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Development of antimicrobial cotton fabrics using herb loaded nanoparticles

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ABSTRACT

In the present work ethanol, methanol, petroleum ether and water extracts of the leaves of *Ocimum sanctum* were screened for their anti-microbial activity by using the agar diffusion method. The minimum inhibitory concentration of the extracts was also measured. The methanol extracts *O. sanctum* proved to have the maximum antimicrobial effect were loaded inside the sodium alginate chitosan nanoparticles by cation induced controlled gelification method and finished on cotton fabric by pad dry cure method. The average particle size of the nanoparticles was calculated using dynamic light scattering technique. The antimicrobial activity of the fabrics was assessed by using the standard AATCC technique (AATCC 100). The quantitative tests proved that cotton fabrics finished with the methanol extract of *O. sanctum* loaded nanoparticles possessed remarkable antibacterial activities with excellent wash durability. The study revealed that the herb encapsulated nanoparticle could act as a biocontrol agent against bacteria in fabrics.

1. Introduction

Textile consumers are now becoming much more aware of the deleterious effect that microorganisms may have upon textiles and human hygiene. In particular, the medical textile sector has welcomed the greater applicability of antimicrobial finishes to stem the possibility of infections arising from the presence of microorganisms. Several researchers have used antimicrobial finishes with barriers against microorganisms (Holme, 2008). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Davis, 1994; Service, 1995). One of the hottest trends in textile industry is 'nanotechnology' which can provide high durability for fabrics as they have a large surface area to volume ratio and high surface energy, thus presenting better affinity for fabrics and leading to an increase in durability of the function.

Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities (Falerio et al., 2003; Hamburger & Hostettmann, 1991; Juliani & Simon, 2002; Kalemba & Kunicka, 2003). The use of herbs and medicinal plants as the first medicine is a universal phenomenon (Lee, Kin, Moon, & Shun, 1998). Antimicrobials of plant origin have enormous therapeutic potential as they are effective in the treatment of infectious diseases while mitigating the side effects of the synthetic antimicrobials (Joshi, Lekhak, & Sharma, 2009; Manandhar, 1987).

Ocimum sanctum (Family Labiatae) is a many branched, erect, stout and aromatic herb about 75 cm high. This small herb is a native of Iran, Afghanistan and India (Mann, Cox, & Markaham, 2000; Volak & Jiri, 1997). The leaves, seeds and root of this plant have been used in indigenous Ayurvedic medicine. It is an aromatic herb used traditionally in the treatment of headache, coughs, diarrhea, constipation, warts, worms and kidney malfunctions (Sikmon, Morales, Phippen, Vieira, & Hao, 1990).

In this study, the herb loaded nanoparticles were synthesized by cation induced controlled gelification of alginate for the development of antimicrobial textiles. The study also deals with the identification of the antimicrobial activity of different extracts of *O. sanctum* against four bacteria (Gram-positive – *Bacillus subtilis*, *Staphylococcus aureus*; Gram-negative – *Escherichia coli* and *Pseudomonas aeruginosa*) and two fungi (*Aspergillus niger* and *Penicillium* sp.,). Internationally recognized tests were used to characterize the antimicrobial efficiency of the plant extracts yielded by methanol, ethanol, petroleum ether and water. Subsequently, the most effective extracts were used for loaded inside the nanoparticles and coated on the cotton fabric by pad-dry-cure method. The antimicrobial efficacy of the fabrics along with wash durability was also evaluated.

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2. Methodology

2.1. Plant materials

The leaves of *O. sanctum* were collected in and around Coimbatore, Tamil Nadu, India, then air-dried and powdered before the extraction.

2.2. Extractions

For aqueous extraction, 10 g of air-dried powder was placed in 100 ml of distilled water and boiled for 6 h and then filtered (De & Ifeoma, 2002). The filtrate was condensed in boiling water bath and used for further analysis.

