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Green synthesis and characterization of copper nanoparticles by *Tinospora cardifolia* to produce nature-friendly copper nano-coated fabric and their antimicrobial evaluation

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ABSTRACT

Metallic nanoparticles such as gold, zinc, copper possess anti-microbial activity. These nanoparticles have a small size which provides a large surface area for the interaction with microbes and there are various mechanisms through which copper nanoparticles (CuNPs) act. The demand of these nanoparticles are increasing in the textile industry as they decrease the catalytic degradation property of various dyes as well as being helpful in the treatment of various topical infections. Our aim is to formulate the copper nanoparticle which is capped with *Tinospora cardifolia* and incorporate these nanoparticles on fabric and to study the anti-microbial activity of these nanoparticles formulated along with their study on the fabric. Formulated nanoparticles were tested for various characterizations such as SEM (Scanning Electron Microscope), TEM (Transmission Electron Microscope) for the microscopical study. The interaction of excipients with the drug was studied using FTIR, XRD, and Raman and the anti-microbial study was studied to determine the activity of the nanoparticles on gram-positive and gram-negative bacteria. Least particle size of 63.3 nm was used as optimized formulation (CuNPs-5) and further used for testing. Laundry durability, ZOI study and %efficacy of copper nanoparticles along with nanoparticle-coated fabric was tested and it was found that fabric was more efficacious for gram-positive bacteria as ZOI for gram positive and gram negative was 21.99 mm and 11 mm. The %efficacy of copper nanoparticle-coated fabric was 101% and 74% at the highest concentration for gram positive and gram negative bacteria respectively.

1. Introduction

The emergence of nanoscience and nanotechnology presents the use of the anti-microbial properties of metallic nanoparticles. The anti-bacterial properties of these nanoparticles are due to the presence of high surface to volume ratio and small size which provides a high surface to interact with a microbial membrane (Subhankari and Nayak, 2013). Among all the methods employed today radiation method, microemulsion method, thermal decomposition method, laser ablation method, and aqueous chemical reduction method (Joshi et al., 1998; Solanki et al., 2010; Kim et al., 2006; Yan et al., 2010; Liu et al., 2012), the aqueous reduction method is the most favored route for its simple and economic strategy, high yield and quality of the desired product and ease of control over particle size and distribution with various experimental parameters (Athawale et al., 2005). Nanoparticles made of inorganic matter possess novelty and enhanced chemical, physical and biological properties, and functionality because of their nanosize. These nanostructured and nanophase materials are widely used due to their selectivity in biological and pharmaceutical applications (Dave et al., 2017). *Tinospora cardifolia* is used as a natural herb in Ayurveda to provide various health benefits such as glucose metabolism, inflammation, immune-system and as an anti-pyretic. It helps in improving the ability of immune cells and macrophages, which makes it useful as an immuno-stimulatory. It is also a potent anti-allergic supplement, as MAO inhibitor, it increases the catecholamine level. It contains phyto-androgen which helps in protecting DNA damage due to radiation therapy or the environment. Formulation of copper nanoparticles using green synthesis is an advanced approach toward

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nanoparticles synthesis in which plant extract which possess different properties can be used to extract a capping agent to protect these nanoparticles and provide various therapeutic benefits. For our study, we used *Tinospora cardifolia* extract which possess anti-microbial properties. Anti-microbial activity is itself provided by copper nanoparticles but the chemical degradation power of copper is very high when they come in contact with air. To protect the property of copper nanoparticles we capped them with *Tinospora cardifolia* plant extract in order to maintain their properties for a long duration of time. Nowadays researchers have been attracted to green synthesis methods because they provide many benefits that can be used as stabilizing agents, capping agents, reducing agents and do not include any harmful or costly substances (Mukherjee et al., 2001). The metal nanoparticles are focused mainly on research work due to their potential applications in different fields such as magnetic recording media or microelectronics, (Saranyaadevi et al., 2014) nanosensors, catalysis, optoelectronics nanoelectronics, and information storage devices. Wastewater from the textile industry containing dyes harmful to waterborne animals has been increasing constantly, making it one of the main causes of severe water pollution worldwide (Hu et al., 2008). Development and synthesis of eco-friendly, biocompatible, clean and non-toxic nanoparticles through the green process has gained attention. Use of environment-friendly substances and cost-effectiveness are the main focus of the green synthesis approach. With nanotechnology green synthesis reduces the disadvantages of synthesis through toxic procedures. The concept of synthesizing green nanoparticles was first established by Raveendran et al., 2003. Synthesis of copper nanoparticles using extracts from microorganisms and plants are currently in use and being explored. Here we will discuss the synthesis of copper nanoparticles with the help of leaves extract of *Tinospora cardifolia*. Various analytical techniques were applied for the conformity of synthesis of copper nanoparticles such as Ultra Violet/ Visible spectrophotometry, FTIR and RAMAN. Morphology of the nanoparticles was studied with the help of TEM and SEM techniques. Other parameters such as anti-microbial study and laundry durability testing were also performed for the testing of copper nanoparticle-coated fabric.

2. Materials and methods

2.1. Materials

White colored plain weaved cotton fabric (100% cotton) was taken for incorporation of copper nanoparticles. Copper chloride (II) was bought from Sigma Aldrich, USA. Leaves of *Tinospora cardifolia* was procured from a medicinal garden of Banasthali Vidyapith, Pharmacy Department. Bacterial strain *E.coli* (MTCC-443) and *S. aureus* (MTCC-3381) were bought from MTCC Chandigarh. All other ingredients were used in high purity and grade.

2.2. Preparation of aqueous extract of leaves of Tinospora Cardifolia

The aqueous extract of leaves of *Tinospora cardifolia* was used as capping and reducing agent for the synthesis of copper nanoparticles. For the preparation of extract 25 g of dried leaves were taken in 50 ml water and heated for 30 min. The colour of solution turns colorless to brown. Then with the help of Whatman filter paper (N0–1), the extract was filtered and was allowed to cool at room temperature (25 °C). This extract was then collected in a glass vial and stored in a refrigerator (4 °C) for further analysis and synthesis of copper nanoparticles.

2.3. Mercerization of the cotton fabric

Various properties of fabric like luster, deposition of dyes, stability, and smoothness were enhanced with the mercerization process or by treating the fabric with various types of strong alkali solution like potassium hydroxide, sodium hydroxide, lithium hydroxide etc. Agents used for mercerization process are recognized as scouring agents. When the fabric was treated with an excess of alkali its structure and properties get changed. Sodium hydroxide (23.5%) and subitol MEZ-N in lukewarm water (20 °C) used as a wetting agent, then the fabric was rinsed with the help of hot water and then by lukewarm water to increase the sudden tension between the strands of fabric. Afterwards, the mercerized cotton fabric was centrifuged at 7000 rpm to remove the untrapped deposits and then dried at room temperature of 30 °C. This pretreated cotton fabric was then utilized for copper nanoparticle impregnation.

