Carbohydrate Polymers 79 (2010) 717–723

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Effective antibacterial adhesive coating on cotton fabric using ZnO nanorods and chalcone

P.M. Sivakumar [a], S. Balaji [b], V. Prabhawathi [a], R. Neelakandan [c], P.T. Manoharan [b,*], M. Doble [a,*]

[a] Department of Biotechnology, Indian Institute of Technology Madras, Adyar, Chennai 600 036, India
[b] Sophisticated Analytical Instruments Facility and Department of Chemistry, Indian Institute of Technology Madras, Chennai 600 036, India
[c] Department of Textile Technology, Anna University, Guindy, Chennai, India

ARTICLE INFO

Article history:
Received 20 August 2009
Received in revised form 23 September 2009
Accepted 29 September 2009
Available online 4 October 2009

Keywords:
Nanorods
Chalcone
SEM-EDAX

ABSTRACT

Chalcone ((E)-1-(3-hydroxyphenyl)-3-(4-methoxyphenyl) prop-2-en-1-one) and ZnO flower-like nanorods were prepared and coated on cotton cloth with acacia as binder. The surface was characterized by FT-IR, AFM, goniometer and SEM-EDAX. The antibacterial activity of the coated cotton was tested against three organisms namely *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* in terms of live bacterial load, as measured by the colony forming units (CFU), adhered on the cotton surface. More than 99% reduction in bacterial load was observed against all three organisms. Viability of the bacterial cells was tested using a dual staining BacLight Kit. Majority of the cells adhered on the coated cotton surface were dead and on uncoated were live. *S. aureus* was found to be most hydrophobic organism. The chalcone showed 48%, 45% and 35% reduction in slime produced by *S. aureus, E. coli* and *P. aeruginosa*, respectively.

1. Introduction

Patients in the critical care setting are more predisposed to a variety of nosocomial or Hospital acquired infections (Dieckhaus & Cooper, 1998), more so with multidrug-resistant bacteria, viral and fungal organisms which pose serious threat to the spread of diseases (Lowy, 2003). The most common pathogens include staphylococci (especially *Staphylococcus aureus*), *Pseudomonas*, and *Escherichia coli*. According to a 2006 report, nosocomial infections are estimated to occur in at least 5% of all patients hospitalized (Nguyen, 2006). Direct contact between host and infected person is recognized to be the most important mode of transmission of nosocomial infection (Borkow & Gabbay, 2008) and the contaminated objects predominantly include cloth materials such as bed linen, towel and clothing (Beggs, 2003). These cloth material might get infected with microbes up to the level of 10^6 to 10^8 colony forming units (CFU) per 100 cm^2 (Blaser, Smith, Cody, Wang, & LaForce, 1984; Tony, Michael, Annette, & Vanya, 2009). The use of chlorine or bromine and high temperature washing kills the microbes but also cause damage to the fabric leading to its replacement (Belkin, 1998). In recent years, there is a growing awareness on the use of antibacterial fabrics in the form of medical clothes, protective garments and bed spreads to minimize the chance of nosocomial infections (Wang et al., 2007).

Using more than one drug to inhibit microbial action is called combination antimicrobial therapy. This combination therapy confers an advantage over single drug therapy by preventing or slowing the emergence of resistant strains and might also help in speeding up the process of bacterial inhibition (Petersdorf, 1975). Hence the present work aims at using a combination of antimicrobial agents in preparing antimicrobial cloth which could be more effective towards multidrug resistant strains.

Nanoparticles (particles less than 100 nm in diameter) are much more active than larger particles because of their higher surface area and they display unique physical and chemical properties which make them suitable for preparing hygienic surfaces (Chen & Chiang, 2008). Textiles coated with silver nanoparticle have become quite common (Chen & Chiang, 2008; Duran, Marcarto, De Souza, Alves, & Esposito, 2007). To our knowledge, the efficiency of ZnO nanoparticle in imparting antibacterial effect to fabric is not yet well established although it is known to strongly resist microorganisms (Sawai et al., 1996). ZnO nanoparticle is currently being investigated as an antibacterial agent both against Gram negative microorganism like *E. coli* and Gram positive microorganism like *S. aureus* in microscale and nanoscale formulations (Applerot et al., 2009). An important aspect of the use of ZnO as antibacterial agent is the requirement that the particles are not toxic to human cells (Huang et al., 2008; Nair et al., 2008). Although the exact mechanism has not yet been clearly elucidated,

* Corresponding authors. Tel.: +91 044 22574938 (P.T. Manoharan), +91 044 22574107 (M. Doble).
E-mail addresses: ptm@iitm.ac.in (P.T. Manoharan), mukeshd@iitm.ac.in (M. Doble).