For organic solvent extraction, 10 g of air-dried powder was placed in 100 ml of organic solvent, namely petroleum ether (distillation range: 60–80 °C), methanol and ethanol in separate conical flasks and kept in rotary shaker at 150 rpm for 48 h (De & Ifeoma, 2002). Then it was filtered and the solvent was evaporated with rotary vacuum evaporator to make the final volume one-fourth of the original volume. It was stored at 4 °C for further studies.

2.3. Microorganisms and inoculum preparation

Clinical isolates of *E. coli*, *B. subtilis*, *P. aeruginosa*, *S. aureus*, *A. niger* and *Penicillium* sp., obtained from the Department of Microbiology, KMCH, Coimbatore were used in this study. All cultures were bio chemically tested for purity.

Clinical isolates were frequently sub cultured and maintained in Nutrient Agar plates. For antimicrobial assay, microbial cultures freshly grown at 37 °C were appropriately diluted in sterile normal saline solution to obtain the cell suspension at 10^5 CFU (colony forming units)/ml.

2.4. Antimicrobial susceptibility testing

The agar well diffusion method was used for antimicrobial susceptibility testing (Odebiyi & Sofowora, 1990). 0.1 ml of diluted inoculum (10^5 CFU/ml) of test organism was spread on MHA (Muller Hinton Agar) plates. Wells of 8 mm diameter were punched into the agar medium and filled with 100 μl of plant (*O. sanctum*) extract and solvent blanks. The plates were incubated for 18 h at 37 °C for bacteria and 72 h at 27 °C for fungal specimens. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. The differences in the zones of inhibition between different extracts of *O. sanctum* were interpreted using ANOVA (analysis of variance).

2.5. Determination of the minimum inhibitory concentration (MIC)

The leaf extracts of *O. sanctum* which showed significant antimicrobial activity in the antimicrobial susceptibility testing were selected for determination of MIC (De & Ifeoma, 2002). A stock solution of 100 mg/ml of the condensed leaf extract was prepared. This was serially diluted to obtain various ranges of concentrations between 5 μg/ml and 100 μg/ml. 0.5 ml of each of the dilutions of different concentrations was transferred into sterile test tube containing 2.0 ml of nutrient broth. To the test tubes, 0.5 ml of test organism previously adjusted to a concentration of 10^5 cells/ml was then introduced. A set of test tubes containing broth alone were used as control. All the test tubes and control were then incubated at 37 °C for 18 h. After the period of incubation, O.D. (optical density) readings of the tubes were measured at 420 nm spectrophotometrically.

2.6. Synthesis of herb loaded nanoparticles

Methanolic extracts of *O. sanctum* loaded alginate nanoparticles were prepared by the principle involving cation induced controlled gelification of alginate. About 0.5 ml of calcium chloride (18 mM) was added to 9.5 ml of sodium alginate solution (0.06%, w/v) containing herbal extract (Ahmad, Pandey, Sharma, & Khuller, 2006). About 20 ml of Chitosan solution (0.05%, w/v) was added followed by stirring the mixture in magnetic stirrer for 30 min and the mixture was kept at room temperature overnight. All the samples were then centrifuged at 1100 rpm for 10 min to remove any large aggregates. Centrifugation under these conditions allowed the aggregates to form pellet, leaving nanoparticles suspended in the supernatant. The particle suspension was then centrifuged at 5000 rpm for 10 min at 25 °C to separate free polymers from nanoparticles. The pellet containing the leaf extract loaded alginate nanoparticles was washed five times and then suspended in distilled water and stored at 4 °C for further analysis. Alginate nanoparticle controls were also prepared by the same procedure without herbal extract loading.

2.7. Characterization of the leaf extract loaded nanoparticle

2.7.1. Dynamic light scattering (DLS)

Dynamic light scattering was done using Malvern Instruments version 2.2. Zeta potential and average particle size of the herb loaded nanoparticles were determined.

