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Antibacterial Cotton Finish Using Green Tea Leaf Extracts Interacted with Copper

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ABSTRACT

Majority of the antimicrobial compounds used for treating textiles are synthetic based and are not considered to be environmental friendly. Therefore, the main aim of this study was to use the extracts from leaves of edible Green tea showing antibacterial properties to treat textile materials. The extracts (GTE) were made interacted with copper (GTC) in the copper vessel for enhancing the antibacterial properties. The extracts were treated onto cotton fabrics using a standard Pad-dry-cure method with and without the cross-linker (Citric acid). Using the standard Agar diffusion test the antibacterial activity and durability of GTE and GTC treated cotton was evaluated before and after wash. After 10 washes, the directly applied GTE and GTC extracts (without cross-linker) do not show much activity (<25% bacterial reduction) when compared to that of GTC treated cotton with citric acid (>50% bacterial reduction). Antibacterial textile finish using the eco-friendly leaf extracts of Green tea could have the possibility to eliminate the properties of bacteria gaining resistance against the oral prophylactic antibiotic drugs.

Key words: Pad-dry-cure method, *Camellia sinensis*, cross-linker, *Escherichia coli*, *Staphylococcus aureus*

INTRODUCTION

In hospitals numerous solid surfaces are likely to be contaminated with microbes including the textile materials in staff uniforms, patient gowns, drapes, curtains, bed sheets, pillow cases and pillows. Due to the suitability of temperature and humidity in the environment exposure to microbial contamination further increases the probability of pathogen acquisition. This is a state in which microbes such as viruses or bacteria invade body tissues and increase the risk of disease (Ray *et al.*, 2002). Treatment of these infections is associated with high complication rates and places an enormous burden on both the patient and healthcare workers (Darouiche, 2004). However, antimicrobials administered systemically or orally often fail to reach the site of infection, again decreasing the success of antimicrobial chemotherapy (O'Gara and Humphreys, 2001).

Treatment of device-related infection is very difficult because of the increasing resistance to antibacterial agents. The development of antibiotic-resistant bacteria has increased at a frightening rate since the introduction of antibiotics in the 1940s. The emergence of resistance among

nosocomial pathogens can also be attributed to the increasing number of immune-compromised patients, the use of invasive procedures and devices and the breakdown of infection and disease-control practices within the hospital environment. Antimicrobial resistance has a significant impact on patients' outcome by enhancing virulence, delaying the administration of appropriate therapy, limiting available therapy and increasing hospitalization time and subsequent recovery, leading to increased morbidity and mortality (Cosgrove and Carmeli, 2003).

At present, conventional systemic therapies, using standard antimicrobial agents, represent the main strategy for the treatment and prevention of medical device associated infection. The limitations of conventional chemotherapy in the treatment of medical device-related infections have prompted the development of novel approaches, complementary to traditional bactericidal or bacteriostatic mechanisms. These approaches focus on the development of bioactive, anti-infective or antimicrobial devices, which inhibit bacterial adherence or growth by the presence or elution of antimicrobial agents (Darouiche, 2004).

On non-implantable materials (textile materials of staff uniforms, patient gowns and wound dressings) different antimicrobial agents (silver, zinc, antibiotics and disinfectants) were treated to retard the transmission of hospital acquired infections (Ray *et al.*, 2002).

The increased use of antimicrobial textiles has raised concerns about resistance and other issues. Dev *et al.* (2009) reported that traces of silver have been found in the blood and urine of the patient who was treated with a dressing containing nano-crystalline silver. The risks of localized argyria and cytotoxicity to keratinocytes and fibroblasts have also been cited (Chen and Schluesener, 2008). Antimicrobial textiles finished with disinfectants like quaternary ammonium compounds (QACs) and polyhexamethylene biguanides (PHMB) leads to bacterial resistance. Bacterial resistance to triclosan has been well documented and is of great concern (Russell, 2004). Furthermore, when exposed to sunlight in the environment, triclosan breaks down into 2, 8-dichlorodibenzo-p-dioxin which is chemically related other toxic polychlorinated dioxins (Larsen, 2006). N-halamine treatment results in a substantial amount of adsorbed chlorine (or other halogens) remaining on the surface of the fabric in addition to the covalently bonded N-halamines. Such residual adsorbed halogen (e.g., chlorine) produces an unpleasant odour and discolours fabrics, which has proven problematic for such a promising antimicrobial system in the textile industry (Li, 2003). Peroxyacids on the fabrics was stable over extended periods during fabric storage; the antimicrobial activity appeared to be diminished after several washing and recharging cycles (Huang and Sun, 2003).