2.4. Green synthesis of copper nanoparticles by a one-step synthesis process and its impregnation on cotton fabric

Greenly synthesized copper nanoparticles were incorporated on pretreated cotton fabric. Copper nanoparticles were prepared by a one-step synthesis process. To synthesize the copper nanoparticles, 0.25 mM solution of copper chloride in 10 ml of distilled water was poured in a round bottom flask with three necks. The heat was then applied to the solution and 2 ml of the extract was added at a temperature of 87 °C. The change in colour was observed from sky blue to dark green which confirmed the formation of copper nanoparticles. Afterwards, the cotton fabric was dipped in the solution and impregnated with copper nanoparticles. When impregnation took place the fabric was washed with distilled water in order to eliminate the residues from the fabric. The fabric was dried at a normal temperature and ironed for further characterization. The amount of copper nanoparticle in the 10 ml solution was found to be 1.78 mg/ml. The non-impregnated copper nanoparticles were centrifuged (REMI centrifuge) at 6000 rpm for 15 min and distilled water washing was done to remove the unbound *Tinospora cardifolia*. After centrifugation, the sample was further lyophilized (Labconco lyophilizer) at a temperature of -85 °C and a pressure of 0.040 mBar vacuum. After complete drying, the lyophilized sample was stored in an airtight container for further characterization.

2.5. Characterization of copper nanoparticle impregnated with cotton fabric

2.5.1. Ultra violet/visible spectroscopy

After visual identification, the confirmation of copper nanoparticles was confirmed by UV Spectrophotometer containing UV Win Software (LABINDIA). The absorption maxima were determined by scanning the sample from 200 to 800 nm. Distilled water was used as a blank. (Dutta et al., 2014; Anandan et al., 2008; Singh et al., 2014; Wesenberg et al., 2003).

2.5.2. Particle size, polydispersity index (PDI) and zeta potential

With the help of dynamic light scattering principle Particle size, the PDI and zeta potential of copper nanoparticles was determined using a Zeta seizer Nano ZS (Malvern Instruments, Malvern UK). Zeta potential determines the stability of the formulation whereas PDI determines the homogeneity of the sample. All the quantifications were done three times for the reproducibility of the data.

2.5.3. Field emission-scanning electron microscopy (FE-SEM), transmission electron microscopy (TEM), energy dispersive X-ray spectroscopy (EDX) and atomic force microscopy (AFM)

The morphological behavior of copper nanoparticles, nanoparticle-coated fabric, and pretreated cotton fabric was determined with FE-SEM, furnished with Mira 3 TESCAN software operated at a power of 5 kV. The transmission electron microscopy (TEM, MNIT-Jaipur) provides a visual and perfect morphology of the synthesized green copper nanoparticles. For the analysis one drop of nanoparticle sample (2 μl) was placed on the conductive copper grid and stained with 2%*w/v* phosphotungstic acid. Further, the sample was dried and surface analysis was performed at a voltage of 200 kV.

To determine or to confirm the presence of copper present on the

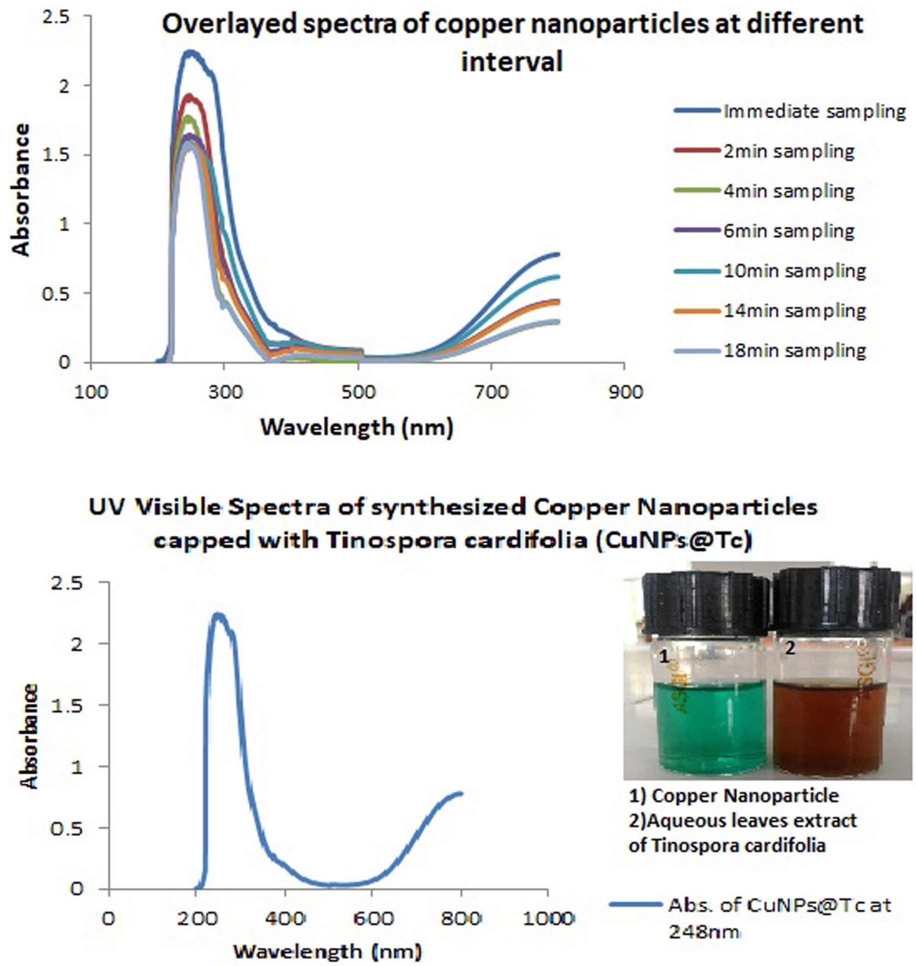

Fig. 1. UV/Visible spectrum of the green copper nanoparticles A) an overlay of UV/Visible spectra showing peaks at different time intervals and B) showing absorption maxima of green copper nanoparticles at 248 nm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Characterization of copper nanoparticles capped with *Tinospora cardifolia*. (CuNPs@Tc).

Formulation	Particle size (d.nm)	PDI	Zeta potential (mV)
CuNP 1	95.86 ± 0.31	0.87 ± 0.56	−21.1 ± 0.24
CuNP 2	72.55 ± 0.53	0.76 ± 1.54	−18.90 ± 1.76
CuNP 3	143.43 ± 0.41	0.54 ± 2.76	−27.09 ± 2.97
CuNP 4	87.22 ± 0.38	0.44 ± 0.98	−13.34 ± 0.76
CuNP 5	63.3 ± 0.64	0.26 ± 1.43	−33.98 ± 0.65
CuNP 6	89.54 ± 0.54	0.72 ± 0.54	−28.09 ± 1.54

cotton fabric, energy dispersive (EDX) spectra were taken with an Oxford instrument furnished with Aztec software and purged with continual a nitrogen rate at 15 kV.To determine the surface property of the synthesized copper nanoparticles, atomic force microscopy was performed (AFM, MNIT-Jaipur) (AIST-NT Model: Smart SPM 1000). AFM determines the height and diameter of the nanoparticle. This was done by spreading 10 µl of nanoparticles on mica slips and incubating for 5 min. To remove the unwanted material sample was washed with water and to produce a thin film of sample it was dried with the help of a spin coater. The dried sample was placed under the lens and investigated at different-different magnifications. An image of the nanoparticles was taken with the help of AIST FP tip number 01 with AC mode.

