -10%, on Oxy Cotton + K-Alum-10% _+ Catechu -30% + Eucalyptus Leaves extract-2** -10% & 2% lemon juice by pad-dry-curing	**4**	**4**	**4-5**	**4**	**3-4**

The addition of 10% eucalyptus extract by pad-dry-cure process on such bio-cationised pre-oxidised oxy-cotton with alum pre-mordanting and dyeing with catechu forms more larger complex of bio-finished said catechu dyed oxy-cotton improving further colour fastness to soap washing, UV-light fading and dry and wet rubbing to increase further ½ to 1 grade particularly best result is obtained for water: ethanol mixture extracted EL bio-finish.

4.4 Qualitative Phytochemical analysis and Quantitative LCMS analysis of 2 types of EL extract

4.4.1 Phytochemical analysis of aqueous and aqua-ethanolic extract of eucalyptus leaves (EL)

The qualitative phytochemical test results are shown in **Table-6**, which indicate the presence of important organo/bio-chemical components of the EL (eucalyptus leaves) extract. The presence of low to moderate amounts of alkaloids, flavonoids, tannins, terpenoids, saponins etc all are well identified and confirmed in less or more quantity in both types of EL extraction mediums (aqueous media and aqua-ethanolic mixture media) and results are shown in **Table-6**. However, the aqua-ethanolic extract of EL shows a higher abundance of the said alkaloids, flavonoids, tannins, terpenoids and saponins than those obtained for only aqueous extract of EL Thus aqueous extract of EL has lesser presence of all these bio-active components. Thus, the content of alkaloids, flavonoids, tannins, and saponin in the aqua-ethanolic extract of EL is relatively much higher than that of aqueous extract as tabulated below and shown in **Table -6.**

Table 6: Qualititative phytochemical analysis of two types of extract of eucalyptus leaves

Compounds	**EL: Eucalyptus Leaves extract**	
	AQ	AQE
Detection of alkaloids (compounds containing nitrogen)		
Potassium bismuth iodide/Dra gen Dorff's reagent (DR)	2^{+}	3^{+}
A mixture of mercuric chloride and potassium iodide (Mayer's reagent)	1^{+}	3+
Iodo-potassium iodide (Wagner's reagent)	2^{+}	3^{+}
2,4,6-trinitrophenol (Picric Acid)	2^{+}	3^{+}
Detection of flavonoids (phenolic compounds)		
Ammonium	2^{+}	3^{++}
Aluminium chloride	2^{+}	3^{+}
Detection of Tannins		
Ferric chloride	2^{+}	3^{++}
Detection of Terpenoids		
Salkowski test	2^{+}	2^{+}
Detection of Saponins		
Emulsion test	2^{+}	3^{++}
EL- Eucalyptus leaves; AQ- aqueous extract; AQE- Aqueous -ethanol (50_50) mixture extract; 0-absence of compound; 1^{+}-presence in low amount; 2^{+}- presence in moderate abundance; 3^{+}- presence in high amount and 3^{++}- presence in much higher amount		

4.4.2 LC-MS Analysis of differently extracted Eucalyptus Leaves

LC-MS Base peak intensity (BPI) of two different extracts (Aqueous extract and Aqua-Alcoholic extract) of Eucalyptus Leaves (EL) are shown in **Fig – 5A and Fig 5B** below shown side by side respectively.

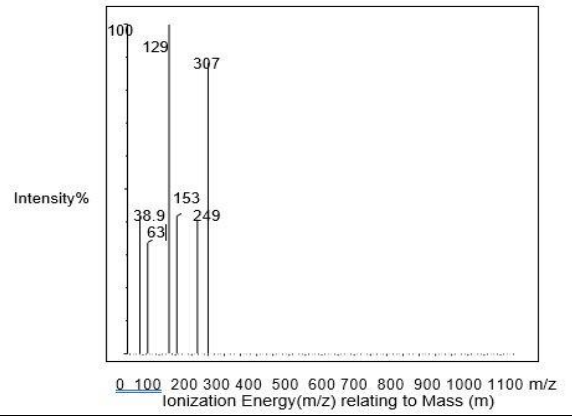


Fig-5A: LC-MS peaks of Aqueous extract of Eucalyptus Leaves *(Eucalyptus Globulus*)