Even though all type of antimicrobial agents used for treating textiles and its fibres were proved to be more effective in antibacterial activity, still due to several above mentioned factors they are not considered as biocompatible. Therefore, in the present research, we treated the cotton fabric materials with an eco-friendly approach using the leaves of Green tea extracts as antibacterial agents. Also for the first time, a synergistic behavior of copper with these leaf extracts was analyzed to increase the antibacterial activity. The synergistic approach in the present study was based on the research work carried out by Tiwari *et al.* (2005). In their research, the extracts of tea were mixed with different antibiotics like chloramphenicol, nalidixic acid and gentamicin to increase the antibacterial activity against *Salmonella typhi*, *Escherchia coli* and *Shigella dysenteriae*.

Since copper also has good antibacterial activity, the main objective of this research was chemically to interact the leaf extracts of green tea (*Camellia sinensis*) with copper in the form of a copper vessel for 14 days to enhance the antibacterial properties. This approach was considered to be a novel method since no where, the literature has been cited so far.

MATERIALS AND METHODS

In the present research leaf extracts of Green Tea and its antibacterial activity were carried out in the Department of Microbiology, Karpagam University, Coimbatore, India from March 2011 to June 2011. Increasing the antibacterial activity studies of Green tea extract with Copper studies and its textile finish studies were carried out in Department of Microbiology, CMS College of Science and Commerce, Coimbatore, India from August 2011 to October 2011.

Textile materials: The fabric from a commercial producer used for various purposes in the healthcare centres was used as the test fabric. 100% cotton (plain weave, 75.50 g m⁻²; ends, 70/inch; picks, 60/inch) was selected and sterilized prior experimentation. The fabric was cut into squares approximately 30×30 cm before being treated. After treatment the swatches were wrinkle removed and sterilized to carry out the antibacterial assay.

Test bacterial strains: Standard reference strains of *E. coli* (MTCC739) and *S. aureus* (MTCC96) were procured from the microbial type culture collection centre (IMTECH, Chandigarh). The strains were selected based on their biological properties of their significance in causing nosocomial infections.

Antibacterial leaf extracts of green tea (*Camellia sinensis*) plant: The leaves were collected from Tea Stanes, Coimbatore, India. Leaf extracts of Green Tea (GTE) were prepared using ethanol (Hi Media, India). In order to increase the antibacterial activity of leaf extracts, the extracts were incubated in the copper vessel by dissolving in distilled water in the ratio of 7:20 (mg mL⁻¹) and incubated for 14 days (GTC). The purpose of this technique was to analyze whether the chemical interaction between the green tea and copper vessel could increase the antibacterial activity? (Both the test extracts were indicated throughout the study as GTE and GTC).

Preparation of medicinal leaf extract: Fifty grams of dried leaves of *C. sinensis* (green tea) was mixed with 250 mL of ethanol and subjected to occasional shaking for 24 h. The extracts were filtered using muslin cloth and concentrated by evaporation at room temperature. The evaporated extracts were screened by phytochemical test.

Phytochemical screening tests: To identify the presence of different phytochemical in the leaf extracts, a standard phytochemical screening test was carried out based on the method proposed by Harbone (1994).

Test for alkaloids

Mayer's reagent: Two milliliter of the leaf extract was treated with 1 mL of Mayer's reagent. Dull white precipitate indicates the presence of alkaloids.

Dragendroff's reagent: Two milliliter of the extract was treated with 1 mL of Dragendroff's reagent. Orange or orange red precipitate indicates the presence of alkaloids.

Test for steroids and sterol

Salkowski's tests: The extract was dissolved in 1 mL of chloroform and equal volume of concentrated sulphuric acid by sides of the test tube. The upper layer turns red, sulphuric acid layer shows yellow with green fluorescence, represents the steroids and sterol compound, in the extract.

Test for flavanoids

Shinoda test: One milliliter of the extract was treated with magnesium turnings and 1-2 drops of concentrated hydrochloric acid. Formation of pink or red color shows the presence of flavanoids.

