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Original Article

Eugenol Treated Wetlaid Nonwoven Web for Antibacterial Applications

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Surface modification of cellulosic wood pulp was carried out using a cationic agent namely CHPTAC (3-Chloro-2-hydroxypropyl trimethyl ammonium chloride). Wet laying of fibers was carried out to develop a web comprising of normal and cationised wood pulp over which eugenol was treated by the dissolution of the essential oil in ethanol. The work focusses on sustained release of eugenol by surface modification of cellulosic wood pulp rather than bulk release. The sustained release of eugenol can be achieved by the formation of a bond between the quaternary ammonium group of cellulose and the hydroxyl group of eugenol oil which was confirmed using FTIR. The developed web offered lower thermal stability due to the volatile nature of the essential oil. The fibrous web comprising higher concentration of eugenol (30 wt%) offered higher antibacterial activity against *E. Coli* with a zone of Inhibition of 20 mm.

Introduction

Eugenol, a plant based aromatic essential oil majorly found in clove (*syzygium aromaticum*) offers a wide range of applications in biomedical areas due to its antibacterial, antioxidant and antiinflammatory properties [1]. Eugenol offers antibacterial activity by disturbing the lipid within the bacterial membrane increasing the permeability thereby causing the leakage of intracellular contents of the bacteria. Further, eugenol interacts with lipopolysaccharide on the bacterial cell wall due to its the hydrophobic nature thereby infiltrating the same. The antibacterial activity of eugenol can be also associated with the hydroxyl group found on the essential oil which inhibits the enzyme activity of the bacteria [2,3]. Incorporation of eugenol into the textile substrate is an interest for biomedical applications as textile forms act as secondary skin for providing a moist environment and protection from environmental pathogens in order to enhance wound healing function. Eugenol based cotton substrate was prepared by coating the eugenol microsphere by padding the textile for controlled release of essential oil for sustained antibacterial activity [4]. Eugenol was spun using electrospinning by incorporation into polyacrylonitrile (PAN) nanofibers for the treatment of candidiasis and it was reported that increasing the concentration of eugenol increased antifungal activity against *Candida albicians* [5]. Eugenol was applied to viscose nonwoven for medical application by modifying the chitosan by chemical and enzymatic ways. It has been reported that the chemically modified samples offered higher antibacterial activity as compared to enzymatically modified samples. The hydroxyl group of eugenol forms an amide bond with the amine group of chitosan [6]. Hence, eugenol can be coated on the surface modified cellulosic material for controlled release of essential oil for antibacterial application using the wet laying technique. The method utilises an aqeous solvent for the dispersion of short fibers which finds application in wound dressing application as it uses a nontoxic solvent [7]. 3-Chloro-2-hydroxypropyl trimethyl ammonium chloride (CHPTAC) cationic agent used for surface modification of cellulose widely as it is economical and offers less disadvantages compared to other cationising agents [8]. The present work is to prepare a fibrous nonwoven web comprising of normal and cationised fibers using the wet laying technique. To the prepared nonwoven, eugenol was treated making the fibrous web suitable for antibacterial application.

Materials and Methods

Wood pulp was purchased from The South India Textile Research Association (SITRA), Coimbatore. CHPTAC (3-Chloro-2-hydroxypropyl trimethyl ammonium chloride) was purchased

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from Sigma Aldrich Corporation, USA. Sodium hydroxide (NaOH) and Eugenol were purchased from Sisco Research Laboratories, Mumbai of Analytical grade.

The preparation of wetlaid nonwoven was carried out as cited in the Literature [9]. In short, the wood pulp was treated in NaOH at a concentration of 18 wt % to increase the smoothness of the fibers. The treated fibers were surface modified using CHPTAC to increase the cationic sites on the cellulosic fibers according to the Literature [10]. A known weight of the fibrous samples were dispersed in water prior to wet laying into nonwoven. The fibrous samples were labelled as normal (NWPWNW), a blend of normal/cationized fibers (50/50) (BWPWNW) and cationized (CWPWNW) wet laid nonwoven. To the prepared web, eugenol was dissolved in ethanol of different concentrations say 10, 20 and 30 wt% and poured onto the nonwoven samples which were placed on the petri dish. Eugenol incorporated samples were prepared by solvent evaporation method by placing at room temperature and the samples were labelled as 10ENWPWNW, 20ENWPWNW and 30ENWPWNW for normal treated fibers whereas 10ECWPWNW, 20ECWPWNW and 30ECWPWNW for cationised fibers respectively.