2.7.2. Antimicrobial finishing of cotton fabric

A fine-medium weight 100% cotton fabric (bleached, plain weave, 20^sK warp and 20^sK weft; ends: 54/in.; picks: 60/in.; 122 cm width) was used for the finishing. The different samples (nanoparticles loaded with *O. sanctum*, methanolic extract of *O. sanctum*, control nanoparticle) was applied on cotton fabric using pad-dry-cure method. The cotton fabric cut to the size of 30 cm × 30 cm was immersed in the extract (20%) and citric acid binder (8%) for 5 min and then it was passed through a padding mangle to remove excess solution. A 100% wet pick-up was maintained for all of the treatments. After padding, the fabric was air-dried and then cured at 140 °C for 3 min. Subsequently, it was immersed in a solution of 2 g/l of sodium lauryl sulfate for 5 min to remove the unbound extract, and then rinsed to remove the soap solution and finally air-dried (Yadav et al., 2006).

2.8. Antimicrobial assessment of finished cotton fabrics

2.8.1. Quantitative bacterial reduction test (AATCC test method 100-2004)

About 1.0 ml of the test inocula (*S. aureus* and *E. coli)* were loaded on the swatches (treated and untreated) of 4.8 ± 0.1 cm diameter. They were then transferred to the respectively labeled sterile AATCC Bacteriostasis broth. After an incubation of 24 h, serial dilutions were made up to 10^{-7} for all the samples. 0.1 ml sample from each dilution were spread plated on to the sterile AATCC Bacteriostasis agar plates and incubated at 37 °C for 24 h. The percentage reduction of bacteria by the treatment can be calculated by the formula (1). The percentages in reduction of the two extracts were interpreted using ANOVA.

$$R = 100\frac{B - A}{B} \quad (1)$$

where R is the reduction in %, A and B are the number of bacteria recovered from the inoculated treated and untreated swatches, respectively.

Table 1
Microbicidal activity of *Ocimum sanctum* and *Ocimum basilicum* extracts produced by water, ethanol, methanol and petroleum ether.

Test organisms	Zone of inhibition (mm) *Ocimum sanctum* extracts			
	Aqueous	Ethanolic	Methanolic	Petroleum ether
B. subtilis	2	8	10	–
S. aureus	3	10	15	5
E. coli	–	9	12	4
P. aeruginosa	1	12	18	4
A. niger	–	–	–	–
Penicillium sp.	–	–	–	–

–, no zone of inhibition.

2.9. Wash durability analysis (Sarkar, Purushottam, & Chauhan, 2003)

Washing was carried out as per test no: 1 of IS: 687-1979 by using a neutral soap (5 gpl) at 40 ±2 °C for 30 min, keeping the material:liquor ratio at 1:50, followed by rinsing, washing and drying. After drying, the test samples were assessed for antimicrobial activity using AATCC 100 procedure up to 30 laundering cycles.

2.10. Surface topographical analysis of the fabric

The topography of the fabrics treated with the leaf extract loaded nanoparticles was studied using FESEM (JEOL/JSM 6701F).

3. Results

3.1. Antimicrobial activity of the extracts

In the present investigation, the crude extracts were derived from *O. sanctum* and their efficacy to inhibit the growth of certain microorganisms (*E. coli*, *B. subtilis*, *P. aeruginosa*, *S. aureus*, *A. niger* and *Penicillium* sp.,) was studied. The results presented in Table 1 showed that the antibacterial activity was found to be the maximum for the methanolic extract, which was followed by ethanol, petroleum ether and aqueous extracts. However, there existed a significant difference between the zones of inhibition produced by different extracts of the same herb (ANOVA, $p > 0.05$).

The methanolic extract of *O. sanctum* exhibited the maximum inhibitory zone against the test organisms followed by ethanol, petroleum ether and aqueous extracts. The maximum inhibition was found against *P. aeruginosa* (18 mm), followed by *S. aureus* (15 mm), *E. coli* (12 mm) and the least antibacterial activity was found to be against *B. subtilis* (10 mm).