Test for saponins: 0.5 g of plant extract was taken with few mL of distilled water, mixed well and kept in steam bath for few minutes. White foam indicates the presence of saponins.

Test for glycosides: 0.5 g of the extract was taken. Two milliliter of glacial acetic acid was added, mixed well. To it add 1 drop of ferric chloride and 1 mL of concentrated sulphuric acid. Formation of brown ring or double layer with violet and brown color indicates the presence of glycosides.

Test for tannins and phenolic compounds: One milliliter of extract was treated with 1 mL of 5% neutral ferric chloride, a dark blue or bluish black color product shows the presence of tannins and another 1 mL of extract was treated with 1 mL of gelatin solution. Formation of white precipitate reveals the presence of phenolic compounds.

Test for terpenoids: The extracts were dissolved in 2 mL of chloroform and 10 drops of acetic anhydride and 5 drops of concentrated sulphuric acid were added. Appearance of red to violet color indicates the presence of terpenoids.

Test for carbohydrates

Fehling's test: 2.5 mL of Fehling's A and 2.5 mL of Fehling's B solution was added to the plant extract and boiled on a water bath. A yellow or red precipitate indicates the presence of reducing sugars.

Benedict's test: Five milliliter of Benedict's solution was added to the leaf extract and boiled in water bath. A red yellow or green precipitate indicates presence of reducing sugars.

Test for amino acid and proteins: 1% zinc sulphate was added to the leaf extract solution by drop wise. Formation of white precipitate indicates the presence of amino acids and proteins.

Presence of copper in the leaf extract using Flame test: The presence of copper in the leaf extract was confirmed using a standard Flame test. Briefly, the sample was made into a paste with concentrated hydrochloric acid (Hi Media, India) on a watch glass. A small amount of this paste was taken at the end of a platinum loop and shown very near a non-luminous Bunsen flame. The blue or bluish green color imparted indicates the presence of copper.

Qualitative analysis of antibacterial extracts: Preliminary screening of the extracts (GTE and GTC) to detect the antibacterial activity against the test strains were carried out using a standard Agar diffusion method as described by Opara and Ansa (1993). Briefly, on a pre-seeded MHA plates (*E. coli* and *S. aureus*) 3 wells (6 mm diameter) were made. The wells were filled with 250, 500 and 1000 μ L of the prepared dilutions of leaf extract (GTE and GTC separately) and incubated at 37°C for 24-48 h. After incubation, the inhibition zone was measured in diameter after subtracting the diameter of the well. Dimethyl sulphoxide were used as a negative control to differentiate from the other two extracts.

Method of imparting antibacterial leaf extracts on cotton fabric: The antibacterial extracts were imparted on to cotton fabric by the method described by Sathianarayanan *et al.* (2010). A fine-medium weight 100% cotton woven fabric was used for the application purpose. Ethanol extracts (GTE and GTC) were applied onto the cotton fabric (30×30 cm) by pad-dry-cure method with material-to liquor ratio of 1:20 at 50°C using 8% citric acid (cross-linker) concentration. Padding was carried out in a pneumatic padding mangle at a pressure of 3 psi to get a wet pick up of 100% on weight of fabric. After padding, the fabric was air-dried and then cured for 3 min at 140°C.

Antibacterial properties of GTE and GTC treated cotton fabrics

Agar diffusion method (Mucha *et al.*, 2002): Bacteriostasis agar was dispensed in sterile petriplates. Twenty four hours broth cultures of the test organisms (*E. coli* and *S. aureus*) were used as inoculums. Using sterile cotton swab the test organisms were swabbed over the surface of the agar plates. The pad-dried cotton swatches were cut in the form of disc (15 mm in dia) prior testing qualitatively. The test fabrics (fabrics treated with GTE and GTC) and Control (untreated fabrics) was gently pressed in the center of the mat culture. The plates were incubated at 37°C for 18-24 h. After incubation, the inhibition zone was measured in diameter after subtracting the diameter of the test fabrics.

Wash fastness test: The treated textile materials were analyzed to investigate the durability of the drugs after undergoing periodical and consecutive washings. The treated textile materials were washed based on the standard AATCC Test Method-124 as described by Thilagavathi *et al.* (2007). The treated and control samples were washed 2, 5 and 10 times, respectively. The evaluation of modified Hohenstein test was made on the basis of the percentage reduction of bacteria for the washed cotton swatches. Percentage reduction was calculated using the following formula:

$$R = \frac{(A-B)}{A}$$

where, R is percentage reduction, A is the number of bacteria in the broth inoculated with treated test fabric sample immediately after inoculation i.e., at zero contact time and B is the number of bacteria recovered from the broth inoculated with treated test fabric sample after the desired contact period (18 h).