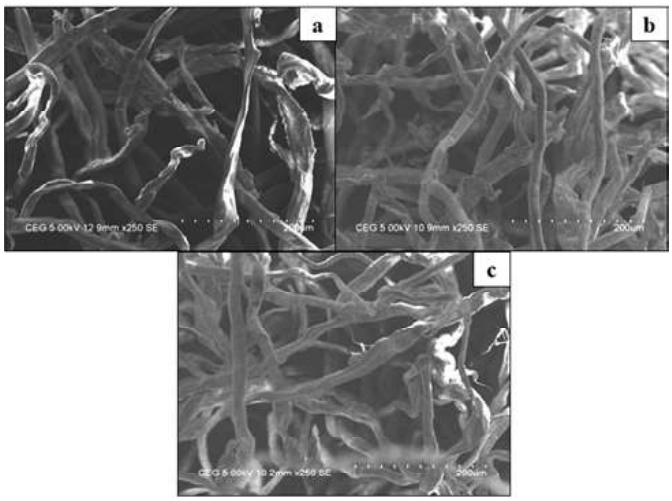

Figure 1 SEM images a) NWPWNW l b) NWPWNW c) 30ECWPWNW

Characterization

The surface morphology of normal, cationized and eugenol treated cationized samples was studied using a Scanning Electron Microscope (SEM, Hitachi Japan). The samples were cut to the dimension and placed on the black carbon film and sputter coated with gold prior to scanning. The thermogravimetry analysis of the developed nonwoven samples was carried out from room temperature to 700 °C under a nitrogen atmosphere with a heating rate of 10 °C and flow rate of 30 ml/min in order to study the thermal stability of the samples after incorporation of eugenol. To study the interaction between the cationising agent (CHPTAC), wood pulp and eugenol essential oil, Fourier Transform Infrared Spectroscopy (FTIR) (Bruker, Japan) was carried out at room temperature a wavelength ranging from 500 cm^{-1} to 4000 cm^{-1}. The tensile strength and elongation of eugenol incorporated cationized samples were carried out according to ASTM D 882-9 using a Universal tensile strength tester (Instron 3369). Phosphate buffer saline percentage (PBS %) of the developed samples was studied by immersing the known weight of the nonwoven in phosphate buffer saline solution for 30 mins and the weight of the sample is measured using weighing balance after removal of surface bound solution by filter paper. The PBS uptake of the developed samples is calculated using equation (1)

$$PBS\ Absorbancy = (W1 - W0)/W1 \times 100 \quad (1)$$

Where W_0 and W_1 are the weight of the samples before and after absorption of PBS by the samples, respectively. The antibacterial activity of the developed samples was analyzed using a agar diffusion test method. Zone of Inhibition (ZOI) was measured against Gram negative bacteria namely *E. coli* for the developed samples. Experiments were repeated thrice and the data were expressed as average ± standard deviation.

Results and Discussion

The SEM micrographs of the normal, cationized and eugenol treated cationized samples are shown in figure 1. The surface morphology of the normal wet laid samples was smooth and regular whereas in the cationized samples, the surface was found to be rough and flattened which was due to the incorporation of NH_3^+ groups (quaternary ammonium) on to the surface on the surface of the cellulosic wood pulp upon treatment with CHPTAC. From the SEM micrograph, it was clear that eugenol dissolved in ethanol was absorbed by the cationized wood pulp wet laid samples representing no changes within the fiber structure.