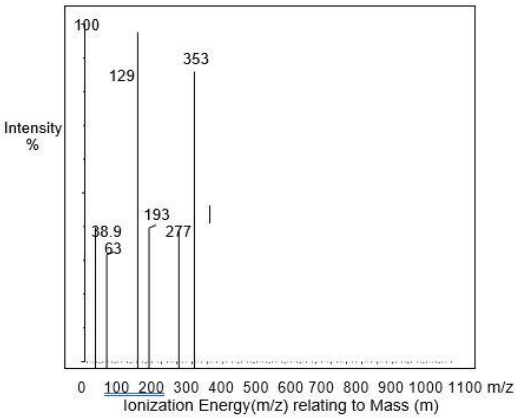


Fig-5B: LC-MS peaks of Aqueous + Ethanolic (50:50) extract of Eucalyptus leaves *(Eucalyptus Globulus)*

BPI (Base Peak intensity) stands major peak intensity of the chromatograms. It is only the intensity of the most intense peak at a given retention time, on summing up intensities of all mass spectral peaks belonging to the same scan. Thus, contrary to the BPI, all ions generated in chromatograms are taken into account. **Tables -7A and 7B** shown here show the BPI data and corresponding identified compounds/ components obtained from the LC-MS scan of EL aqueous extract and aqua-ethanolic 50:50 extract of EL respectively, showing major data and corresponding major LC-MS peak energy corresponds to the identified compounds present in both types of EL extracts.

Table 7A: LC-MS Major Peak energy of Aqueous extract of Eucalyptus leaves:

Srl. No.	**Peak Energy (m/z)**	**%BPI**	**Identified Compounds**
1	**38.9/39**	41.91	Citric acid
2	**62.9/63**	9.74	Ethanol amine
3	**128.9/129**	28.87	Gallic acid & Ethyl gallate
4	**152.9/153**	12.05	Quinic acid
5	**249**	11.78	Phloroglucinols & Flavonol O-glycosides
6	**307**	25.47	Ellagic acid & Quercetin 3-glucuronide Q

Table -7B: LC-MS Major Peak energy of Aqua-Ethanolic (50:50) extract of Eucalyptus Leaves

Srl No.	Peak Energy m/z	% BPI	Identified Compounds
1	**38.9 /39**	37,80	Citric acid
2	**62.8 /63**	**11.67**	Ethanol amine
3	**128.9/129**	**28.87**	Gallic acid &Ethyl gallate
4	**193**	55.08	Ferulic acid
5	**277**	65.84	Rutin / Hydroxy octa-deca dienoic acid
6	**301/307**	57.33	Ellagic acid and Quercetin 3-glucuronide
7	**353**	65.98	Eucalyptol

It is reported in the literature that 1,8-cineole (eucalyptol) and polyphenolic flavonoids present in eucalyptus, fruit, bark and leaves have a strong microbial-resistant effect against many bacteria (Cristóbal et al 2020) and (Sluiter et al -2008). Amongst all components of EL extract, Eucalyptol i.e. alpha-cineole / 1,8-cineole is identified as a component of Aqua -ethanolic extracts of eucalyptus fruits, barks and leaves having more diverse antibacterial effects. It is reported that EL extracts have MIC (minimum inhibitory concentration) values of 12.5 µm/ ml and 6.25 µm/ ml for Streptococcus mutans to Streptococcus sobrinus and MBC (minimum bactericidal concentration) of ethanolic extract. Eucalyptus leaves contain gallic acid (2.81%) and Quinic acid (2.40%), which indicates its good resistance towards E. coli, Salmonella sp, S. aureus and L. innocua and Other microbial Species (Cristóbal et al 2020) and (Sluiter et al -2008) as reported and summarized here giving the data below:

	MIC (mg/mL)	MBC (mg/mL)
E. coli	45	55
Salmonella sp	40	50
S. aureus	30	35
L. innocua	25	30

Another study reported in the literature indicates that the extract of eucalyptus globulus fruits exhibited reasonable inhibition of microbes by lipid peroxidation with linoleic acid emulsion (51.34 ± 0.72%) for its high reducing power (IC_{50} = 39.52 µg/mL µg/mL) showing high antimicrobial activity against B. subtilis and S. aureus with minimum inhibitory concentration (MIC) values of 30 µg/mL and 80 µg/mL, having strong antibacterial and antioxidant properties (Lila B-Makhlouf et al -2013). The present study also indicates a similar property for both types of EL extracts. Moreover,hydroxy-tyrosol has been identified as a good antibacterial agent against Propionibacterium acnes, S. aureus, and S. epidermidis (Ghalandari, M, N et al -2018).