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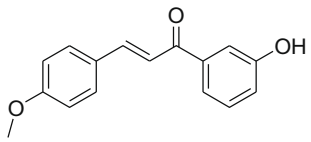
doi:10.1016/j.carbpol.2009.09.027

the suggested mechanisms include, the role of reactive oxygen species (ROS) generated on the surface of the particles (Applerot et al., 2009; Sawai et al., 1996, 1997, 1998), zinc ion release (Yang & Xie, 2006), membrane dysfunction (Yang & Xie, 2006; Zhang, Jiang, Ding, Povey, & York, 2007), and nanoparticle internalization (Brayner et al., 2006).

Chalcones are antibacterial agents which exert bactericidal activity by damaging the bacterial membrane. Since thee chalcone used, ((E)-1-(3-hydroxyphenyl)-3-(4-methoxyphenyl) prop-2-en-1-one), exerts its influence externally to the microorganism by damaging the delicate cell membrane, it need not be dissolved in solution to produce the killing.



Chalcone

This is an added advantage if it is used to impart antibacterial action to cloth. Moreover chalcones also possess slimicidal and bactericidal properties which are helpful in preventing biofilm formed by the microorganism. Formation of biofilm is a prerequisite for bacterial adhesion on surfaces, subsequently leading to infection on implanted devices (Pavithra & Doble, 2008).

Gum arabic is a complex and variable mixture of arabinogalactan oligosaccharides, polysaccharides and glycoproteins. The simultaneous presence of hydrophilic carbohydrate and hydrophobic protein enable their emulsification and stabilization properties.

The aim of the present study was to produce ZnO nanoparticle, chalcone and acacia coated fabric, and estimate its antibacterial property against infectious strains namely *S. aureus*, *Pseudomonas aeruginosa*, and *E. coli*. These microorganisms are the common cause of nosocomial infections. While chalcone is expected to have bactericidal property, ZnO can act as antibacterial agent and is also believed to act as a carrier to transport chalcone. In combination therapy, the concentration of individual compound is less thereby reducing the toxicity level. Moreover the synergistic activity in combination therapy helps in combating their drug resistance compared to single drug therapy.

2. Materials and methods

2.1. Experimental methods

All the chemicals were purchased from Sigma–Aldrich (St. Louis, USA) and SRL (Mumbai, India). The bacterial strains (*S. aureus* NCIM5021, *E. coli* NCIM2931 and *Pseudomonas areuginosa* NCIM2901) were purchased from National Chemical Laboratory, Pune, India.

2.2. Synthesis of ZnO flowers-like nanorods (NRs)

3.2925 g of Zn $(CH_3COO)_2{\cdot}2H_2O$ (0.5 M), 6 g of NaOH (5 M) were added along with 10 ml of butyl amine. The suspended mixture was transferred to a 300 ml Teflon coated autoclave and the volume was made up to 80% by adding 170 ml of distilled water. The pH of the final solution was measured to be 11.8. The contents of the autoclave were heated in an oven to 160 °C for 12 h. The product was cooled to room temperature and centrifuged. The precipitate was thoroughly washed first with distilled water and then with methanol. The final product was dried in a vacuum oven at 80 °C for 3 h.

2.3. Chalcone synthesis

The synthetic procedure for chalcone is adopted from Lin, Rivett, and Wilshire (1977). The product was characterized by FT-IR, NMR, and mass spectrometry.

2.4. Coating procedure

A 100% (by weight) cotton woven fabric containing 140 grams per square meter of plain weave, 20 ends/cm and 16 picks/cm was used in the current study. The cotton fabric was cut to the size of 10 sq. cm and was immersed in the solution containing 20% by weight of ZnO nanoparticles, 20% by weight of chalcone and 60% by weight of acacia for 5 min, and then was passed through a padding mangle (Electronic and Engineering Company, Bombay, India), run at a speed of 15 m min^{-1} and pressure of 25 kg cm^{-2} to remove excess solution. The Material (cloth) to Liquor (ZnO nanoparticle, chalcone and acacia) ratio was kept at 1:20. A 100% wet pick-up (wetness) was maintained for all of the treatments. The fabric was then passed through padding mangle to give uniform coating and was dried to remove excess solution.

