Mariappan Gopalakrishnan*, Dhandapani Saravanan

Bannari Amman Institute of Technology,
Sathyamangalam, Tamil Nadu, India-638401
* E-mail: gokin_m@yahoo.co.in

Antimicrobial Activity of *Coleus ambonicus* Herbal Finish on Cotton Fabric

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Abstract
An extract obtained from Coleus ambonicus was applied on cotton fabric by means of the exhaust, micro encapsulation and nano encapsulation methods, and the antimicrobial activity of the finished fabric assessed quantitatively by the AATCC test method 100 against gram positive (Staphylococcus aureus) and gram negative (Escherichia coli) microbes. The finish applied on the the samples using all three methods exhibit a good bacterial reduction percentage. The finish applied on the samples using all three methods possesses a higher bacterial reduction percentage against gram positive microbes than gram negative, even after washing. The method of washing conforms to ISO method 3. The wash durability of the antimicrobial activity was assessed by the bacterial reduction percentage after washing. The wash durability of the samples using the direct exhaust method was very poor and it lost its antimicrobial activity after 10 wash cycles. The wash durability of the samples using the micro encapsulated method shows antimicrobial activity up to 10 wash cycles, dropping gradually to very low levels at 20 wash cycles. The wash durability of the samples using nano encapsulation shows good antimicrobial activity against both gram positive and gram negative microbes even after 30 washes.

Key words: *Coleus ambonicus, cotton, micro encapsulation, nano encapsulation, antimicrobial activity, bacterial reduction %, FTIR spectrum, wash durability.*

Introduction

Natural textile fibres like cotton are prone to microbe growth. The moisture regain of cotton, the climate conditions in India and the cellulose present in cotton favour microbe growth [1, 2]. Microbial growth on textiles may cause foul smells, skin irritation, cross infection, discolouration and colour stain. It also affects the functional properties of fabric like strength and elastic properties [3].

A wide range of synthetic antimicrobial agents like triclosan, metals and their salts, organometallics, phenols and quaternary ammonium compounds have been applied to cotton [4]. Synthetic antimicrobial agents have excellent antimicrobial efficacy for a wide range of microbes, but issues of eco-friendliness & biodegradability limit the use of synthetic antimicrobial agents [5]. Natural antimicrobial agents like neem, pomegranate fruit, tulsi leaf and etc., possess very good resistance to most microbes [6-10]. Most of the natural antimicrobial agents are available in abundance and are eco-friendly.

The encapsulation process increases the life cycle of the finish by a controlled release mechanism. The nano encapsulation method of finishing provides a high surface area and extends the shelf-life of the finish on cotton [11, 12].

Coleus ambonicus is a medicinal plant found abundantly in India. Six major compounds account for 97% of the composition of the *coleus ambonicus* extract. Of these six, thymol is the major compound and has antimicrobial, antifungal and insecticidal properties [13]. The other flavonoid and phenol functional groups present in the herbal extract may also contribute to antimicrobial activity [14]. In this work, the antimicrobial activity of *coleus ambonicus* herbal finish on cotton fabrics was analysed using the direct, micro encapsulation and nano encapsulation methods.

Materials and methods

Materials

In my study, 100% cotton fabric with a plain weave construction was used. The fabric has 32 threads per centimeter in the warp and weft directions. The yarn count used for this purpose was 15 tex in the warp and weft directions. 115 grams per square meter fabric density was used. The fabric was desized, scoured and bleached with the standard recipe.

Coleus ambonicus used as natural antimicrobial agents was collected locally from the Sathyamangalam village, Erode district, Tamil Nadu, India.

Preparation of herbal *Coleus ambonicus* extract

Collected *Coleus ambonicus* herbal leaves were shade dried to reduce the moisture content of the leaf to below 10%. Unwanted materials like dirt and other parts of plants were removed manually by hand. The dried herbal leafs were crushed into small pieces and made into powder, using a mixer. The ground herbal powder was then sieved using fine mesh fabric to remove unground portions. 20 grams of the fine herbal powder was refluxed in a Soxhlet apparatus (Borosil, India) in 200 ml of methanol. The resultant supernatant was filtered with Whatman No.1 filter paper and the filtrate was maintained at room temperature to remove organic solvents. The fabric to be treated with the herbal extract was scoured with soap at 60 °C to remove any existing surface finish. The soap treated fabric was washed with distilled water and air dried. The herbal extract prepared was applied to the cotton fabric using the exhaust method with a 1:30 material to liquor ratio at 60 °C for 30 minutes, after which the fabric was washed and dried in air.