4.5 Analysis of Antimicrobial and UV-resistant properties

The antibacterial properties of all the pre-oxidized and chitosan-based bio-cationized /potash-alum mordanted, catechu-dyed and EL extract bio-finished cotton fabrics were carried out as per the AATCC-100-2012 test method using *Klebsiella pneumonia (*AATCC 4352: a gram-ve bacteria) and *Staphylococcus aureus (*AATCC 6538: a gram+ve bacteria) and the results are shown in **Table-8.**

UV resistant property in terms of UV-Protection Factor (UPF) and transmission % of UV-A and UV-B part were assessed as per the **AATCC-183-2004/2010** method and corresponding test data are shown in **Table 8**

Table -8: Antimicrobial and UV-resistance properties for Chitosan-based Bio-Cationised/ Alum Pre-mordanted /Catechu dyed and Eucalyptus leaves bio-finished cotton.

Sl. No	Sample Type and Treatments	Bacterial Reduction % (AATCC-100-2012 method)		UV Resistance (UV-Tr % and UPF) (AATCC-183-2004/2010 method)		
		Klebsiella pneumonia **(gram -ve) AATCC 4352**	***Staphylococcus aureus*** **(gram +ve) AATCC 6538**	**Tr % of UV-A**	**Tr % of UV-B**	**UPF Value**
1.	Scoured and bleached control cotton (No pre-treatment and dyeing)	**No reduction**	**No reduction**	**14.5**	**16.1**	**05**
2	1% $K_2S_2O_8$ Oxidised control cotton (Oxy-Cotton) fabric (No Dyeing)	**No reduction**	**No reduction**	**15.2**	**17.11**	**05**
2	**5%** Chitosan treated bio-cationized oxy-cotton (No dyeing)	43.0	**66.5**	8.46	7.66	10
4	10% Chitosan treated bio-cationized oxy-cotton (No dyeing)	**72.0**	**74.2**	**7.10**	**6.52**	**15**
5	10% Chitosan treated bio-cationized oxy-cotton dyed with 30% catechu extract	**74.2**	**78.4**	**5.85**	**4.77**	**20**
6	Only 10% K-alum pre-mordanted cotton (No dyeing)	**90.2**	**94.6**	**8.20**	**7.10**	**10**

7	10% K-alum pre-mordanted cotton dyed with 30% catechu extract	**80.20**	**84.40**	**6.01**	**5.20**	**15**
8	10% Chitosan treated bio-cationized + 10% K-alum pre-mordanted oxy-cotton (No dyeing)	**87.30**	**90.20**	**4.35**	**3.55**	**25**
9	10% Chitosan + 10% K-Alum + 30 % catechu dyed oxy-cotton	**80.20**	**85.40**	**4.15**	**3.28**	**30**
10	10% Chitosan on Oxy Cotton + K-Alum-10% _+ Catechu -30% + Eucalyptus Leaves extract-1* -10% with 2% lemon juice	**84.56**	**88.34**	**4.78**	**3.31**	**35**
11	10% Chitosan on Oxy Cotton + K-Alum-10% _+ Catechu -30% + Eucalyptus Leaves extract-2** -10% with 2% lemon juice	**88.45**	**92.49**	**3.08**	**2.81**	**40**
***Eucalyptus leaves extract-1: Aqueous extract of Eucalyptus leaves, **Eucalyptus leaves extract-2: Aqua-Ethanolic (50:50) extract of Eucalyptus leaves**						

Only Scoured and bleached control cotton or 1% $K_2S_2O_{8\ \text{pre-oxidised}}$ control cotton (Oxy-Cotton) fabric has no antibacterial properties. Only 10% K-alum pre-mordanted cotton without any dyeing shows a very good antimicrobial effect (**Table-8**) showing 94.6 % bacterial reduction against *Staphylococcus aureus* bacteria (gram +ve) no. AATCC 6538 and 90.2 % bacterial reduction against *Klebsiella pneumonia* bacteria(gram -ve) *no.* AATCC 4352. but though only potash alum treatment before dyeing has good antimicrobial character, while after catechu dyeing of alum, pre-mordanted cotton shows that the said antibacterial effect is partly reduced probably due to consumption of alum in fixing the catechu dye forming cellulose-alum -catechu dye complex. This antimicrobial effect of fitkari i.e. K-alum (Bnyan A Ilham et al -2014) and (Shahriari Reza et al,2017), is a well-known common antiseptic and antimicrobial agent.