2.5. Zone of inhibition

Agar diffusion test was used to assess the antimicrobial activity of the treated cloth sample (Vaideki, Jayakumar, Rajendran, & Thilagavathi, 2008). The zone of inhibition of the test sample was measured in mm, and it was a measure of the antimicrobial activity of the treated cloth. Zone of inhibition around the test sample was measured in mm, and it was a measure of the antimicrobial activity of the treated cloth.

2.6. Bacterial adhesion

Adhesion of bacteria on compound coated and untreated control cloths were studied in triplicate. *S. aureus, P. aeruginosa* and *E. coli* were subcultured and maintained in nutrient agar plates. The adhesion experiments were carried out based on the method suggested by Zhao et al. (2007) with slight modifications. A single colony from an agar plate was inoculated into 20 ml tryptic soy broth (TSB) and grown for 16 h in a shaker at 180 rpm at 37 °C until the cultures reached mid-exponential phase. The culture was centrifuged at 8000 rpm (6000*g*) at 4 °C for 10 min. The pellets were suspended in 0.9% saline and adjusted to an optical density of 0.1 at 660 nm, and this gave approximately 1×10^7 cells/ml. The treated and control cloths were immersed in 25 ml of the above made bacterial suspension and incubated in static condition at 37 °C for 2 h. At the end of this time period, the samples were transferred into 25 ml of fresh tryptic soy broth and incubated for 24 h at 37 °C at 120 rpm. After the incubation period of 24 h, the samples were removed using sterile forceps and washed twice in sterile water to remove non-adherent bacteria. The adherent bacteria were then removed from the cloth surface by water-bath ultrasonication (sonication was for a minute with 1 min interval break for a total 10 min sonication). After sonication, the colony counts of viable cells present in it were determined by spreading it in tryptic soy agar plates (TSA).

2.7. Assessment of hydrophilicity

Hydrophilicity of the treated and untreated cloth sample was assessed using static immersion test reported in ATCC Technical Manual 2001. This is a test used to measure the amount of water absorbed by the fabric. Coated and uncoated cloth sample were weighed and immersed to a depth of 10 cm in a beaker containing 250 ml of distilled water. The cloth was removed after 20 min and

tapped ten times to remove excess water and then weighed once again. The absorption percentage was determined by the following formula (Vaideki et al., 2008).

$$\text{Absorption percentage} = (\text{mass of water absorbed/original mass}) * 100$$

2.8. BacLight assay

The bacterial cell membrane damaging activity of the compound mixture was determined as per the method reported by Hilliard et al. (Hilliard, Goldschmidt, Licata, Baum, & Bush, 1999), using BacLight Kit (Invitrogen, USA). The kit contains a mixture of two nucleic acid staining dyes namely, SYTO9 which stains all live cells and PI dye which enters only dead cells i.e. membrane damaged cells. Both cloths exposed to bacteria for 24 h were used for this test. After adhesion experiments the test and control cloths were washed with distilled water and 20 μl of the dye mixture was placed on the surface and incubated in the dark for 10 min. Excess of dye was washed with distilled water and the materials were viewed under fluorescence microscope (Leica DM5000, Germany) with a blue filter at an excitation of 475 nm. Live cells fluoresce green and dead cells fluoresce red.

2.9. Slimicidal activity

Reduction in slime production by these three microorganisms after treatment with chalcone was evaluated based on the protocol suggested by Tsai, Schurman, and Smith (1988). This chalcone at its MIC concentration was added to a glass tube containing 1 ml of tryptic soy broth supplemented with 10% (v/v) glucose. A single colony of the bacteria was inoculated into this broth and was incubated without any agitation at 37 °C for 24 h. A control was maintained without the compound. The supernatant containing the culture was decanted and the biofilm sticking onto the wall of the test tube was washed twice with 1 ml of water and reacted with Carnoy's solution (containing abs. ethanol: $CHCl_3$: Glacial acetic acid at a ratio of 6:3:1, respectively) for 10 min. One milliliter of saffranin was added to the tube and then was gently rotated to uniformly coat the walls with the adherent material. Excess stain was removed by washing twice with 3 ml of water. One milliliter of 0.2 M NaOH was added to the tube and the sample was heated for 1 h at 85 °C. Then it was vortexed, cooled at room temperature and the OD was measured at 530 nm. The percentage reduction in slime was calculated using the following formula,

$$\%\ \text{slime reduction} = \frac{(\text{Control OD} - \text{OD after treating with compound}) \times 100}{\text{Control OD}}$$