2.5.4. Attenuated total reflection- Fourier transform infrared spectroscopy (ATR-FTIR)

Attenuated total reflection- Fourier transform infrared spectroscopy (Bruker EQUINOX 55 FTIR) was furnished with mercury cadmium telluride detector which operates at room temperature with a resolution of 2 cm^{-1}. Functional groups present on the surface of copper nanoparticle and other samples were determined with the help of this study. As internal reflection element diamond was used, located at an angle of $45^o$with 32 times scan and given one reflection that is equal to 21 resolutions. Spectra were scanned in the region of 4000–400 cm−1, innovative ATR rectification was applied to the selected spectra and peak fitting was completed with the help of opus software (Saranyaadevi et al., 2014).

2.5.5. Raman spectroscopy

The samples were scanned through a Raman spectrophotometer (Thermo-scientific instrument (DxRxi), furnished with OMINICxi-Analysis software). Raman spectroscopy helps to provide the information on rotational, vibrational and various other frequency modes in the system. A laser beam of 532 nm along with a laser power of 5-100 mW was used to scan the synthesized copper nanoparticles and copper nanoparticle-coated fabric. The spectra were taken in the range of 125-4000 cm^{-1} (Cho, 2007; Colomban et al., 2009).

2.5.6. Powder X-ray diffraction (PXRD) study

With the help of an X-ray diffractometer, (Bruker AXS D8,

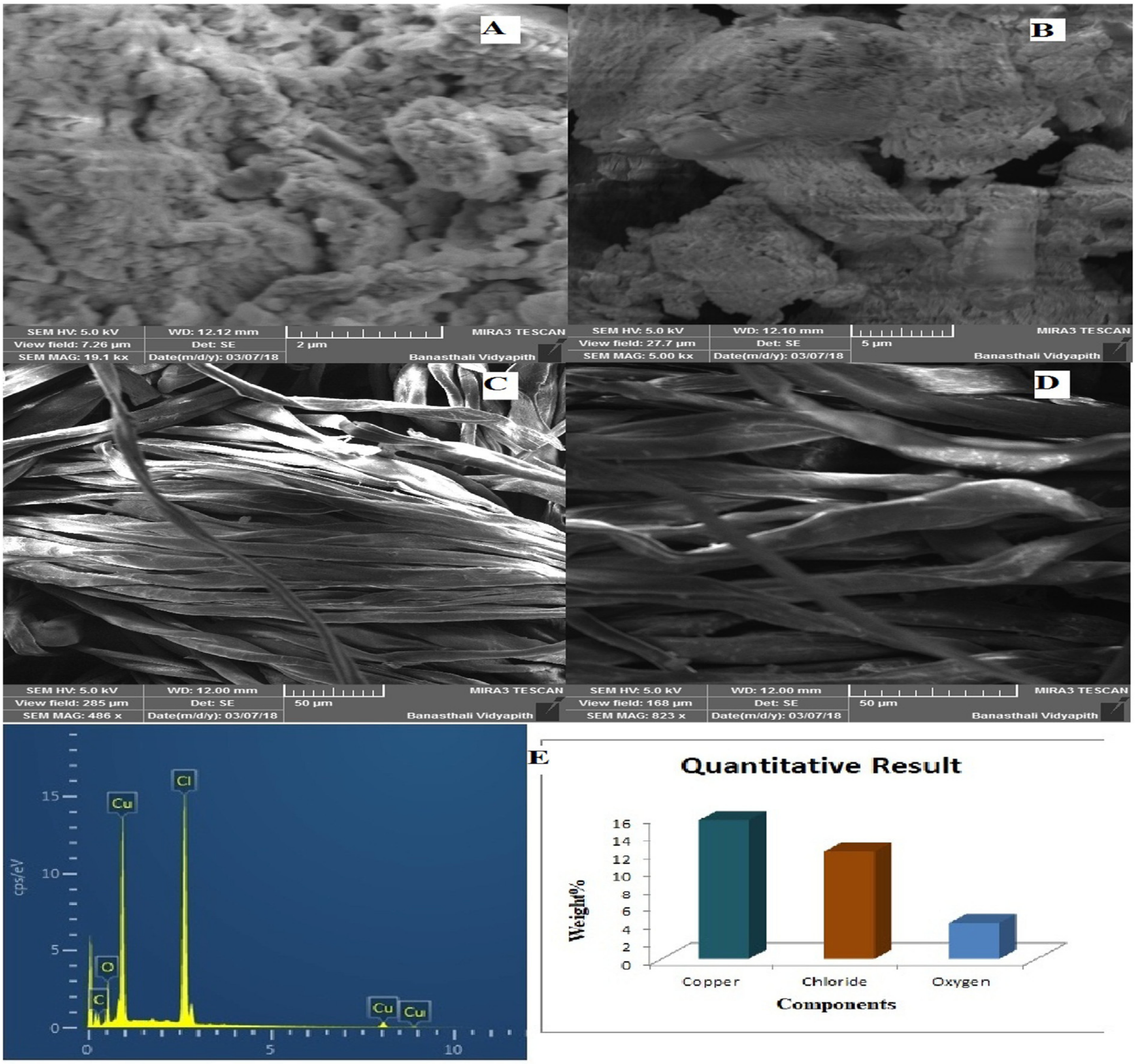

Fig. 2. Morphology study using SEM. A) and B) copper nanoparticles, C) non-coated fabric, D) copper nanoparticle-coated fabric, E) EDX and quantification data of copper nanoparticles.

controlled by Diffrac plus XRD commander software with 5 s/scan speed) the XRD arrangement of the copper nanoparticles coated fabric was studied. The samples were prepared by spreading powder sample on polymethyl methacrylate (PMMA) specimen holder rings. The 2θ value for copper nanoparticles ranged from 10-60° with 7 s/scan speed. Further, the crystallinity of the green copper nanoparticles coated on the fabric was determined with the help of the Debye-Scherrer method.

2.5.7. Determination of antimicrobial activity of greenly synthesized copper nanoparticle-coated cotton fabric

Synthesized green copper nanoparticles and copper nanoparticle-coated fabric were tested for anti-microbial activity. Testing was done with two strains of bacteria i.e. gram-positive (*S. aureus* -MTCC 3381) and gram-negative bacteria (*E.coli*-MTCC 443) at IC50 (minimum concentration to inhibit 50% of bacteria growth) calculated for each strain. IC50 value for copper nanoparticles was found to be 200 μg/ml for *S. aureus* and 125 μg/ml for *E.coli* using turbidimetric method. Further, ZOI was also calculated for copper nanoparticles using cup plate method through nutrient agar plate method. The study of copper nanoparticles was done against the standard drug (Ampicillin) in the concentration range of 50–175 μg/ml for *S. aureus* and 50–300 μg/ml for *E.coli*. Similarly, copper nanoparticle-coated cotton fabric ZOI was studied against the non-impregnated cotton fabric in standard drug solution for the same concentration range (Sharma et al., 2017a, 2017b). Methylene blue was used as a dye to study the zone inhibited by nanoparticles. After preparation of plates, microbes were streaked on the plate using an inoculation loop and incubation was done at a temperature of 37 °C in an incubator.