Relevant results of bacterial reduction % (**Table -8**) also indicate that Chitosan-based bio-cationized cotton further pre-treated with K-alum mordant showed good antimicrobial protection. Chitosan-based bio-cautioned cotton with or without alum pre-mordanting shows medium to good antibacterial and UV-resistant properties too.

But in all cases, the lesser antibacterial strength is shown for gram -ve bacteria than that for gram +ve bacteria, which is presumed to be due to the repulsion of anionic catechu dye against gram -ve bacteria (Klebsiella pneumonia) as a reason for lesser antibacterial effect.

The antimicrobial effect of chitosan is said to be due to positive charges of a free amino group of chitosan (or positive charges of an aldimine group formed in chitosan-treated bio-cationized cotton) in an acidic medium, strongly interacting electrostatically with negatively charged surface membrane component of microbes, facilitating killing/preventing microbial growth for chitosan treated bio-cationized cotton/oxy-cotton fabrics with or without salt-free dyeing with catechu in acidic media.

Considering UV resistant Property, UPF value up to 25 is obtained for dyeing with catechu after K-alum pre-mordanting, but chitosan based bio cationized cotton samples with K-alum pre-mordanting and catechu dyeing show somewhat higher antimicrobial and UV-resistance property showing maximum 80.2 % reduction in gram -ve bacteria growth and maximum 85.4% reduction in gram +ve bacteria growth and showing UPF value up to 30.

Relevant UPF data in Table-8, show gradual enhancement in UPF value for treatment with chitosan, and also with potash alum mordant and finally more increase for dyeing with catechu, showing that each of these agents has an important role in increasing UV protection criteria.

Potash alum is known to be antimicrobial (Bnyan A Ilham et al,2014) and (Shahriari R et al,2017), and also has some sort of UV protection capacity for its scavenging action of aluminium metal during photo exposure, chitosan is also known to be antimicrobial and UV protective too for the participation of its -NH_2 group in scavenging action (Yan, D et al – 2021,) on photo exposure for UV protection and finally, catechu dye i. e. catechin being polyphenolic mordantable dye structure, it can act as UV blocking agent (Jose Seiko et al -2022), The presence of saponin, tannin, and polyphenolic catechins present in catechu play a major role in imparting both antimicrobial and UV resistance by free radical scavenging action of these (Lee, X. Z et al,2022) ., Presence of Phloroglucinols & Flavonol O-glycosides (flavonoids) as evident from LC-MS data in **Table -7A** for aqueous EL extract and additional presence of Eucalyptol and Rutin in water: ethanol mixture extract of EL, as evident from LC-MS data in **Table 7B**, plays important role in imparting both antimicrobial and UV resistant free radical scavenging action of these, improving 90.0- 92.0 % reduction in bacterial growth and achieving UPF value upto 40 after both catechu dyeing and eucalyptus leaves (EL) extract bio-finishing of pre-oxidized -Chitosan based bio-cationized cotton.

5.0 Conclusions:

Amino group (attached in a ring structure in chitosan), particularly when the -NH_2 group of chitosan (attached to glucoside ring structure is less labile to convert to

cationic ammonium structure) is reacted with only when -CHO group of cotton aldehyde is generated in pre-oxidized cotton called oxy-cotton, which can easily forms aldimine group by addition of Cotton-Cellu-CHO group and NH_2 group of chitosan keeping free sec-OH in a chitosan ring structure, which (aldimine group) however later forms $-N=H_2^+$-R- cationic group (having lesser pk_a values than free $-NH_3^+$ group) producing quaternary ammonium-based newer cationic group incorporated in chitosan-based bio-cationized cotton) hence chitosan based bio-cationised cotton attracts easily anionic catechu dye, facilitates salt-free dyeing with catechu dye and is amenable also for subsequent bio-finishing with any of the two types of eucalyptus leaves extracts for bio-finishing for enhancing its antimicrobial properties and UV resistance properties to a very large extent. Thus, these bio-pre-treatments, natural catechu dyeing and EL-based natural bio-finishing facilitate the development of naturally coloured cotton fabric-based bio-medical textiles usable for improving human wellness behaviour of wearer using chitosan, potash alum, catechu and subsequent finishing treatment with EL extract as natural bio-finishing agent.