2.10. Organism hydrophobicity

BATH test (Rosenberg, Gutnick, & Rosenberg, 1980) was performed to determine the hydrophobicity of the bacteria. Bacterial cells have greater affinity to hydrocarbon such as hexadecane. The more hydrophobic the microorganism, greater is its affinity to hydrocarbon, which results in transfer of cells from aqueous phase to organic phase leading to a reduction in the turbidity of the former phase. The bacteria were cultured in tryptic broth medium till the growth reached mid-logrithmic phase. At this stage, the broth was centrifuged and the cells were washed twice with Phosphate–Urea–Magnesium (PUM) buffer containing 17 g K_2HPO_4, 7.26 g KH_2PO_4, 1.8 g urea and 0.2 g $MgSO_4 \cdot 7H_2O$ per liter. The washed cells were resuspended in PUM buffer to reach 1.0 OD at 400 nm. Aliquots of 1 ml of this suspension were transferred to a series of test tubes. Increasing volumes of hexadecane (0.0–0.2 ml of hexadecane in steps of 0.5 ml) were added to these test tubes. The samples were mixed well for 10 min and allowed to stand for 2 min to facilitate phase separation. OD of the aqueous phase was measured at 400 nm, while the cell-free buffer was used as blank. A graph was plotted between OD and different concentrations of hexadecane. If the OD decreases with increasing hexadecane concentration, it means that the microorganism is hydrophobic (since it prefers the hydrocarbon) and the reverse trend means that the microorganism is hydrophilic.

2.11. Scanning electron microscopy

The surface of the coated and uncoated cloth was observed using Scanning electron microscope (SEM) before and after the adhesion experiments. The chemical coating on the cloth was confirmed using SEM-EDAX. After adhesion experiment, the cloth was washed with distilled water and then fixed using 3% glutaraldehyde (in 0.1 M phosphate buffer at pH 7.2) for an hour. Later it was washed twice with phosphate buffer, once using distilled water and dehydrated using alcohol of various gradients (20%, 50%, 70% and 90%) for 10 min. The samples were dried overnight in a dessicator. These biomaterials were coated with platinum at 30 mA for 1 min and were viewed under a scanning electron microscope (Jeol JSM 5600 LSV model) at a magnification of X3000.

2.12. Instrumentation

FEI Quanta 200 environmental scanning electron microscope (ESEM) with EDS was used for measuring ZnO nanorods. The X-ray powder diffraction was measured with Bruker Discover D8 diffractometer. The step width is 0.1 degree and X-ray source was Cu K_α for diffraction at 1.54 nm wavelength. Perkin Elmer Spectrum 1 FT-IR with KBr pellet model was used for analyzing the ZnO, chalcone and acacia in the range of 450–4500 cm^{-1}.

3. Results and discussion

3.1. Characterization of zinc oxide, chalcone and acacia

The powder XRD pattern of ZnO nanorods is shown in Fig. 1. The result shows the presence of good crystalline material and is well indexed to infer it to be hexagonal wurtzite when compared with

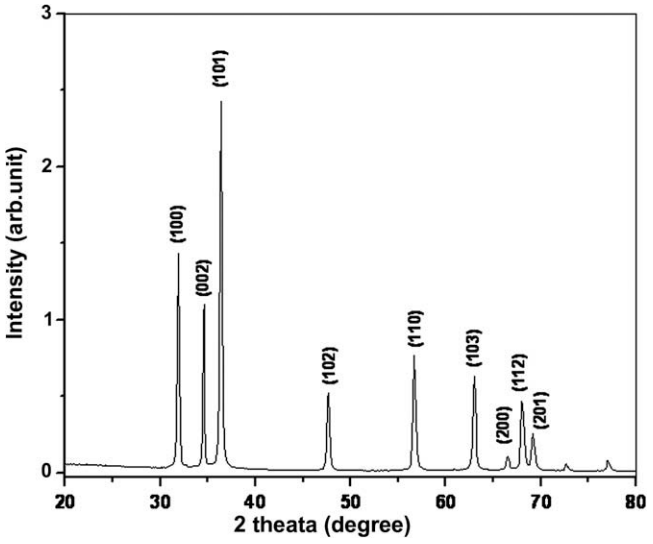


Fig. 1. Powder XRD pattern of ZnO nanorods in hexagonal phase with orientation of (1 0 1) plane.