2.5.8. Study of bacterial growth dynamics of greenly synthesized copper impregnated nanoparticle-coated cotton fabric against S. aureus

Growth dynamic study was carried out on *S. aureus* (gram-positive bacteria) because nano coated cotton fabric was found more effective on these bacteria. To observe the effect of nanoparticle on growth dynamic, firstly the nutrient culture media having different concentration of test compound i.e. 50–175 μg/ml was incubated at 37 °C for 72 h. The optical density of the samples was measured at regular intervals with the help of UltraViolet-Visible spectrophotometer at 600 nm. The experiment was performed in triplicates (Wesenberg et al., 2003; Sharma et al., 2017a, 2017b).

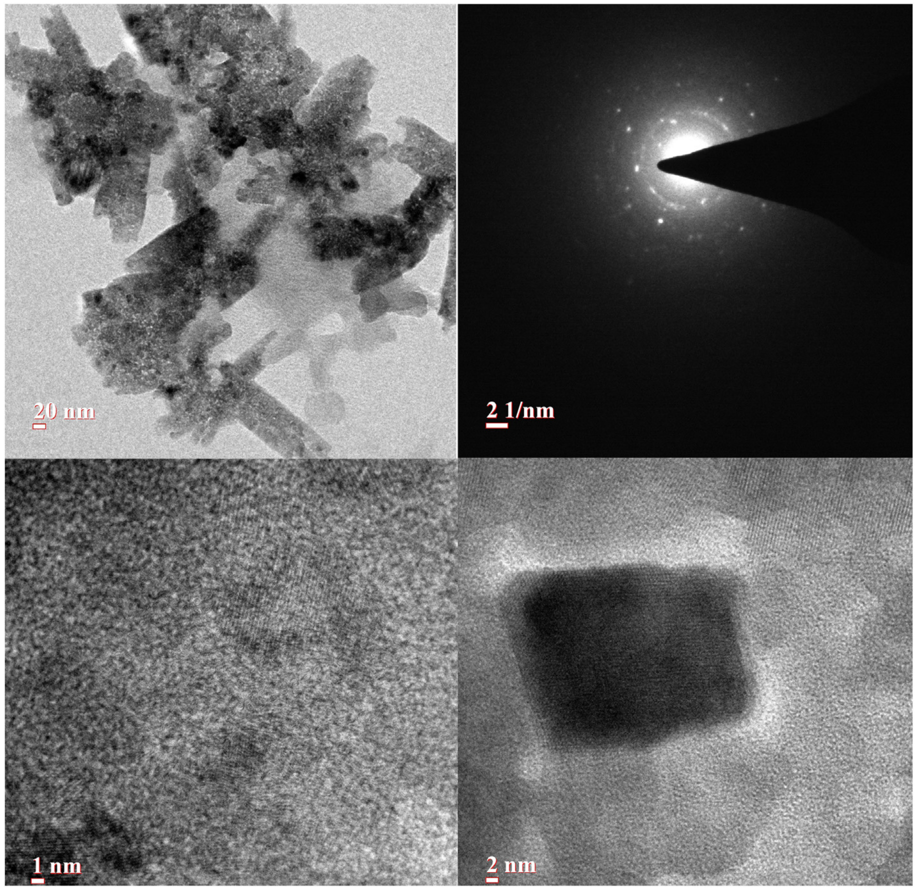

Fig. 3. TEM image of copper nanoparticles.

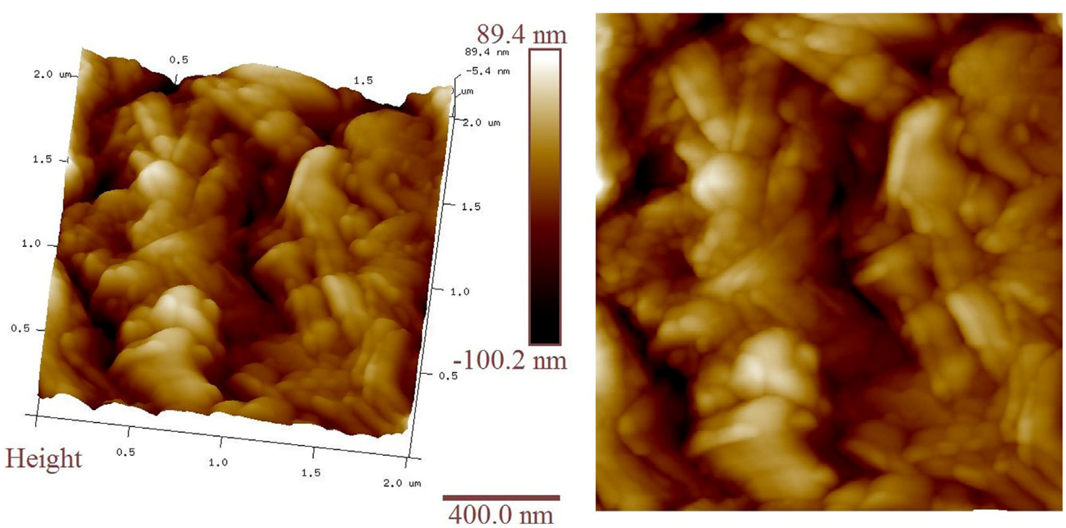

Fig. 4. AFM of green copper nanoparticles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.6. Laundry durability assessment of copper nanoparticle-coated cotton fabric

With the help of the American Association of Textile Chemist and Colorists test method 61–2006 laundry durability of copper nanocoated fabric was performed and by using without optical brightener, detergent in the concentration of 0.15% *w*/w and 50 stainless steel balls in warm water at 80 °C testing condition was kept. To perform the experiments accelerated laundering rounds were used in which 1 round is equal to 5 rounds. Several numbers like 0, 2, 5 and 10 of laundry rounds were taken which shows the outcome of accelerated stress testing measure in 0, 10, 25, and 50 numbers of laundry rounds. The holding capacity of copper nanoparticles in each round was measured and results were reported (Zhang et al., 2014; Kim et al., 2011).

3. Results

3.1. UltraViolet/visible spectroscopy

Synthesis of copper nanoparticles was confirmed by a UV visible

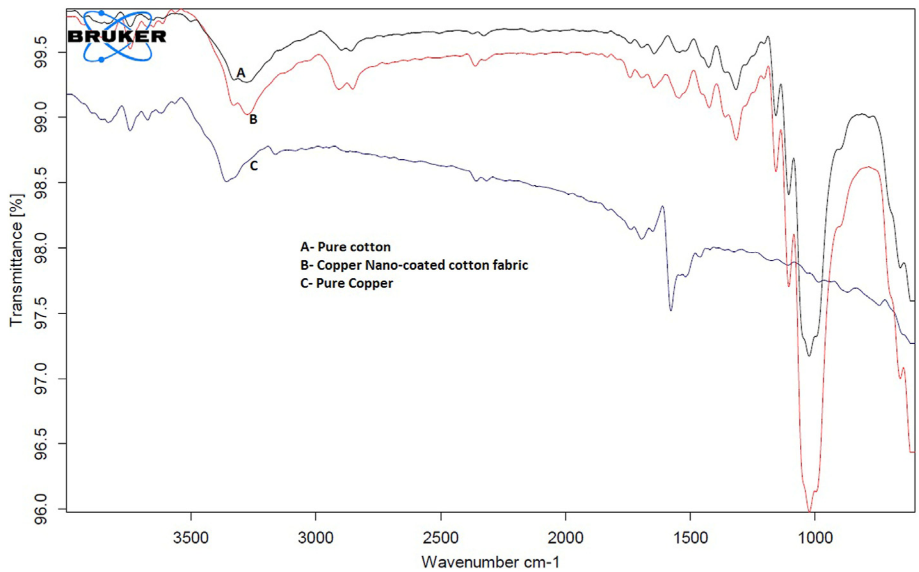

Fig. 5. An ATR-FTIR spectrum of A) pure cotton B) green copper nanoparticles-coated cotton fabric, and C) pure copper chloride. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