RESULTS AND DISCUSSION

Phytochemical screening tests of the leaf extracts of green tea: The presence of different phytochemical elements in the leaf extracts was identified using a standard phytochemical screening test proposed by Harbone (1994). From the Table 1, it was understood that different phytochemicals were evident in the extracted Green tea leaves. The major components present in green tea leaf extracts was Alkaloids, Steroids and Sterol, Cardio glycosides, Flavanoids, Saponins, Glycosides, Amino acids and Proteins, Tannins and phenolic compounds, Terpenoids and Carbohydrates. These compounds have been found to possess antibacterial and antiviral action as well as anti-carcinogenic and anti-mutagenic properties (Kuroda and Hara, 1999). Similar phytochemical analysis reported by Kuroda and Hara (1999) showed that the green tea extracts contains catechin and polyphenols.

Table 1: Phytochemical screening of *Camellia sinensis*

Components	Results
Alkaloids	-
Steroids and sterol	-
Cardio glycosides	+
Flavanoids	+
Saponins	-
Glycosides	+
Amino acids and proteins	+
Tannins and phenolic compounds	+
Terpenoids	+
Carbohydrates	+

Table 2: Antibacterial activity of GTE and GTC

Volume of the extracts (μL)	Zone of inhibition			
	GTE*		GTC*	
	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>
250	8.5	8.8	9.2	9.5
500	9.2	9.1	10.3	10.8
1000	8.6	9.5	8.6	9.2

*Mean value (tested in triplicates). GTE- Green Tea Extracts, GTC- Green Tea with Copper

Antibacterial activity of GTE and GTC: The result of the study showed that the leaf extract (GTE and GTC) of *C. sinensis* produced zones of inhibition against both the representative bacterial strains. This indicates the presence of potent antibacterial activity, confirming its use as anti-infective. Although both GTE and GTC extract of *C. sinensis* produced inhibitory actions against *E. coli* and *S. aureus*, GTC extracts showed more inhibitory effects than the GTE extract. From the Table 2, it was evident that both *E. coli* and *S. aureus* were highly susceptible to fabric treated with GTC extract than the fabric treated with GTE extract. Maximum zone of inhibition (*E. coli*- 10.3 mm and *S. aureus* 10.8 mm) was obtained for GTC extract (at 500 μL concentration) when tested against the test organisms (Fig. 1). This tends to show that the active ingredients in the leaves were chemically interacted with copper in the vessel to produce more antibacterial activity than the GTE samples. Similar synergistic results were also obtained by other researchers indicated that the results were supportive to the present investigations.

Chakraborty and Chakraborti (2010) reported the antibacterial activity of methanolic extract of green tea leaves (*C. sinensis*) against four different bacteria namely *E. coli*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *S. aureus* and two fungi of *Aspergillus* species. Antibacterial activity was assigned by measuring the Zone of Inhibition (ZOI) as well as Minimum Inhibitory Concentration (MIC) at four different concentrations of the methanolic extract (10, 25, 50 and 100 mg mL^{-1}) and erythromycin (10 mg mL^{-1}) as the antibiotic control. HPTLC analysis of the extract was identified as catechin. Zone of inhibition was observed in most of the organism. Alcoholic extract of the leaves of *C. sinensis* was found to be most effective against *B. cereus*.

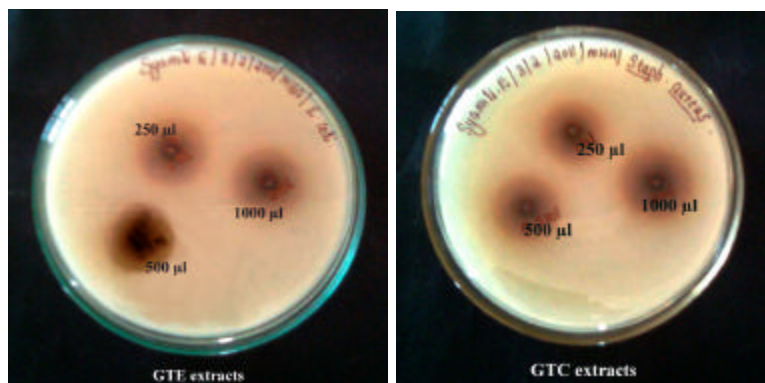


Fig. 1: Antibacterial activity of GTE and GTC on test bacteria. GTE extracts showing antibacterial activity against test bacteria-*E. coli* and GTC extracts showing antibacterial activity against test bacteria-*S. aureus*