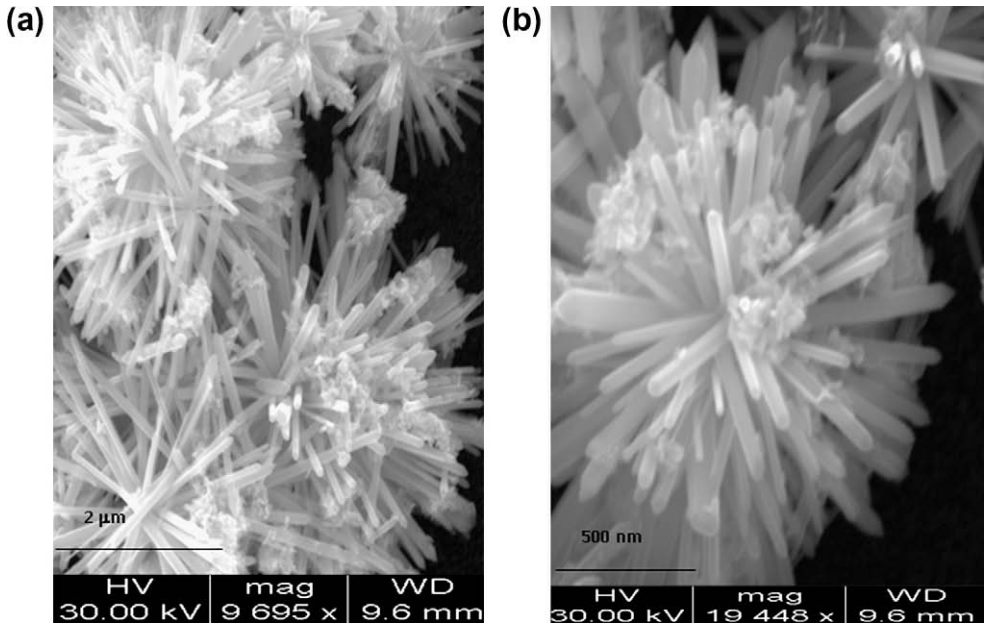


Fig. 2. (a and b) Scanning electron microscopy picture of ZnO.