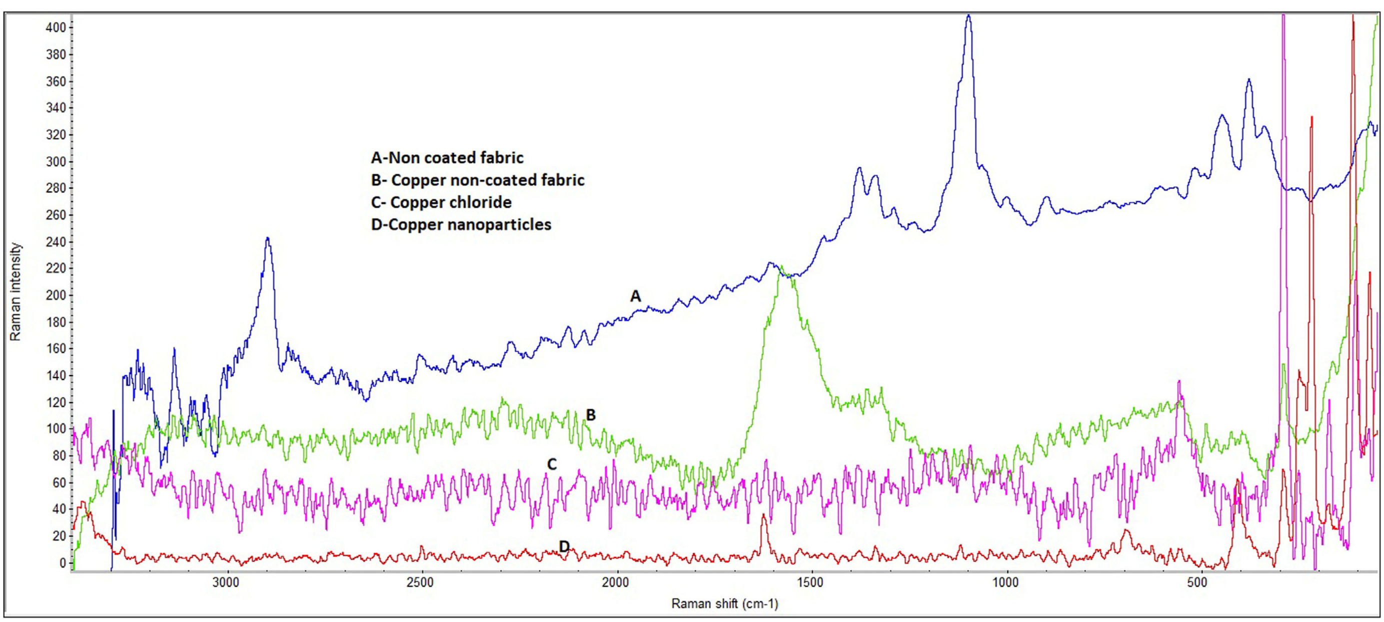

Fig. 6. Raman shift spectra of A) un-treated cotton fabric, B) green copper nanoparticle-coated fabric, C) copper chloride, and D) green copper nanoparticles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

analytical method(Athawale et al., 2005). During the synthesis process, sampling was done at different time intervals until there was a complete colour change in the solution and these samples were examined through UV Visible spectrophotometer. Each sample was scanned from 200 nm to 800 nm. The UV peak was shown in Fig. 1.

3.2. Particle size, PDI and zeta potential

Using Malvern Zeta sizer particle size, PDI and zeta potential of the formulation was studied. In this study 5 ml of the sample was diluted with distilled water and a reading was taken. The particle size, PDI and zeta potential were found in the range of 63.3-143d.nm, 0.26–0.87 and − 13.34 - -33.98 mV respectively, as showed in Table 1. Optimized formulation was found to be CuNPs-5 with a particle size of 63.3d.nm, PDI value of 0.26 and zeta potential of −33.98 mV.

3.3. SEM (scanning Electron microscopy), TEM (transmission Electron microscopy), energy dispersive X-ray spectroscopy (EDX) and atomic force microscopy (AFM)

Morphology of copper nanoparticles, cotton fabric, and copper coated cotton fabric was studied using SEM. Images of SEM confirmed that the copper nano coated cotton fabric contains nanoparticles compared to non-coated fabric, shown in Fig. 2A. Through TEM the shape of the green synthesized copper nanoparticles were observed. It was seen that the optimized nanoparticle samples were spherical in shape with a smaller diameter. The result was shown in Fig. 3. To confirm the presence of copper, Energy Dispersive X-Ray spectroscopy (EDX) was performed. The presence of different elements were confirmed by demonstrating strong spectra along with certain elements for example carbon, oxygen, and chlorine. The peak of Copper was found to be 15% weight which was dominant to other peaks. Peaks of oxygen, chlorine,

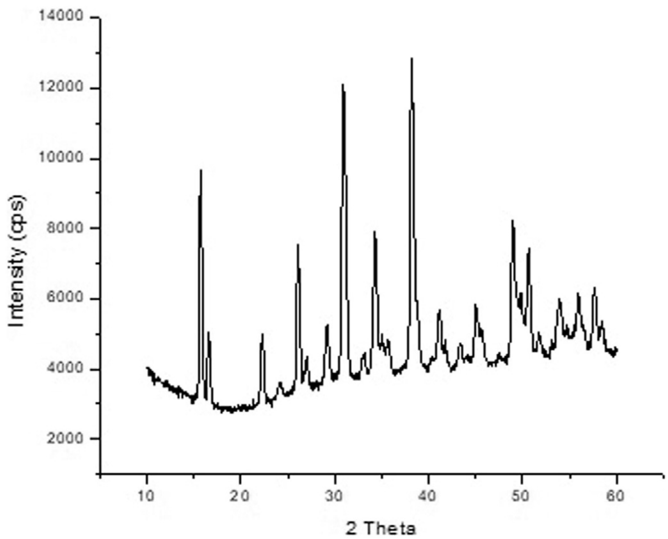

Fig. 7. XRD of green copper nanoparticles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

carbon were found due to the capping of nanoparticles with *Tinospora cardifolia*. The peaks of these inorganic materials were found due to the presence of these materials in leaves extract of *Tinospora cardifolia* which capped the nanoparticles (Fig. 2C). Atomic force microscopy image helped to determine the surface property of nanoparticles. The AFM image of the nanoparticle formulation is shown in Fig. 4. Height and surface area of the nanoparticle was found to be 178 nm and 27,912,430 nm^2 which demonstrated that the nanoparticles were smooth and spherical in shape. No visible cracks or pinholes were visualized in the nanoparticles.