Jazani *et al.* (2007a) evaluated the antibacterial activity of water soluble green tea extracts on isolates of *Acinetobacter*. The susceptibilities of isolates to different antibiotics were tested using agar disk diffusion method. Antibacterial activity of water soluble green tea extract was measured by Minimum Bactericidal Concentrations (MBCs). In their study 75% of isolated strains showed resistance to at least 12 antibiotics or more and all the strains were Multi-drug Resistant (MDR) strains. The average MBCs of the extract against all strains of *Acinetobacter* were $387.5 \pm 127.6 \mu\text{g mL}^{-1}$. Their study finally suggests that green tea has significant bactericidal action on multi-drug resistant strains of *Acinetobacter*.

Aboulmagd *et al.* (2011) aimed to evaluate the antibacterial activity of imipenem-green tea extract combination against methicillin-resistant *S. aureus* (MRSA) isolates using disc diffusion technique, checkerboard titration method and time-killing assay. The researchers concluded that, the combined effect of imipenem and green tea extract seems to be significantly synergistic and may be eligible for further evaluation *in vivo* against MRSA infections. In addition, extension of the Post-Antibiotic effect (PAE) duration of imipenem-green tea extract combination compared with imipenem alone may have significant impact on the effect of consumption of green tea extract on the dosing regimen of imipenem.

Jazani *et al.* (2007b) aimed to study the evaluation of the synergistic effect of sub-MIC doses of ciprofloxacin with water soluble green tea extract against urinary tract isolates of *E. coli*. The amounts of MIC and MBC for green tea water extract, ciprofloxacin or a mixture of green tea with sub-MIC doses of ciprofloxacin were determined and three groups were compared. The Synergism between ciprofloxacin and water soluble green tea extracts were seen for 93.7% (15 of 16 tested) of bacterial isolates. This study suggests that combination of water soluble green tea extracts and ciprofloxacin has *in vitro* synergistic effect on urinary tract isolated *E. coli*. All the obtained results were similar to our present research analysis which indicates that the study was supportive to the cited articles.

Antibacterial properties of GTE and GTC treated cotton fabrics: In the preliminary screening method, it was shown that both GTE and GTC extracts contain the antibacterial properties against the two test strains (Table 2). With respect to these results, the extracts were

Table 3: Antibacterial properties of GTE and GTC treated cotton fabrics

Cotton+extracts	Zone of inhibition* (mm)	
	<i>E. coli</i>	<i>S. aureus</i>
GTE	25.5	24.8
GTC	28.1	27.4

*Mean value (tested in triplicates). GTE: Cotton treated with Green Tea Extracts, GTC: Cotton treated with Green Tea Copper extracts

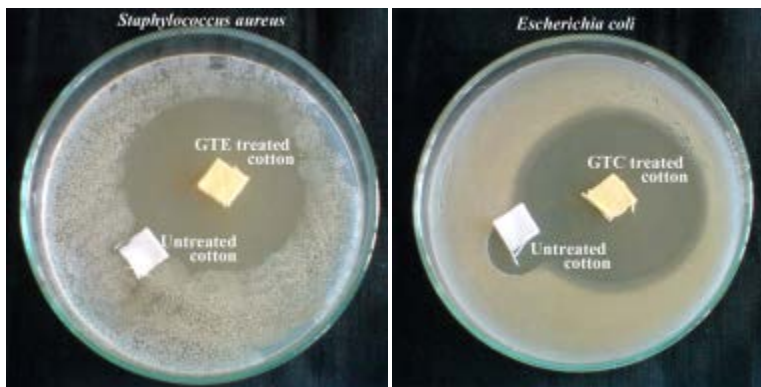


Fig. 2: Antibacterial activity of GTE and GTC treated cotton fabrics. Cotton treated GTE extracts showing antibacterial activity against test bacteria-*S. aureus* and cotton treated GTC extracts showing antibacterial activity against test bacteria-*E. coli*. GTC treated cotton showed more inhibitory zone than GTE