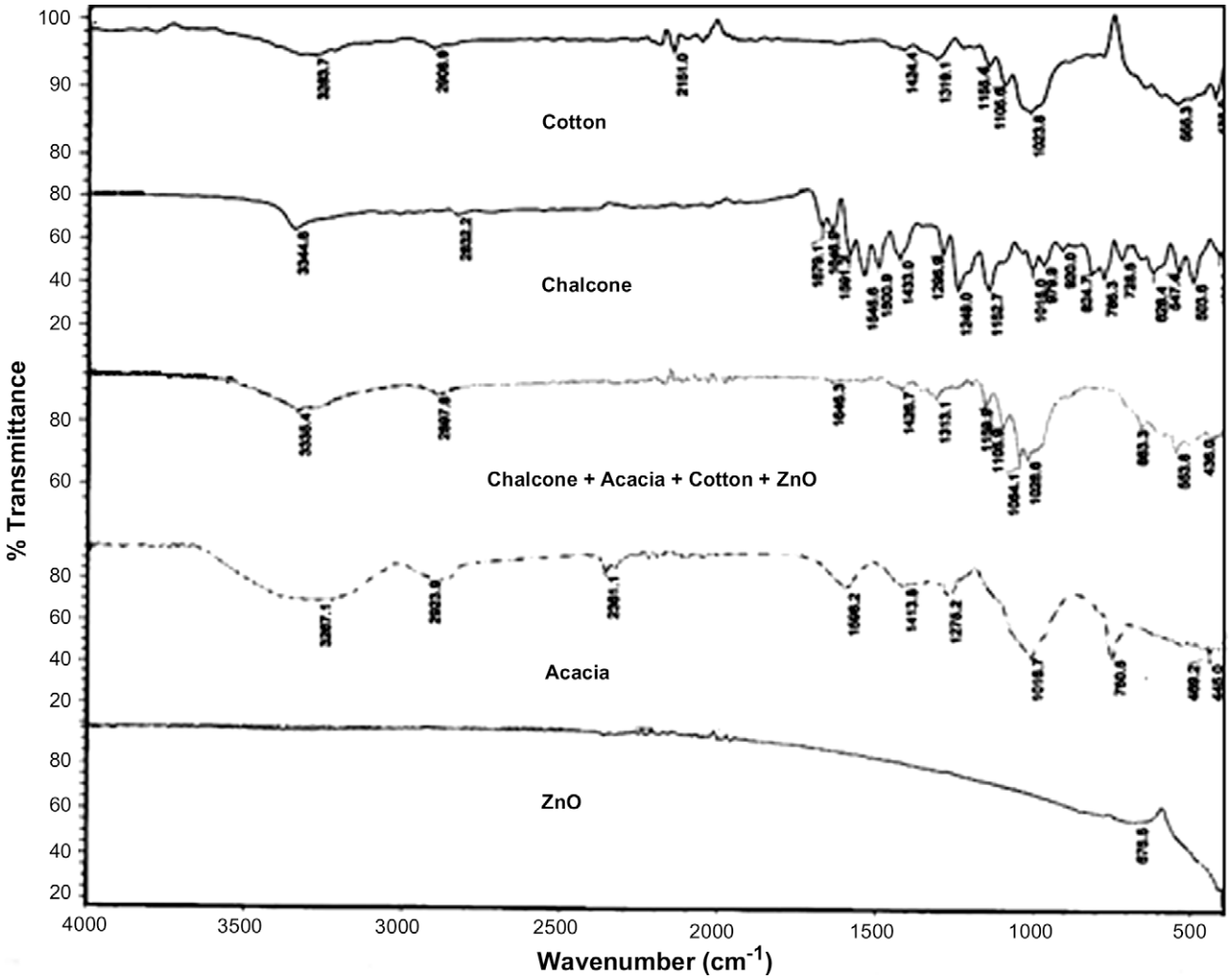


Fig. 3. FT-IR spectrum of cotton, chalcone, coated cotton, acacia and ZnO nanorods.

bulk material (JCPDS 36-1451). These nanorods show their orientation of crystal growth along (1 0 1) plane.

The typical SEM images of ZnO nanorods are shown in Fig. 2a and b. They have well defined structure with some imperfection. Their width varies from 80 to 150 nm and the length from 500 nm to few micrometers. EDAX pattern of ZnO (Fig. 1S) shows sharp peaks corresponding to Zn (L) and O (K). The Zn:O composition was found to be 50.21:49.79 confirming its purity and stoichiometry within experimental error.

High concentration of NaOH and the consequent pH of the reaction mixture decide the morphology of the nanorods. The reaction mixture contains $(Zn(OH)_4)^{2-}$ as soluble species. This complex ion gets converted to $Zn(OH)_2$ which then decomposes to form ZnO nanorods. Initially formed such ZnO nanorods act as nucleus which allow the deposition of subsequently formed ZnO particles on the circumference of the former. Such a growth seems to be responsible for the formation of flower-like nano structures (Kale, Hsu, Lin, & Lu, 2007).

The synthesised chalcone was characterized by NMR and FT-IR spectroscopy (Pavithra & Doble, 2008). Fig. 3 shows the individual FT-IR spectrum of chalcone, ZnO and acacia.

The strong peak at 3344 cm^{-1} in chalcone is due to the presence of hydroxyl group. The peak at 1646 cm^{-1} reveals the presence of C=O stretching (α,β-unsaturated carbonyl system) vibration. A broad peak observed in the region 575–675 cm^{-1} indicates the formation of ZnO (Wua, Wua, Panb, & Kong, 2006). Both IR and EDAX of ZnO indicate the lack of any contaminants. Acacia shows a broad peak in the region of 3700–2800 cm^{-1} due to the combination O–H stretching and C–H symmetry and asymmetry stretching. In addition, peaks around 1598 and ~1413 cm^{-1} indicate COO asymmetry and symmetry stretching, respectively (Cuia, Phillipsb, Blackwellc, & Nikiforukc, 2007).

3.2. Characterization of the coating

The chalcone, ZnO and acacia coated cloth are characterized by FT-IR and SEM-EDAX. The coated cotton shows the peak around 663 cm^{-1} revealing the presence of ZnO as in Fig. 3. The region at 1646 cm^{-1} indicates the C=O stretching of α,β-unsaturated carbonyl system in the chalcone. The broad region from 3500–3200 cm^{-1} is responsible the hydroxyl groups present in chalcone, acacia and cotton.