Micro encapsulation of herbal extract

Gum acacia was taken as wall material for encapsulation of the *Coleus ambonicus* extract. 10 grams of gum acacia was allowed to swell for half an hour by mixing with 100 ml of hot water. 50 ml of hot water was added to this mixture and stirred for 15 minutes at 40 °C to 50 °C

temperature. 10 ml of core material *Coleus ambonicus* was added to this mixture and stirred at 300-500 rpm for 15 minutes. 10 ml of 20% sodium sulphate solution was added slowly drop wise for 5-10 minutes. 5 ml of 17% formaldehyde was added at a slower stirrer speed. The stirrer was then stopped and the mixture was freeze dried. The cotton fabric was immersed in the micro encapsulated herbal extract using the exhaust method with a 1:20 material to liquor ratio at 55 °C for 30 minutes and then dried at 80-85 °C in an oven.

Nano encapsulation of herbal extract

The herbal extract prepared was encapsulated with bovine serum albumin as the wall material by the coacervation process, followed by cross linking with glutaraldehyde. The *Coleus ambonicus* herbal extract was incubated for an hour at room temperature with the 2% bovine serum albumin protein powder required. A digital pH meter was used to adjust the pH to 5.5, by means of a 0.1M HCL. Ethanol was added slowly at 1 ml per minute into the solution to obtain a 2:1 ratio (v/v). The required amount of 25% glutaraldehyde was added and kept for two hours to allow the cross linking of protein. A rotary vaccum evaporator was used at reduced pressure to remove organic solvents. The resultant nano particles were purified by centrifuging at 4 °C and 10,000 rpm. The resulting pellets formed of nano capsules were suspended in a phosphate buffer (pH: 7.4, 0.1M) and lyophilised with 2% w/v mannital. The nano capsules thus obtained were further dried by lyophilisation. The resultant nano encapsulated herbal powder was applied on cotton by the exhaust method. Citric acid was used as a binder with a 1:20 material-to-liquor ratio at 55 °C temperature for 30 minutes. Antimicrobial activity of the finished fabric was assessed by the AATCC 100 test method.

Test methods

Antimicrobial activity of the treated and untreated samples was assessed quantitatively by the agar diffusion test method (AATCC 100). 4.8 $\pm$ 0.1 cm circular cut treated, untreated and control samples were inoculated with the required quantity of AATCC specified gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) test organisms. 100 $\pm$ 1 ml of neutralising solution distilled water was added and incubated at

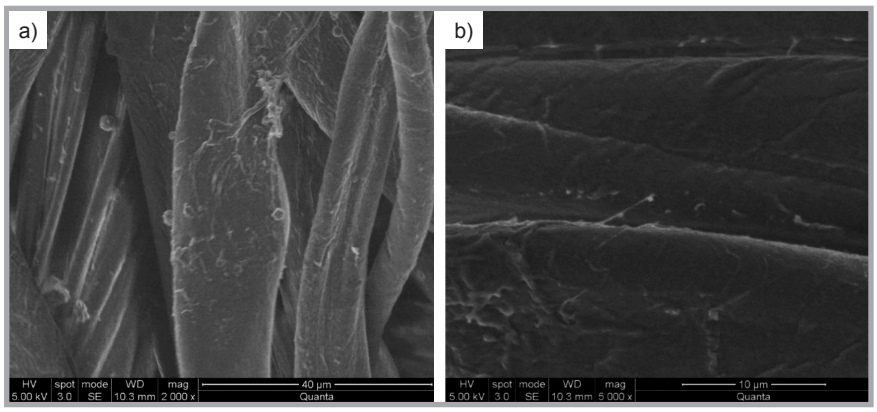

Figure 1. *SEM photographs of fabric treated with nano encapsulated Coleus Ambonicus at magnifications: a) 2000, b) 5000.*