The FTIR spectrum of the developed samples is shown in figure 2. The characteristic peak around 3331 cm^{-1} represents the stretching of –OH groups and 2871 cm^{-1} corresponds to the stretching of –CH groups in the cellulose structure. The spectral peak around 1700 cm^{-1} and 1200 cm^{-1} corresponds to the stretching of C=O and C-N groups in normal and cationized wood pulp samples. In the case of cationized wet laid samples, a new peak was seen around 1490 cm^{-1} which indicates the modification of cellulose in the presence of CHPTAC, as it represents the stretching of quaternary ammonium groups on the cellulosic structure. The new peak in the FTIR spectrum was seen in Eugenol treated wet laid samples. The peak around 1512 cm^{-1} represents the signature peak of eugenol

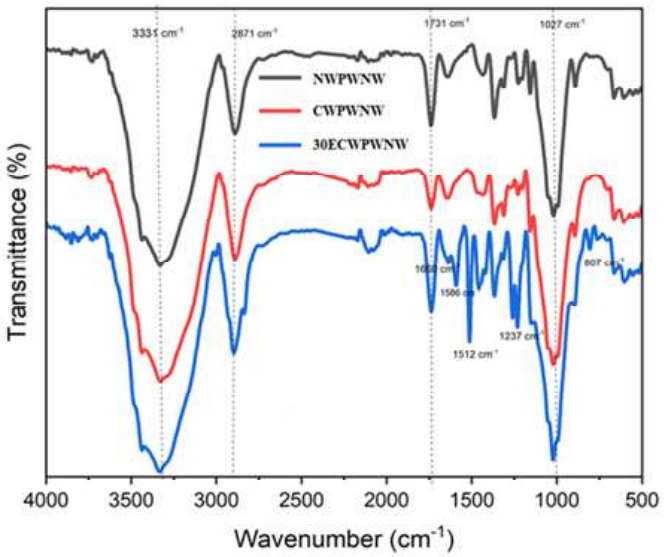

Figure 2: FTIR of developed samples

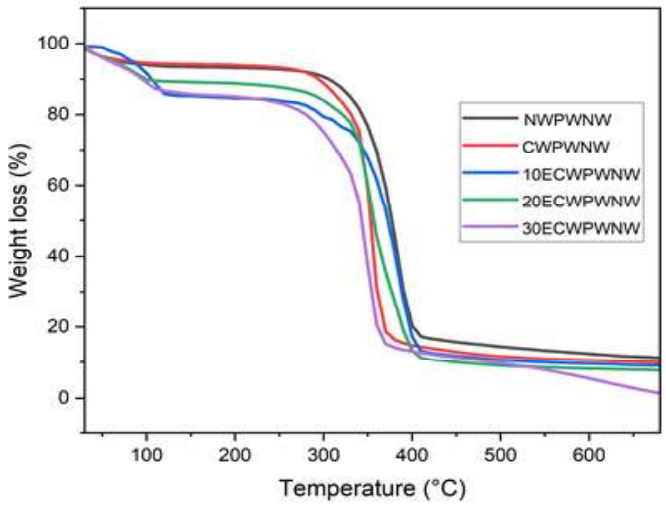

Figure 3: TGA of developed samples

Figure 4: Antibacterial activity of the developed samples

which corresponds to the stretching of C=C of the aromatic moiety. The peak around 1237 cm^{-1} and 807 cm^{-1} corresponds to the asymmetric stretching of C-O-C groups and out-of-plane bending of C-H groups respectively. The peak around 1586 cm^{-1} is related to the presence of aromatic groups and also asymmetric stretching of $–CO_2$ in eugenol structure [11,12]. The spectral peak around 1650 cm^{-1} corresponds to the formation of an amide bond between the quaternary ammonium group of cationized wood pulp and the hydroxyl group of eugenol. From the spectrum, the presence of eugenol was confirmed in the developed samples and chemical interaction was seen between the cationized wood pulp and eugenol.

The thermogram of the normal, cationized and different concentrations of eugenol treated cationized samples is shown in figure 3. It was seen that the developed samples showed three stages of weight loss such as evaporation of moisture, and breakage of amorphous region followed by the crystalline structure of the cellulose. In the case of normal and cationized wood pulp, the onset degradation began at 110°C which was due to the presence of moisture within the cellulosic structure and the weight loss corresponding to 5 %. But, in eugenol treated samples, the weight loss was about 15 % which was due to the evaporation of moisture along with the volatile essential which was dissolved in ethanol. The second onset degradation began around 340 °C representing the decomposition of the cellulosic samples in the case of normal wetlaid samples whereas, in the cationized samples, the degradation began around 300°C representing the breakage of the bond within the cellulosic structure due to CHPTAC. The third onset degradation began around 390°C representing the decomposition of the cellulosic backbone. It was clear from the spectrum that by increasing the concentration of eugenol from 0 to 30 wt %, the degradation began earlier and weight loss (%) was higher compared to the normal and cationized wetlaid samples due to the evaporation of eugenol at higher temperatures [4]. The developed samples offered lower thermal stability after the incorporation of eugenol by a solvent evaporation method.