3.4. Attenuated total reflection- Fourier transform infrared spectroscopy (ATR-FTIR)

The presence of functional groups was detected through ATR-FTIR. Different functional groups present in the leaves extract of *Tinospora cardifolia* was accountable for stabilizing and capping copper nanoparticles. The nanoparticles possess absorption peaks at 3329 cm^{-1}, 1620 cm^{-1}, 1395 cm^{-1}, 1319 cm^{-1} and 1049 cm^{-1}. These bands are responsible for different functional groups present in a sample such as 3329 cm^{-1} corresponds to OH stretching vibration of alkaloids and steroids, 1620 cm^{-1}, 1395 cm^{-1} absorption peak is due to C═O stretching of fatty acid and carboxylic O─H bending vibration of fatty acid while 1319 cm^{-1} and 1049 cm^{-1} bands are due to C─O and C─O─C stretching respectively. The result of ATR-FTIR is shown in Fig. 5.

3.5. Raman spectroscopy

Raman spectroscopy technique relies on the electric polarizability of the molecule present in the sample (Miranda et al., 2011; Wartewig and Neubert, 2005). Raman spectra of the copper Nanoparticle-coated fabric is shown in Fig. 4. The peaks were found at 1600 cm^{-1} which indicates that there is aromatic bending of the alkene (C=C) group. More peaks were found at 125–500 due to the presence of functional groups C─O─C and C═O and another undefined? functional group present in the plant extract *Tinospora cardifolia*. The result of RAMAN spectra was shown in Fig. 6.

3.6. Powder X-ray diffraction (PXRD) study

To analyze whether the sample is amorphous or crystalline, an X-ray diffraction study was performed. The foremost Bragg's diffraction peaks were detected in the XRD of copper nanocoated fabric at an angle of 2ϴ and the value was 34.91, 38.18 and 48.99 which were similar to the diffraction peaks observed by numerous researchers. These peaks suggested that the copper nanoparticle indexed to face central to the cubic assembly. Various unassigned peaks (Fig. 7) suggest that some of the phytoconstituents were present on the copper nanoparticles over the synthesis process of the copper nanoparticle.

3.7. Determination of antimicrobial activity of greenly synthesized copper nanoparticles and nanoparticle-coated cotton fabric

The anti-microbial activity of copper nanoparticles was estimated through the turbidimetric method and cup plate method. The IC50 value of copper nanoparticles was estimated against the standard drug (Ampicillin) using a turbidimetric method. The UV Visible spectrophotometry system was used to scan the sample and the inhibitory concentration to kill 50% of microbes (IC50) was calculated for two strains of bacteria i.e. *S. aureus* and *E.coli* which are gram positive and gram negative bacteria respectively.

The IC50 value of the ampicillin in gram positive and gram negative bacteria was found to be 50 μg/ml and 62 μg/ml respectively. It was observed that the copper nanoparticles with *Tinospora cardifolia* (CuNPs@Tc) were more effective for gram-positive bacteria as compared to gram-negative bacteria. Further, our study was conducted on copper nanoparticles and nanoparticle-coated cotton fabric, for which nutrient agar media was prepared to study (ZOI) Zone of Inhibition. The result was depicted in Fig. 8 and the graph was depicted in Fig. 9. The nano coated fabric was found to be effective for both *S. aureus* and *E.coli* but the maximum length of the ZOI was found for *S. aureus*. The ZOI value of nanoparticles for *S.aureus* was in the range of 7.01 mm to 16.62 mm for the concentration range of 50–175 μg/ml and for *E.coli* the range was 5.34 mm to 10.54 mm for a concentration range 50–300 μg/ml. Efficacy of the nanoparticles was calculated using % efficacy and found to be increasing from 61% to 75% for *S.aureus* and 59% to 71% for *E.coli*. The ZOI value of nanoparticle-coated fabric for gram-positive bacteria i.e. *S.aureus* was 11.43 mm to 21.99 mm for the same concentration range 50–175 μg/ml and ZOI value for gram-negative bacteria i.e. *E.coli* was 5.47 mm to 11 mm for concentration range 50-300 μg/ml as shown in Fig. 10. The %efficacy of gram-positive bacteria for nanoparticle-coated fabric was 97%–101% and 60%–74% for gram-negative bacteria. In Table 2 and Table 3 ZOI of *S.aureus* and *E.coli* using nanoparticles and copper nanoparticle-coated fabric with % efficiency are represented respectively.

3.8. Study of bacterial growth dynamics of greenly synthesized copper impregnated nano-coated cotton fabric against S. aureus

The effect of copper nanoparticles on the growth of *S.aureuss* was studied between the concentration ranges of 50–175 μg/ml and scanned in UV Visible spectrophotometry. Different concentrations of copper nanoparticles effectively decrease the growth of *S.aureus* and by increasing the concentration and exposure time, the proportionate decrement in the growth of *S.aureus* was observed as shown in Fig. 11.

3.9. Laundry durability assessment of copper Nanoparticlecoated cotton fabric

The laundry durability assessment was accomplished in accordance with the AATCC test method 61-2006. The results of laundry durability testing of copper nanoparticle-coated fabric were demonstrated in Fig. 12. Initially, the content of copper nanoparticles impregnated on the cotton fabric was 58 μg/ml. After the rounds of 10, 25 and 50 laundry rounds some amount of copper nanoparticles leached out. The leaching of copper nanoparticles from nanoparticle-coated fabric was shown as a decrement in the entrapment of the nanoparticles. The