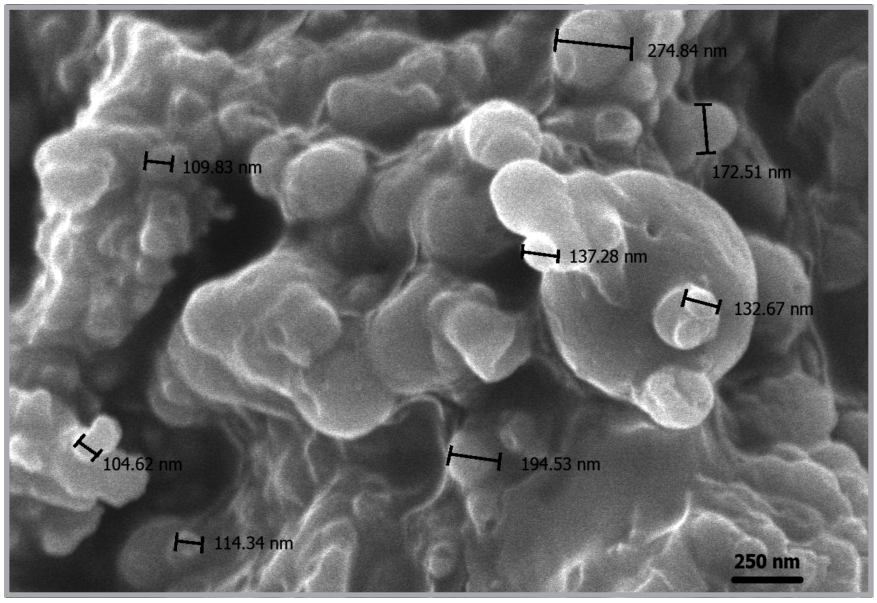

Figure 2. *Particle size of nano encapsulated Coleus Ambonicus herbal powder.*

37 $\pm$ 2 °C for 24 hours. After incubation, the number of bacteria present in the solution and percentage reduction (R) were calculated using the following formula:

$$R = 100(B\text{-}A)/B$$

Where, A is the number of bacteria recovered from inoculated treated test specimen swatches in a jar incubated over the contact period desired, and B the number of bacteria recovered from inoculated treated test specimen swatches in a jar immediately after inoculation (AATCC test method). The durability of the finishing was assessed by analysing antimicrobial activity of the washed samples. The washing of the sample was done in a laundrometer with the ISO 3 wash test method. After the specified number of washes, the test samples were taken and washed thoroughly for further assessment. A scanning electron microscope was used to analyse the binding of nano encapsulated particles on the fabric. An FTIR8400S Spectrophotometer (Shimadzu, Japan) was used to obtain FTIR spectra to discover functional groups present in the sample.

Result and discussion

SEM analysis

Figure 1 shows SEM photographs of nano encapsulated *Coleus ambonicus* at different magnifications. It is clear that the nano encapsulated *Coleus ambonicus* was attached to the fibre surface firmly.

Figure 2 shows the diameter of the nano encapsulated powder. The average diameter of the nano encapsulated powder is around 155.07 nm.