Figs. 2S and 3S show the EDAX of the uncoated and coated cloth, respectively, and Fig. 4 shows the corresponding SEM. Table 1 presents the weight percentage of the uncoated and coated cloth. Uncoated cloth shows only C and O. Au is also observed since it is used as a coating for making the cloth conducting.

Table 1
Results of EDAX from uncoated and coated cloth.

Element	Uncoated		Coated	
	Weight percentage	Atom percentage	Weight percentage	Atom percentage
C	42.68	63.42	41.84	63.7
O	30.63	34.16	28.66	32.75
Au[a]	26.69	2.42	25.19	2.3
Zn			4.31	1.21

[a] Au is used for coating to make the material conducting.

Presence of Zn in the coated cloth indicates that ZnO is deposited on it. Since both chalcone and acacia contains only C and O, no new peaks were observed in the EDAX. SEM images (Fig. 4) of the coated cotton fibers show the presence of compound coating on the fibers.

3.3. Antibacterial activity of coated cotton

Coated material showed more than 99% reduction in bacterial adhesion. The reductions were 99.58%, 99.99% and 99.89% for *S. aureus, E. coli* and *P. aeroginosa*, respectively, as reported in Table 1S. It is noteworthy to mention that all these are slime producing bacterial strains. The reduction of the bacterial load on the coated cloth surface is also seen in the SEM image (Fig. 5B).

Both coated and uncoated cloths after the exposure to the microorganism were treated with BacLight Kit® (as described before). The images showed green and red cells, indicating the presence of live and dead cells respectively. More (red) dead cells were found on the coated cloth (Fig. 6B) and (green) live cells were found on the uncoated cloth. Propidium Iodide (PI) penetrates only damaged cells and binds to the DNA producing red colour, whereas SYTO9 dye remains on the exterior of the undamaged cell walls producing a green colour. The mechanism of chalcone is probably to damage the bacterial cell membrane and it is in accordance with our earlier findings (Sivakumar, Priya, & Doble, 2009).

3.4. Hydrophobicity of the cloth

We related the bacterial adhesion and hydrophilicity of the coated and uncoated cotton. The hydrophilicity of the cotton was measured by static immersion test. The uncoated cotton fibers showed 171% and the coated cotton fibers showed 157% absorption of water (Fig. 4S). This clearly shows an increase in the hydrophobicity of the coated when compared to the uncoated cotton fibers.

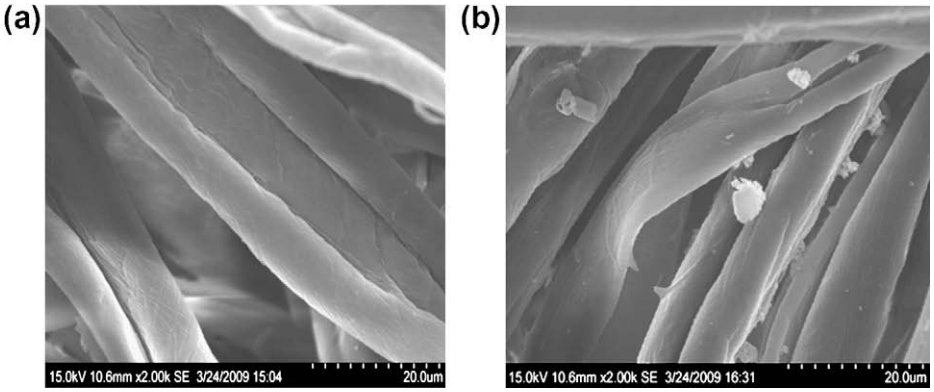


Fig. 4. Scanning electron microscope photomicrographs of (a) uncoated and (b) coated cotton fibers.