3.2. Minimum inhibitory concentration of the methanol extracts

Since the previous antimicrobial susceptibility tests clearly proved that the methanol extracts can be characterized by the highest antimicrobial activity against the test microorganisms, this was the reason why the minimum inhibitory concentration of the crude methanol extracts was determined. The results in Table 2 reveal that the crude methanol extract of the leaves of *O. sanctum* inhibited and fully prevented the growth of *P. aeruginosa* at a concentration of 30 μg/ml, *S. aureus* at a concentration of 40 μg/ml, *E. coli* at 60 μg/ml and *B. subtilis* at a concentration of 80 μg/ml.

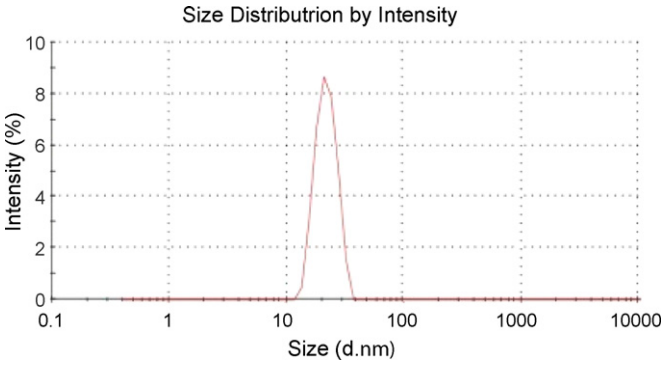

Fig. 1. Average particle size of the *Ocimum sanctum* leaf extract loaded nanoparticles by dynamic light scattering technique.

3.3. Characterization of the herb loaded nanoparticles – dynamic light scattering technique

The average particle size and zeta potential of the herb extract loaded nanoparticles were studied using dynamic light scattering technique. The average particle size and zeta potential of the *O. sanctum* loaded nanoparticles were found to be 33.2 nm and −41.6 mV as shown in Figs. 1 and 2 respectively. The particles were found to be in the nano regime with the highest negative potential which proved the even distribution and stability of the nanoparticles in the suspension.

3.4. Quantitative assessment of the antibacterial activity of the fabrics

The quantitative assessment of antibacterial activity of cotton fabrics finished by different treatments (*O. sanctum* leaf extract loaded nanoparticles, *O. sanctum* leaf extract, control nanoparticle) was assessed by percentage reduction test, according to AATCC 100 standard and shown in Table 3. The *O. sanctum* leaf extract loaded nanoparticles coated fabrics showed 100% inhibition against all the test organisms except against *E. coli*. While the leaf extract of *O. sanctum* reduced 92% of the growth of *P. aeruginosa*. On comparing the antibacterial activities of the extract coated fabrics, the inhibition was greater against *S. aureus* (98%) than against *E. coli* (81%). The rate of reduction of bacteria exhibited by the nanoparticles and the extract were found to be significantly different (ANOVA $p > 0.05$).

Table 2
Minimum inhibitory concentration (MIC) of the methanol extract of *Ocimum sanctum* against some selected microorganisms.

Test organisms	Concentration of extract (μg/ml)								
	100	80	60	50	40	30	20	10	5
Bacillus subtilis	0.01	0.01[a]	0.03	0.04	0.09	0.15	0.26	0.30	0.39
Staphylococcus aureus	0.01	0.01	0.01	0.01	0.01[a]	0.02	0.02	0.05	0.07
Escherichia coli	0.01	0.01	0.01[a]	0.03	0.06	0.10	0.15	0.22	0.30
Pseudomonas aeruginosa	0.01	0.01	0.01	0.01	0.01	0.01[a]	0.02	0.04	0.08
Control	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

[a] MIC.