Finally, improvement of both antimicrobial and UV resistant properties is observed after bio-finishing the said chitosan bio-cationized and catechu dyed oxy - cotton fabrics bio-finished with 10% Water" ethanol mixture extracted EL in acidic media (using lemon juice as catalyst) by pad -dry-cure process, showing about 90.92% reduction in bacterial growth, achieving UPF value upto 40.The antimicrobial cotton thus developed may be used as surgical gown or for preparing a multi-layer wound healing pad .The UV resistant such bio finished cotton fabric may be used for wearer of Vitamin D deficient patient requiring sun bath every day medically.

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REFERENCES:

1. **AATCC-100-(2010/2012)** –Antibacterial Finishes on Textile Materials and Assessment, AATCC Technical Manual,. Vol 85, 2010. P-142, American Association of Textile Chemists and Colorists. Research Triangle Park, NC 27709, USA.
2. **Aranaz Inmaculada, Alcantara R Andres, Civera C M, Arias C, Elorza B, Caballero H A and Acosta N, (2021)**, Chitosan: An Overview of its Properties and Applications, *Polymers*, 13 (19), Sep-2021, 3256-3260-
3. **Bnyan Abass Ilham, Alta'ee Abdulsamie Hassan and Hassan Kadhum, Nadia, (2014)**, Antibacterial Activity of Aluminum Potassium Sulfate and Syzygium Aromaticum Extract Against Pathogenic Microorganisms, *J Natural Sc Res*,4(15) 2014,11-14
4. **Cristóbal Cara, Inmaculada Romero, , Eulogio Castro and Beatriz Gullón (2020)** ,Valorisation of Exhausted Olive Pomace by an Eco-Friendly Solvent Extraction Process of Natural Antioxidants , 2020, *Antioxidants*, Vol -9, 2020,1010/1-18. doi:10.3390/antiox9101010.
5. **Datta, Mahadeb., Konar, Adwaita., Samanta, A. K. and Datta, S, (2014),** Natural Dyeing of Jute Fabric with Catechu, *Int. J. Appl. Eng. Res*. 9(4), 2014, 451-467
6. **F.Mizi, D. Dai, and B. Huang, (2012)**, Fourier Transform Infrared Spectroscopy for Natural Fibres in "Fourier Transform-Materials Analysis"' ed S. Salih, InTech Open, London, UK (2012)., 45-68
7. **Ghalandari, M., Naghmachi, M.\ Oliverio, M.Nardi, M.Reza, H.Shirazi, G.and Eilami, O., (2018),** Antimicrobial effect of hydroxytyrosol, hydroxytyrosol acetate and hydroxytyrosol oleate on Staphylococcus Aureus and Staphylococcus Epidermidis. Electron. *J. Gen. Med*. 2018, 15, em46.
8. **Gies, P.H., Roy, C.R., Holmes, G. (2000)**, Ultraviolet radiation protection by clothing: Comparison of in vivo and in vitro measurements. Radiat. Prot. Dosim. 91(1-3), 2000, 247-250
9. **ISI (BIS): Handbook of textile testing SP: 15-1981. 1st edn. (1982)** , Bureau of Indian Standards, New Delhi, 1982, 221-280
10. **ISO-2469 & 2470, (1977),** (International Organization for Standardization, Switzerland), 1977, 1-3
11. **J.B. Harborne, (1988) (revised edn. 1998)**, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman & Hall Publications,
12. **Jose Seiko, Thomas S. Sankaran A., and Medha K, (2022)**, Functional Aspects of Natural Dyes, Textile Dyes and Pigments: A Green Chemistry Approach, 2022, 267-294.
13. **K. Djebaili, Z. Mekhalif, A. Boumaza, and A. Djellouli, (2015)**, XPS, FTIR, EDX, and XRD Analysis of Al2 O3 Scales Grown on PM2000 Alloy, J. Spectroscopy, Article ID 868109,(2015),1-16 https://doi.org/10.1155/2015/8681
14. **Khan, M.I., Ahmad, A., Khan, S.A., Yusuf, M., Shahid, M., Manzoor, N. and Mohammad, F. (2011)**. Assessment of antimicrobial activity of catechu and its dyed substrate. *Journal of Cleaner Production*, 19(12), 1385-1394.
15. **Kubelka P. (1948)**. New Contributions to the Optics of Intensely Light-Scattering Materials. Part I, JOSA, 38 (5), 1948, 448–451.
16. **Kubelka P. (1954)**. New Contributions to the Optics of Intensely Light-Scattering Materials Part II: Nonhomogeneous Layers, JOSA, 44 (4), 1954, 330-335