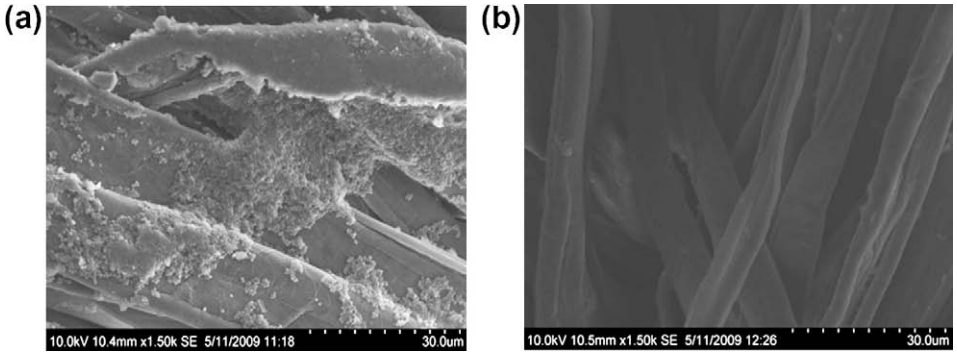


Fig. 5. SEM photomicrographs of (a) biofilm on uncoated cloth and (b) its absence on coated cotton fibers.

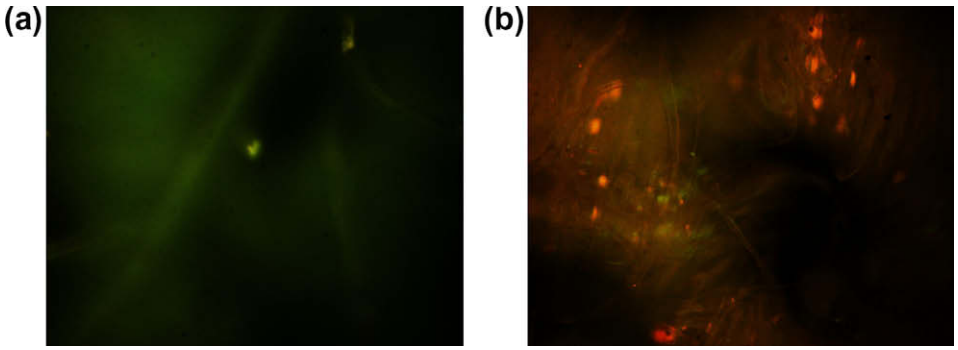


Fig. 6. Live (green) and dead (red) *S. aureus* on- (a) uncoated (control) and (b) coated cloth. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

The hydrophobicity of the organisms, were determined by measuring the OD at 400 nm at different volumes of hexadecane ranging from 0.05 to 0.2 ml (Sivakumar et al., 2009). It was found that *S. aureus* is more hydrophobic. The decrease in OD was 40% with the addition of 0.2 ml 0f hexadecane due to the migration of the cells from the aqueous phase to non aqueous hexadecane phase. *E. coli* and *P. aeruginosa* are relatively hydrophilic, since the OD decreased by only 20% for the same amount of hexadecane (Fig. 5S).

Our earlier research (Sivakumar et al., 2009) exposed the slimicidal (which limits the biofilm formation) activity of chalcone. When the slimicidal activity of chalcone was checked against these three organisms, maximum reduction of 50% was observed in slime produced by S. *aureus* (Fig. 6S).

4. Conclusion

S. aureus, *E. coli* and *Pseudomonas areuginosa* are the predominant nosocomial infection causing organisms. They are also responsible to produce multidrug resistance so single compound therapy will not be effective. Hence here we attempted a multicomponent therapy. To our knowledge, this is the first elaborative report on antimicrobial effect of chalcone, acacia and ZnO nanoparticle coated cotton cloth. The ZnO nanoparticles were produced and characterized by FT-IR, XRD, SEM and SEM-EDAX studies. Since ZnO is used in the form of nanoparticle, it will have very good absorption, penetration and availability. The It is shown that 99.99% reduction in bacterial adhesion on coated cloth against these three organisms. Baclight studies showed that the coating activity exhibit membrane disruption activity which is an added advantage.

Experimental data for compound **24**: Yield: 72%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.84 (s, 3H), 6.90–6.92 (m, 2H), 7.14 (ddd, J = 8, 2.4, 0.8 Hz, 1H), 7.36 (dd, J = 8.4, 7.6 Hz, 1H), 7.38 (d, J = 15.6 Hz, 1H), 7.54–7.58 (m, 3H), 7.66 (dd, J = 2.4, 1.6 Hz, 1H), 7.79 (d, J = 16 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 55.42, 114.45, 115.25, 119.58, 120.33, 120.79, 127.47, 129.83, 130.41, 139.73, 145.45, 156.56, 161.84, 191.00.

Acknowledgements

PTM thanks the DST, Govt. of India for the Ramanna Fellowship (SR/S1/RFIC-02/2006) and the JNCASR, Bangalore for the Honorary Professorship.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2009.09.027.

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