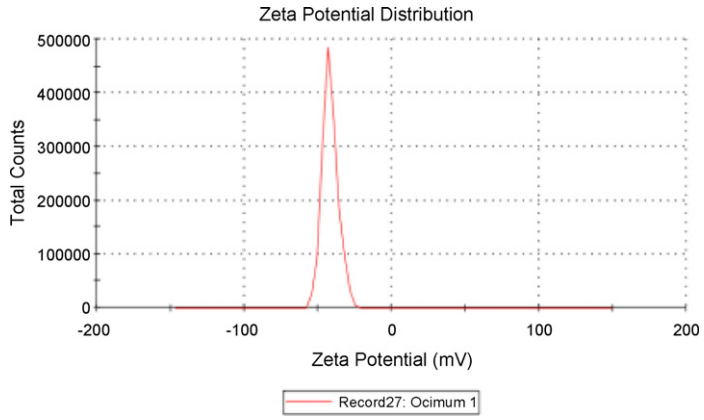

Fig. 2. Zeta potential of the *Ocimum sanctum* leaf extract loaded nanoparticles by dynamic light scattering technique.

Table 3
Quantitative assessment of antibacterial activity of the cotton fabrics finished with methanol extract of *Ocimum sanctum* and *Ocimum basilicum* by percentage reduction test (AATCC 100).

Plant extract	Test organisms	Bacterial reduction (%)
Ocimum sanctum	*Bacillus cereus*	72
	Escherichia coli	81
	Pseudomonas aeruginosa	92
	Staphylococcus aureus	98
Ocimum sanctum leaf extract loaded nanoparticle	*Bacillus cereus*	100
	Escherichia coli	98
	Pseudomonas aeruginosa	100
	Staphylococcus aureus	100
Control nanoparticle	*Bacillus cereus*	40
	Escherichia coli	24
	Pseudomonas aeruginosa	20
	Staphylococcus aureus	37

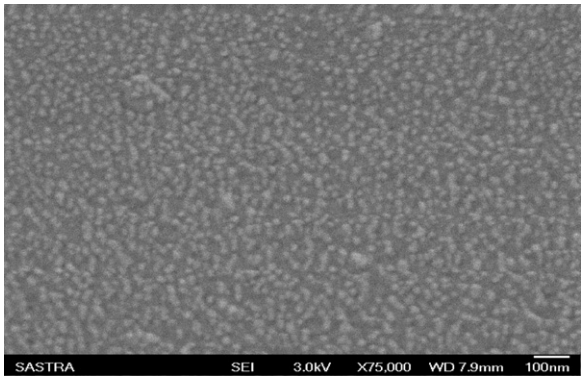

Fig. 3. FESEM micrograph of the fabric treated with the *Ocimum sanctum* encapsulated nanoparticle.

3.5. Wash durability

The bacterial reduction percentage until 30 laundering cycles were determined using laundry test. The results were calculated and tabulated in Table 4. It could be conferred from the table that the *O. sanctum* encapsulated nanoparticle treated fabrics sustained antibacterial activity against all the test bacteria until 20 washes. After that there occurred a slight reduction in the activity of the fabric coated with the herb encapsulated nanoparticles due to the uniform coating, better affinity and sustained release of the nanoparticles. While the fabrics treated with *O. sanctum* extract showed antibacterial activity until 5 washes after which it started reducing owing to the fact that the extracts does not possess sustained release of the antimicrobial compound like that of nanoparticles. However, the meagre reduction of bacteria in the initial laundering cycles of the fabric treated with the bulk extract could be attributed to the presence of the binding agent (citric acid) in the fabrics.

3.6. Surface characterization of fabrics

The nanoparticles loaded with *O. sanctum* leaf extract were observed on the fabrics in a particle magnification of 75,000× operating at 3 kV and represented in Fig. 3. The FESEM micrograph of the fabric treated with the *O. sanctum* leaf extract encapsulated nanoparticles revealed that the synthesized particles were in nanosize. The particle was roughly spherical in shape. The particle size was found to be approximately in the range of 35 nm on the

Table 4
Wash durability analysis of the treated fabrics.