imparted onto the cotton fabric swatches using Pad-dry-cure process to analyse the antibacterial finishing properties of GTE and GTC. From the Table 3, it was evident that both *E. coli* and *S. aureus* were highly susceptible to GTC extract than the GTE extract. Maximum zone of inhibition (*E. coli*-28.1 mm and *S. aureus*-27.4 mm) was obtained for GTC extract (at 500 μ L concentration) when tested against the test organisms (Fig. 2). Similar synergistic results were also obtained by other researchers indicated that the results were supportive to the present investigations. Synergistic combinations of two different antibacterial compounds were used in the study was mainly based on the concept described by Saginur *et al.* (2006). They reported that the accepted clinical practice to treat biomedical-associated infections was the use of combination therapy in which two or more antimicrobials are blended at different combinations. So that broader spectrum of activity is achieved at a lower concentration resulting in more effective therapy and decreased resistance.

Sathianarayanan *et al.* (2010) used tulsi and pomegranate extracts to treat cotton fabrics. In their study, all the treated fabrics showed a zone of inhibition ranging from 9.9 to 12.5 mm for *S. aureus* and 4.9 to 6.6 mm for *Klebsiella pneumoniae*. Compared to these reports in our research the GTE and GTC extracts produced more inhibitory zones against similar type of test bacteria. It indicates that the synergism of copper and tea extracts influences the enhanced properties of antibacterial activity. In another synergistic study, Elayarajah *et al.* (2011) reported that the synergistic activity of ofloxacin and ornidazole could increase the activity of antibacterial textile

Table 4: Wash durability test

Samples	Bacterial reduction (%)			

	No. of washes			
	0	2	5	10
GTE	96.6	63.4	42.5	23.6
GTC	98.5	68.5	47.8	22.3
Untreated	0	0	0	0
GTC (with cross-linker)	99.6	88.5	67.8	52.3

*Mean value (tested in triplicates). GTE: Green Tea Extracts, GTC: Green Tea with Copper

finishes. They made the drugs reactive and imparted onto polyester and nylon fabric materials using reactive dye method. In their study they obtained more than 88% of bacterial reduction when tested quantitatively. In our research, similar bacterial growth reduction was reported qualitatively for GTC treated cotton fabric swatches. All the obtained results were similar to our present research analysis which indicates that the study was supportive to the cited articles.

Wash fastness test: Table 4 shows the antibacterial efficacy in terms of bacterial reduction percentage for the extracts (GTE and GTC) applied onto fabric without cross-linker (96.6 and 98.5%) and the extracts (GTC) with cross linker (99.6%). It is clear that the extracts (GTE and GTC) directly applied and GTC with cross linker possess better activity against *E. coli* and *S. aureus* before wash. Whereas, after 10 washes, the directly applied GTE and GTC extracts (without cross-linker) do not show much activity (<25% bacterial reduction) compared to that of GTC treated fabric with citric acid (>50% bacterial reduction). This may be because of the extracts that might be coated only on the surfaces without any firm bonding and gets removed by washing. In the case of GTC extracts applied with citric acid, the samples possess activity after 10 washes as the active substances are cross-linked to the cotton fabric.

CONCLUSIONS

The process of applying the antibacterial finish through padding and drying is easy and economical. The antibacterial agents (GTE and GTC) examined in this research were selected based on their eco-friendly properties. In this study, one type of Gram-positive bacteria (i.e., *S. aureus*) and one type of Gram-negative bacteria (i.e., *E. coli*) were examined. However, many forms of bacteria are found in the healthcare environment. More studies are needed to determine how well these antibacterial agents could reduce various microorganisms such as *Candida albicans* and other Gram-positive, Gram-Negative organisms. In addition to various microorganisms, different viruses should also be tested due to an increasing concern among health care workers. This research only explored antibacterial agents of Green tea leaf extracts reusable healthcare uniforms made of cotton. These antibacterial agents may have different activities on disposable materials. A similar experimental design is suggested to be conducted to investigate the antibacterial activity on nonwoven materials. In addition, other materials used to make reusable textile materials in the hospital zones such as cotton blended polyester, nylon and viscose could be studied because they are also often used by the patients and healthcare workers.

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