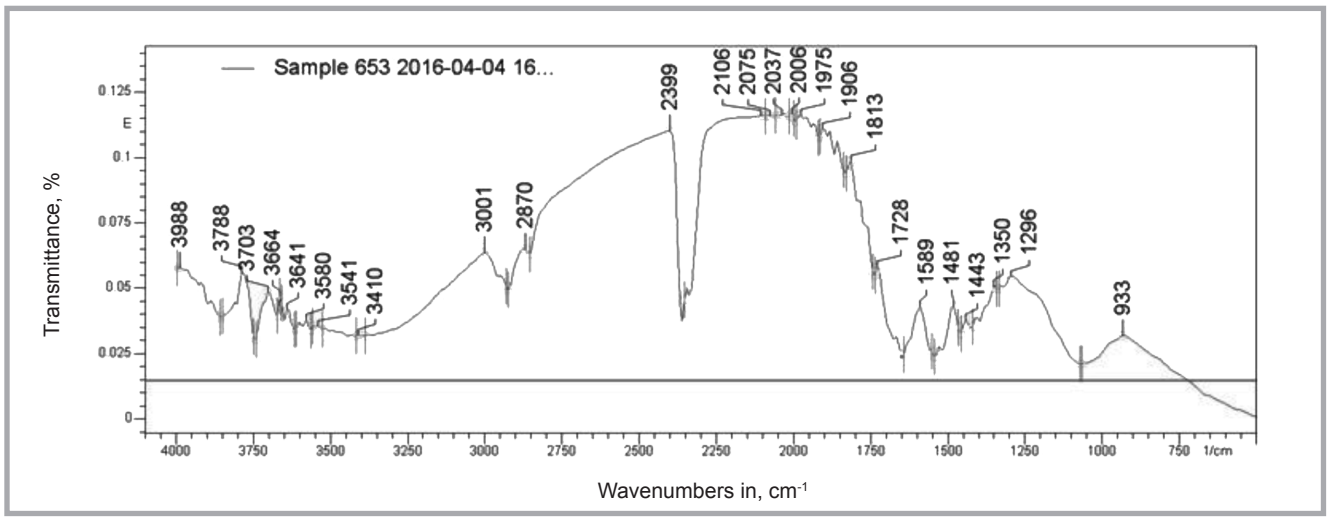

Figure 3. *FTIR Analysis of Coleus Ambonicus.*

FTIR spectrum analysis

The peaks in the FTIR spectrum confirm functional groups present in the herbal powder. ***Figure 3*** shows the FTIR spectrum obtained from the *Coleus ambonicus* herbal extract. The peaks 1580-1590 cm^{-1} substantiate the presence of primary alkyl amide, 3600-3700 cm^{-1} – the existence of O-H phenolic compounds, and 3000-2900 cm^{-1} revealed the presence of C-H in the aromatic phenolic functional groups. Peaks at 2000-2200 cm^{-1} confirm the presence of flavonoids [15]. The presence of flavonoids provides the natural colour of the herbal extract and antimicrobial properties [16]. The existence of these functional groups in *Coleus ambonicus* is responsible for the antimicrobial activity of the sample.

Antimicrobial activity

The bacterial reduction percentage of the sample finished using the direct exhaust method, micro encapsulation method and nano encapsulation method was assessed quantitatively by the AATCC agar diffusion test method 100, the results of which are given in ***Table 1***. The samples finished with *Coleus ambonicus* in all three methods show good antimicrobial activity. This may be due to the rich content of thymol in *Coleus ambonicus* herbs. Sivropoulou A et al. reported the antimicrobial activity of *Coleus ambonicus* due to the presence of thymol [13] and consolacion. Y. Ragasa et al. reported three flavones: *salvigenin, cirsimaritin and chrysoeriol* present in *Coleus ambonicus* herbs and that the presence of these flavones contributes to the antimicrobial activity of the herbs [14].

The samples finished by the direct exhaust method show a better degree of bacterial reduction percentage for both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) than the micro encapsulation and nano encapsulation methods before washing. The samples finished by the direct exhaust, micro encapsulation and nano encapsulation methods possess a higher bacterial reduction percentage against gram positive microbes than gram negative. This indicates that *Coleus ambonicus* herbs are very active against gram positive rather than against gram negative.

Wash durability of finishing

Table 2. show the wash durability of the samples finished by the direct, micro encapsulation and nano encapsulation methods. A marked reduction in the bacterial reduction percentage was observed in the sample finished by the direct exhaust method when increasing the wash cycles. The bacterial reduction percentage was 15 and 12 for *S. aureus* and *E. coli,* respectively, after 10 wash cycles, confirming that the direct method of application has poor wash durability of finishing.

The micro and nano encapsulated samples show better resistivity in both gram positive and gram negative microbes than for the direct method after washing. It is evident that the nano encapsulated and micro encapsulated samples have restricted release of anti microbial agents.

The samples finished by the micro encapsulation method exhibit good resistance to both gram positive and gram negative

Table 1. *Bacterial reduction percentage of the sample at zero wash cycles.*

	Zero wash	
	S. aureus	***E. coli***
Direct method	100	100
Micro encapsulation	87	82
Nano encapsulation	85	81

Table 2. *Bacterial reduction percentage of samples at different wash cycle.*

	Zero wash		5 wash		10 wash		20 wash		30 wash	
	S. aureus	***E. coli***	***S. aureus***	***E. coli***	***S. aureus***	***E. coli***	***S. aureus***	***E. coli***	***S. aureus***	***E. coli***
Direct method	100	100	56	51	15	12	0	0	0	0
Micro encapsulation	87	82	78	73	65	58	18	11	0	0
Nano encapsulation	85	81	80	76	75	71	58	54	38	31

microbes up to 10 wash cycles. After that, the antimicrobial activity deteriorated, was very low at 20 wash cycles and with no activity at 30 wash cycles. It is also evident that the micro-encapsulated samples exhibit good resistivity against both gram positive and gram negative microbes up to 10 wash cycles, which dropped at 20 wash cycles [17].

The nano encapsulated method displays good antimicrobial activity in terms of bacterial reduction percentage up to 20 wash cycles and shows moderate antimicrobial activity after 30 wash cycles, with both gram positive and gram negative microbes. It reveals that the nano encapsulated sample exhibits good wash durability in comparison to the micro encapsulation and direct exhaust methods.

Conslusions

Coleus Ambonicus finished fabric samples show good microbial reduction percentage in both *staphylococcus aureus* and *Escherichia coli* microbes in all three methods at zero washes. The nano encapsulated fabric sample exhibits good wash durability in terms of the bacterial reduction percentage even after 30 wash cycles, whereas the direct method and micro encapsulated method of finishing showed less durability after 10 and 20 wash cycles, respectively.

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