17. **Lee, X. Z., Y. R. Liang, H. Chen, J. L. Lu, H. L. Liang, F. P. Huang, and E. G. Mamati, (2008),**. Alleviation of UV-B stress in Arabidopsis using tea catechins. *African Journal of Biotechnology* 7: 2008., 22-30
18. **Lila Boulekbache-Makhlouf, Sakina Slimani and Khodir Madani, (2013),** Total phenolic content, antioxidant and antibacterial activities of fruits of Eucalyptus globulus cultivated in Algeria, *Industrial Crops and Products*, 41, January 2013, 85-89
19. **Meng Pan,Qicheng Lei,Ning Zang andHong Zhang, (2019)**, A Strategy Based on GC-MS/MS, UPLC-MS/MS and Virtual Molecular Docking for Analysis and Prediction of Bioactive Compounds in Eucalyptus Globulus Leaves, ***Int. J. Mol. Sciebce***. Aug-2019, 20 (16),3875-3890; https:// doi.org/10.3390/ijms20163875
20. **Samant, L., Jose, S., Rose, N. M., Shakyawar, D. B, (2022)**, Antimicrobial and UV Protection Properties of Cotton Fabric Using Enzymatic Pretreatment and Dyeing with Acacia Catechu. *J. Nat. Fibres* 19(6), 2022, 2243-2253.
21. **Samanta A K, (2020)**, Bio-Dyes, Bio-Mordants, and Bio-Finishes: Scientific Analysis for their Application on Textiles, in a book on *'Chemistry and Technology of Natural and Synthetic Dyes and Pigments'* Ed by Samanta A K et.al -(InTech Open publisher, London, UK), June-2020, Ch-1, 3-39.
22. **Samanta Ashis Kumar, (2024),** Advanced Methods and Tools for Color Measuring and Matching: For Quality Check of Colored Products of Textiles and Apparel Industry, Book Chapter, Ch-1, in International Book on Advances in colourimetry, Ed by Ashis Kr Samanta, Intech open, London and Croatia, UK, on line published in March- 2024, Ch-1, 01-30
23. **Samanta Pubalina, Mukhopadhyay Asis, and Konar Adwaita, (2024),** Eco-Friendly Dyeing, and Finishing, for Improving Colour Fastness and Wellness Properties of Cotton, In Springer's Book of "Functional Textiles and Clothing 2023", Springer, Singapore, Ed by Gupta, D., Majumdar, A., Gupta, S. (eds), *ICFTC 2023. Springer Proceedings in Materials*, 42. March 2024, 113-146,. https://doi.org/10.1007/978-981-99-9983-5-9
24. **Samanta, A.K., Agarwal, P., Singhee, D., Dutta, S.:, (2009),** Application of single and mixtures of red sandalwood and other natural dyes for dyeing of jute fabric: Studies on colour parameters/colour fastness and compatibility. J. Text. Inst. 100 (7), 565-587 (2009)
25. **Shah H S & Gandhi R S, (1990),** *Instrumental colour measurements and Computer Aided Colour Matching for Textiles* (Mahajan Book Distributors, Ahmedabad, India), 1990, 76, 116
26. **Shahid, M. and Mohammad, F., (2013)**. Recent advancements in natural dye applications: a review. *Journal of Cleaner Production*, *53*, 310-331.
27. **Shahriari Reza, Salari Saeed, Shahriari Saeed, (2017),** In vitro study of concentration-effect and time-course pattern of white, *African J Traditional Complementary and Alternative Medicine*, 14(2):,2017,311-318, doi:10.21010/ajtcam.v14i2.32
28. **Sluiter, A.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D, (2008)**, Determination of Extractives in Biomass; NREL/TP-510-42619; *National Renewable Energy Laboratory*: Golden, CO, USA, 2008.
29. **Yan, D.; Li, Y.; Liu, Y.; Li, N.; Zhang, X.; Yan, C. (2021**, Antimicrobial Properties of Chitosan and Chitosan Derivatives in the Treatment of Enteric Infections, *Molecules*, 26, 2021, 7136-7173. https://doi.org/10.3390 molecules26237136