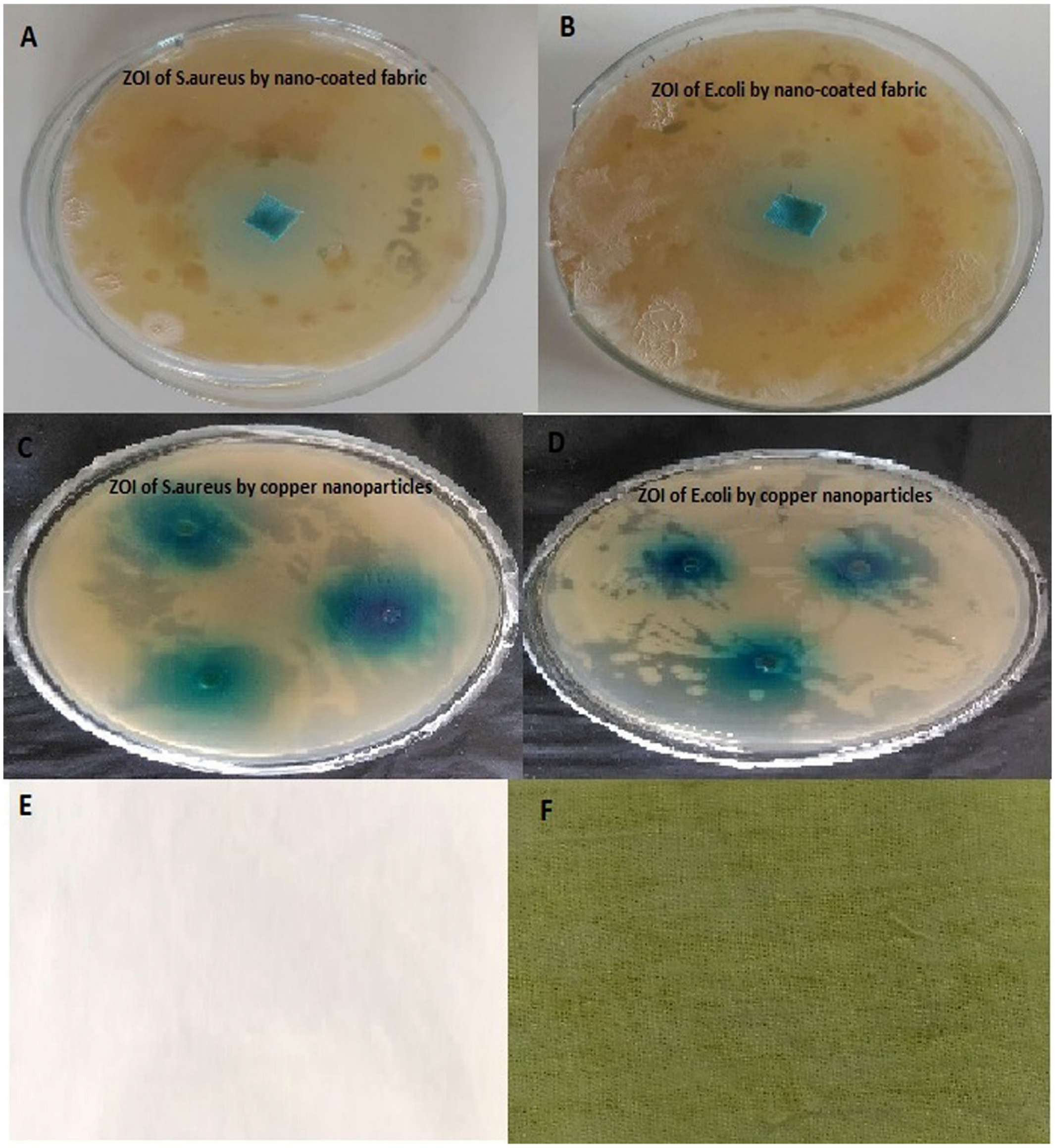

Fig. 8. ZOI study of A) copper nanoparticle-coated fabric on *S. aureus*, B) copper nanoparticle-coated fabric on *E. coli*, C) green nanoparticles on *S. aureus*, D) green nanoparticles on *E. coli*, E) un-treated fabric, and F) copper nanoparticle-coated fabric. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

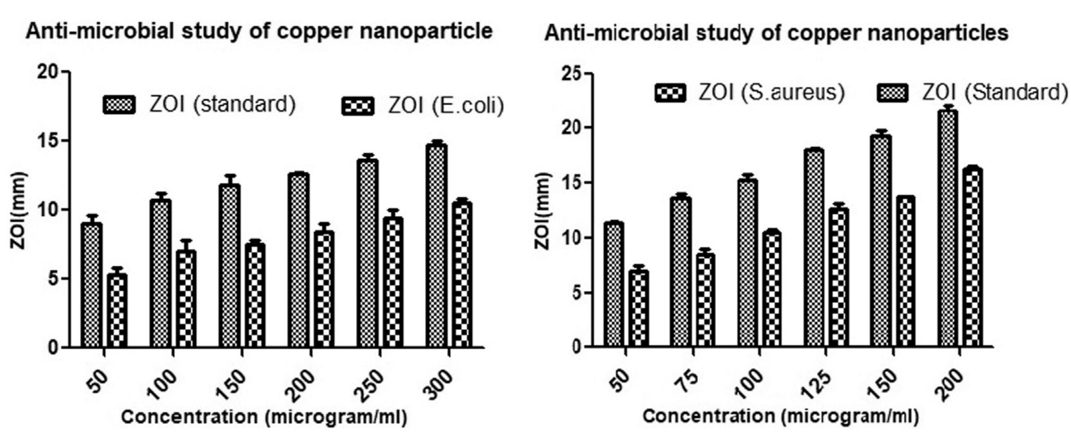

Fig. 9. Graph of ZOI study of A) copper nanoparticles on *E.coli*, B) copper nanoparticles on *S. aureus*.

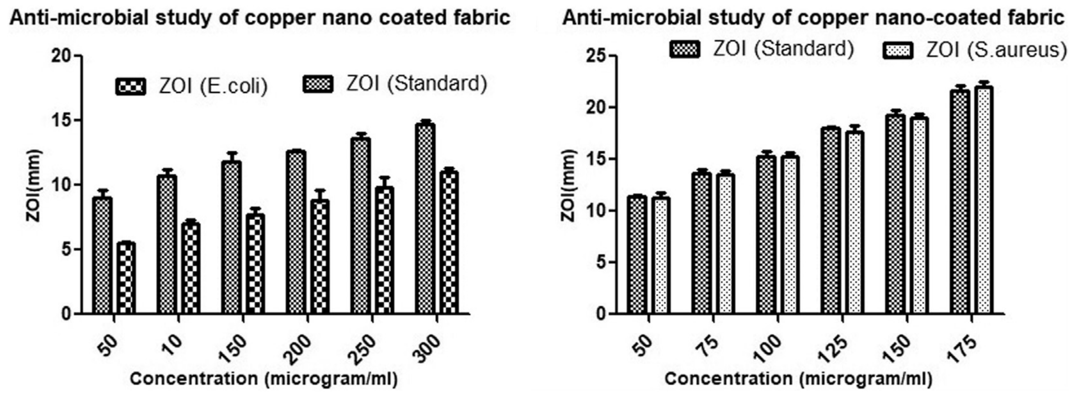

Fig. 10. Graph of ZOI study of A) copper nanoparticles-coated fabric on *E.coli*, B) copper nanoparticles-coated fabric on *S. aureus*.

Table 2
Antimicrobial study of green nanoparticles and green copper impregnated cotton fabric using gram positive bacteria (*S.aureus*).

No of sample	Concentration (μg/ml)	ZOI of standard (mm)	ZOI of green nanoparticles (mm)	%Efficacy
S.aureusS.aureus				
CuNP 1	50	11.66 ± 0.11	7.01 ± 0.53	61
CuNP 2	75	13.64 ± 0.43	8.5 ± 0.53	62
CuNP 3	100	15.32 ± 0.42	10.5 ± 0.32	68
CuNP 4	125	17.96 ± 0.21	12.64 ± 0.54	70
CuNP 5	150	19.21 ± 0.50	13.72 ± 0.11	71
CuNP 6	175	21.65 ± 0.54	16.32 ± 0.21	75
No of sample	Concentration (μg/ml)	ZOI of standard (mm)	ZOI of nanoparticle-coated fabric (mm)	% Efficacy
S.aureusS.aureus				
C-CuNP 1	50	11.66 ± 0.11	11.43 ± 0.38	98
C-CuNP 2	75	13.79 ± 0.43	13.46 ± 0.42	97
C-CuNP 3	100	15.32 ± 0.42	15.25 ± 0.83	99
C-CuNP 4	125	17.96 ± 0.21	17.59 ± 0.65	97
C-CuNP 5	150	19.21 ± 0.50	19.04 ± 0.32	99
C-CuNP 6	175	21.65 ± 0.54	21.99 ± 0.54	101

Table 3
Antimicrobial study of green nanoparticles and green copper impregnated cotton fabric using gram negative bacteria (*E.coli*).