S. no.	Fabric treatments	No. of laundering cycles	Antibacterial activity (bacterial reduction %)			
			E. coli	*P. aeruginosa*	*B. cereus*	*S. aureus*
1	*Ocimum sanctum* leaf extract	5	75	82	69	84
		10	53	70	58	75
		15	22	52	29	49
		20	–	24	–	15
		25	–	–	–	–
		30	–	–	–	–
2	*Ocimum sanctum* leaf extract encapsulated nanoparticle	5	97	100	100	100
		10	97	100	100	100
		15	95	100	100	100
		20	90	100	100	100
		25	87	98	97	99
		30	87	98	95	98

fabric surface. The even distribution of the nanoparticles on the fabric surface was also visualized.

4. Discussion

In this research, different solvents i.e. ethanol, methanol, petroleum ether and water were used to extract the active agents from the leaves of *O. sanctum*. The antimicrobial properties of the extracts were widely characterized by using standard, well-known and internationally recognized methods. The extract with maximum antibacterial activity was loaded inside the nanoparticles and characterized using dynamic light scattering method. They were then coated on cotton fabric by the pad-dry-cure technique and their antimicrobial properties were characterized by percentage reduction and laundering durability. Finally the topographical characterization of the nanoparticles coated fabric was also performed.

Of the four extracts, methanol extracts proved to have the most significant antimicrobial effects. The extracts yielded by ethanol, petroleum ether and water also possessed antimicrobial properties but to a less extent which might be attributed to the polarity of the solvent. The minimum inhibitory concentrations of the crude methanol extracts were in the range of 30–80 μg/ml for *O. sanctum* indicating the efficient extraction process and the high active agent concentration of the extracts. Cotton fabrics finished by the nanoparticles of either *O. sanctum* exhibited significant antimicrobial properties against all test organisms.

The leaf extracts of *O. sanctum* were reported to possess a potent antibacterial activity (Jamine, Xisto, Orionaida de, Jose, & Pedro, 2005; Loughrin & Kasperbauer, 2001). The present finding supports the results of Joshi et al. (2009) and Mann et al. (2000) that crude extracts of *O. sanctum* is effective against the *S. aureus* and other selected Gram positive microorganisms. Mahmood, Yaqoob, and Bajwa (2008) opined that *O. sanctum* extract exhibited maximum inhibitory effect against *S. aureus*, and marked antibacterial efficacy against hospital pathogens, *P. mirabilis*, *P. aeruginosa*, *Klebsiella* sp. and *E. coli* at a concentration of 10 μl. In a similar study, Cock (2008) reported the antimicrobial activity of *O. sanctum* leaves against bacteria and yeast at 22 μl concentration. These differences may be attributed to the differences in the presence of antibacterial component in varieties.

Recently, the mechanism of the antimicrobial effect on textiles fibers was studied. Research has indicated that the antimicrobial agents attach to the fiber surface by bond formation and then disrupt the cell membrane of the microorganisms through physical and ionic phenomenon (Sarkar et al., 2003). In addition, the agent penetrates and disrupts the cell wall of the microorganisms by an electrochemical mode of action, resulting in the leakage of metabolites. That can probably prevent the microorganisms from functioning or reproducing (Jothi, 2009).

The nanoparticles loaded with the herbal extracts exhibited superior antimicrobial activity than the herbal extracts and also showed better laundering durability in the treated fabrics. This could be attributed to the smaller size and controlled release of the nanoparticles. Du, Niu, Xu, Xu, and Fan (2009) and Rajendran et al. (2012) also have reported that the higher antimicrobial activity and laundering durability of the nanoparticles was due to the smaller particle size, uniform coating and controlled release of the nanoparticles.

5. Conclusion

The use of nanotechnology in encapsulating the herbal extracts have paved way for the better antimicrobial activity and the sustained release of the nanoparticles which was evident from the antimicrobial assessment of the treated fabrics and laundering durability analysis respectively. Although, this research was successful in preparing cotton fabrics with remarkable antimicrobial activity, more work is needed for a better understanding of the mode of action of the herb loaded nanoparticles on the cotton fabrics. Further, a deep research on the phytochemical analysis will also be done. Also, a combination of herbs could be used to load inside the nanoparticle and their synergistic activity will also be studied.

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