The tensile strength and elongation (%) of the developed samples are given in table 1. The wetlaid samples comprising normal wood pulp offered higher strength and elongation percentage compared to cationised samples as the latter was surface modified in the presence of CHPTAC as it breaks the bond in the cellulosic polymer chain by the substitution of the cationic group. Eugenol interacts with amorphous region of wood pulp which increases the space between the polymeric chains which reduces the orientation thereby reducing the strength of the fibrous nonwoven. Incorporation of eugenol have less significant change in tensile strength in tensile performance of the developed samples. The wound dressing should absorb the excess wound extrude from the wounds for faster wound healing. The PBS uptake of the developed samples is shown in Table 1. The normal wetlaid nonwoven offered a higher absorbency of 723 % representing the hydrophilic nature of the wood pulp whereas the cationized samples showed slightly lower PBS uptake due to the substitution of NH_3^+ (cationic group) on the primary hydroxyl group of cellulose which makes –OH group unavailable for a water molecule. After incorporation of eugenol in the cationized samples showed lower PBS uptake representing the hydrophobic nature of the essential oil [8].

Eugenol offers higher antibacterial activity against a wide range of bacteria. The antibacterial activity of eugenol incorporated samples was shown in table 2 with increasing the concentration from 0 to 30 wt % which increased antibacterial activity. From figure 4, the cationized samples offered antibacterial activity by inhibiting the growth of the bacteria underneath the sample which was due to the positively charged surface of cellulose which interacts with bacteria by increasing the reactive oxygen groups causing cell lysis

Table 1: PBS (%) and tensile strength of the developed samples

Sample	PBS (%)	Tensile strength (MPa)	Elongation (%)
Normal (NWPWNW)	623 ± 3	0.050 ± 0.03	2.5 ± 1
Blend (BWPWNW)	504 ± 3	0.045 ± 0.05	2 ± 1
Cationised (CWPWNW)	494 ± 3	0.044 ± 0.03	1 ± 0.5
10 % Eugenol (10ECWPWNW)	424 ± 2	0.043 ± 0.02	1 ± 0.5
20 % Eugenol (20ECWPWNW)	411 ± 3	0.044 ± 0.05	0.5 ± 0.1
30 % Eugenol (30ECWPWNW)	398 ± 5	0.043 ± 0.04	0.5 ± 0.1

Table 2: Antibacterial activity of the developed samples

Sample	Zone of Inhibition (mm) against *E. Coli*			
	Control	10 wt % Eugenol	20 wt % Eugenol	30 wt % Eugenol
Normal	0	11 ± 3	13 ± 2	15 ± 3
Blend	0	10 ± 3	13 ± 2	17 ± 2
Cationised	5 ± 1	12 ± 2	15 ± 1	20 ± 3

[13,14]. Eugenol offers antibacterial activity by damaging the cytoplasmic membrane thereby penetrating the lipopolysaccharide membrane and cytoplasm of the bacteria due to its hydrophobic nature. Thus, eugenol treated cationized samples offered higher antibacterial activity of 20mm against Gram negative bacteria such as *E. coli*.

Conclusion

The wetlaid samples comprising eugenol were prepared by surface modification of cellulosic fibers using CHPTAC. The developed samples offered higher antibacterial activity against Gram negative bacteria such as *E. coli*. The bond formation between the cationized web and eugenol and the cationisation of fibrous wood pulp was confirmed using FTIR by the formation of peaks around 1650 cm^{-1} and 1490 cm^{-1} respectively. The interaction between the wood pulp, cationising agent and eugenol offers sustained release of eugenol rather than burst release of agents. The thermal stability of the developed web was found to be lower due to the volatile nature of the essential oil, eugenol. The zone of inhibition of eugenol treated samples was 20 mm as it interacts with bacteria causing cell lysis finding the potential in antibacterial application.

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