No of sample	Concentration (μg/ml)	ZOI of standard (mm)	ZOI of green nanoparticles (mm)	%Efficacy
E.coli				
CuNP 1	50	9.04 ± 0.54	5.34 ± 0.45	59
CuNP 2	100	10.74 ± 0.5	6.98 ± 0.87	64
CuNP 3	150	11.84 ± 0.65	7.56 ± 0.21	63
CuNP 4	200	12.62 ± 0.11	8.43 ± 0.54	66
CuNP 5	250	13.65 ± 0.32	9.45 ± 0.53	69
CuNP 6	300	14.76 ± 0.21	10.54 ± 0.29	71
No of sample	Concentration (μg/ml)	ZOI of standard (mm)	ZOI of green nanoparticles (mm)	%Efficacy
E.coli				
C-CuNP 1	50	9.04 ± 0.54	5.47 ± 0.19	60
C-CuNP 2	100	10.74 ± 0.5	6.99 ± 0.32	65
C-CuNP 3	150	11.84 ± 0.65	7.68 ± 0.58	64
C-CuNP 4	200	12.62 ± 0.11	8.83 ± 0.8	69
C-CuNP 5	250	13.65 ± 0.32	9.78 ± 0.81	71
C-CuNP 6	300	14.76 ± 0.21	11 ± 0.32	74

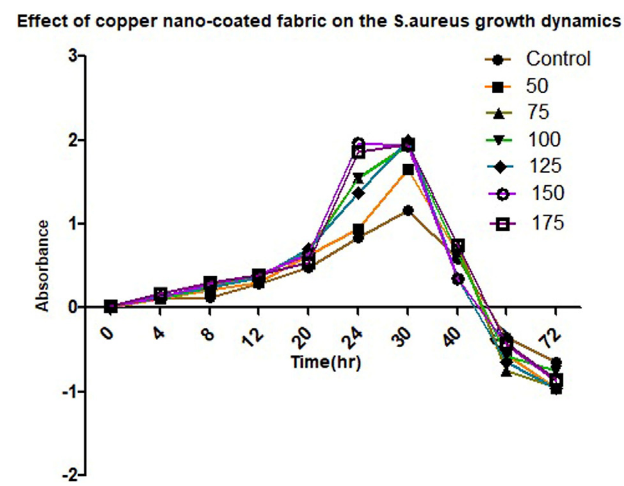

Fig. 11. Effect of copper nanoparticles-coated cotton fabric on *S. aureus* growth dynamics.

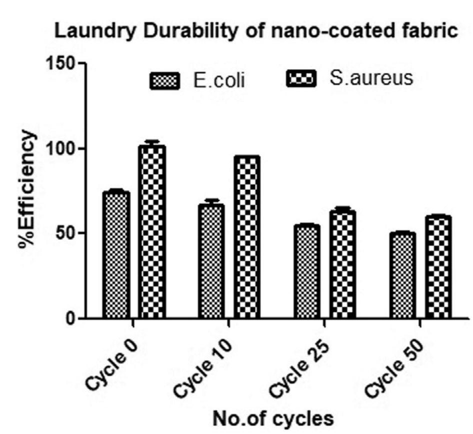

Fig. 12. Percentage efficacy of the green copper nanoparticle-coated cotton fabric after various laundering cycles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

decrement was seen after the 50 laundry rounds in 500 ml of distilled water. The entrapment of copper nanoparticles on cotton fabric in the case of *S. aureus* (gram positive) was found to be 37.7 μg/ml while in the case of (gram-negative bacteria) *E.coli* it was 29 μg/ml. There were significant changes in the concentration of copper nanoparticles incorporated on cotton.

4. Discussion

Surface Plasmon's resonance (SPR) is defined as collective excitation of negatively charged ions (electrons) in a conduction band around the surface of synthesized copper nanoparticles. Electrons present in the nanoparticles were confined to have an exact vibration mode because of their particular shape and size (Shantkriti and Rani, 2014). Plasmon's Resonance band was found at 248 nm. This confirms the presence of copper nanoparticles.From the outcomes of Particle size, it was concluded that the least particle size of copper nanoparticles possessed maximum surface area which provides a large area for the interaction with the microbes. The optimized formulation was used for the incorporation of copper nanoparticles on the cotton fabric.

From the results of SEM, TEM, and AFM it was determined that the nanoparticles were smooth and spherical in shape. No visible cracks or pinholes were visualized in the nanoparticles.

With the help of ATR-FTIR, and RAMAN, it was observed that there is was no interaction between the chemicals used in the preparation of nanoparticles or with the extract. With ATR-FTIR it was concluded that the functional groups present in the copper nanoparticles which were incorporated on the cotton fabric were accountable for stabilizing the copper nanoparticles. Using RAMAN it was concluded that the functional groups present in the fabric indicated that copper nanoparticles were coated with *Tinospora cardifolia* and were present on the coated fabric. The XRD pattern showed the coexistence of two crystalline phases i.e. metallic copper and copper oxide in the nanoparticles.

From the anti-microbial study conducted for copper nanoparticles and copper nanoparticle-coated fabric, it was found that nanoparticles and nanocoated fabric both possessed anti-microbial activity. The anti-microbial activity was found to be more effective toward gram-positive than gram-negative bacteria. The difference in the activity is partly due to the complexity in the structure of gram-negative bacteria. The thickness of the gram-positive cell wall is less than the gram-negative cell wall (Sung and Lee, 2008; Valle et al., 2016). Due to the thickness in the cell wall compounds get entrapped in the cell wall reducing the effect. From the growth dynamics study, it was concluded that the nanoparticle-coated fabric carries anti-microbial property by preventing the growth of microbes.

From the Laundry durability assay, it was concluded that cotton coated copper nanoparticles were efficacious after several accelerated laundry rounds. The efficacy of coated cotton against the gram-negative bacteria was 65% and 50% toward the gram-negative bacteria which might be probable because of the capping of copper nanoparticles with *Tinospora cardifolia*. The laundary durability assay indicates that when more copper nanoparticles adhere to the greater the antimicrobial activity of the cotton cloth. Thus *S.aureus* nano coated fabric showed more antimicrobial activity because it showed less leaching of copper nanoparticles from the cotton when compared to *E.coli*.

Copper nanoparticles possess anti-microbial property which makes them useful in the treatment of various topical infections caused by various bacteria. This approach is an advance in the textile industry for the prevention of airborne topical diseases. There are various methods through which copper nanoparticles can be synthesized such as chemical and physical methods but there are some drawbacks associated with them, so by employing green synthesis processes, we can minimize the environmental hazard by reducing the waste. Further characterization of the nanoparticle was performed through UV0Vis-Spectrophotometer, ATR-FTIR, RAMAN, SEM, EDX, TEM, AFM. The anti-microbial study of copper nanoparticles and the copper nanoparticle-coated fabric was evaluated to support the study that copper nanoparticles contribute to anti-microbial properties. Laundry durability assessment was performed in order to determine the retention power of copper nanoparticles on the fabric. It was found that fabric was 65% efficacious for gram-positive bacteria and 50.5% efficacious for gram-negative bacteria.

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