Synthesis of Silver Nano Particles Using Plectranthus Ambonicus and Its Antimicrobial Activity on Polypropylene Non Woven Surgical Mask

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Abstract: ***Textile materials are good carriers of various types of micro organisms and can cause health related problems to the wearer. Application of natural antimicrobial agents on textile dates back to antiquity, when the ancient Egyptian used. Herbs were used to inhibit the growth of bacteria on textiles. Silver is a renowned antimicrobial agent used on textiles from very ancient time till date. A very small quantity of silver is required to inhibit a very wide range of pathogenic microorganisms. Application of silver in nano size on textile substrate has enhanced the antimicrobial efficiency of textiles. Biosynthesis of silver is simple and less hazardous and this green technology is having advantage over chemicals methods. Present study focused on the synthesis of silver nanoparticles by silver nitrate and utilizing the leaves extract of plectranthus amboinicus and treatment of polypropylene nonwoven fabrics with nanoparticles, assessment of their antimicrobial efficiency, size of nanoparticles were confirmed by Scanning Electron Microscope (SEM) and presence of nanoparticles on the surface of fabrics were confirmed by Fourier Transform-Infrared Spectroscopy (FTIR) and Energy Dispersive X-ray (EDX) analysis.***

Keywords: Antimicrobial, microorganisms, polypropylene nonwoven fabric, plectranthus amboinicus, silver nanoparticles

1. Introduction

Medical textiles present a wide variety of opportunities for manufacturers and applications for end users. A medical textile often used interchangeably with "healthcare textiles" is any textile that provides a medical benefit. These textiles run the gamut: surgical gowns, divider curtains in hospital rooms, antimicrobial upholstery fabrics, adult briefs, gauze, implantable structures, face masks and arm and knee braces [1].

With growth in world population and the spread of disease, the number of antibiotic resistant micro-organisms is rising along with the occurrence of infections from these micro-organisms [2]. Antimicrobial textiles have been tested for use in the medical industry for some time. Currently, the only antimicrobial textiles being used in the field of medicine are disposable and nonwoven [3]. Some of the treatments being used are harmful to our environment not only because of the chemicals used in the treatments but also because the treated textiles are not reusable [4].

The need for and microbial textiles goes hand-in-hand with the rise in resistant strains of microorganisms [5]. Since the only antimicrobial textiles currently on the market are either disposable or used primarily for odor control, the availability of a reusable and durable antimicrobial textile effective against harmful pathogens will not only be beneficial to both medical industry workers and patients but to the general public as well [6].

Metal nanoparticles have a high specific surface area and unique physicochemical properties [7]. Synthesis of novel metal nanoparticles for the applications such as catalysis, electronics, environmental and biotechnology is an area of constant interest [8]. Silver and its compounds have strong broad spectrum of antimicrobial activities for bacteria, fungi, and virus since ancient times. However, use of silver nanoparticles in textile applications has its own restrictions, as particle size below 50 nm can have a harmful impact on humans and the environment. Use of materials like plant leaf extract, bacteria, fungi and enzymes for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol [9].

Plectranthus amboinicus or locally known as bangun-bangun, bebangun, sedingin or hati-hati hijau, is an indigenous vegetable which can be freshly eaten [10]. The leaf of Plectranthus amboinicus has many medicinal uses, especially for the treatment of common illnesses such as of cough, stomachache, headache, skin infection, asthma and urinary conditions [11]. The plant extracts especially the volatile essential oils from the leaves have been reported to possess antioxidant, antibacterial, antimicrobial, anti-inflammatory and fungi toxic activities but due to the geographical region and variety, the activity and composition of essential oils may be vary [12]. It is important to access the local Plectranthus amboinicus to screen the potential biological activity especially antimicrobial properties and volatiles components of the plant [13].

Surgical masks are primarily used to trap respiratory secretions (which may include bacteria and viruses) expelled by the wearer and prevent disease transmission to others [14]. Surgical masks consist of a very fine middle layer of extra fine glass fibers or synthetic microfibers covered on both sides by either an acrylic- bonded parallel- laid or a wet- laid non woven fabric [15]. The application requirements of such masks demand that they have a high filter capacity and a high level of air permeability, are lightweight, and are non- allergenic[16].

In the present study leaves extract of plectranthus amboinicus was synthesized using silver nitrate and finally antimicrobial effect of silver nanoparticle treated fabrics were assessed against *Bacillus subtilis* and *Pseudomonas aeruginosa.*

2. Materials and Methods

2.1. Materials

2.1.1. Substrate

Melt blown polypropylene non woven fabric with 25 GSM was purchased from SITRA, Coimbatore for the study.

2.1.2. Medicinal Plant

Fresh leaves of plectranthus ambonicus were collected from hilly areas around Salem district, Tamilnadu.

Figure 1: Plectranthus Amboinicus

2.1.3. Microorganism

The strains of bacillus subtilis and pseudomonas aeruginosa were used.

2.1.4. Media and Chemicals

Muller Hilton Agar (MH Agar) procured from Hi-media was used to assess antimicrobial activity. Silver nitrate ($AgNO_3$) was obtained from Department of biotechnology, Periyar University, Salem.

2.2 Methods

2.2.1. Preparation of crude extract

About 25grams of the powdered plant material was taken in dry 250ml conical flask, and then 100ml methanol was added and allowed to macerate overnight. The next day the mixture was vigorously stirred for 10 minutes and allowed to settle. The supernatant liquid was filtered using Whatman no.1 filter paper. The residual plant material was extracted twice using 100ml methanol. The three filters were combined and the solvent was evaporated in vacuum using rotary evaporation. The residual plant material was dried well and again extracted with ethyl acetate and ethanol using the same procedure above. Then the crude extracts were kept at 4°c.

2.2.2. Synthesis of silver nano particles

2.2.2.1. Preparation of plectranthus ambonicus leaves extract

20 g of plectranthus ambonicus leaves were washed thoroughly with distilled water and dried for 24hrs at room temperature. The extract solution was prepared by boiling dried leaves in Erlenmeyer flask with 100 ml of distilled water for ten minutes at 100◦C. Freshly prepared aqueous extract was then used for synthesis of silver nano particles (Figure: 2).

Figure 2: Plectranthus Ambonicus plant extract and silver nitrate aqueous extract

2.2.2.2. Preparation of synthesized silver nitrate aqueous solution

5 ml of fresh leaves extract was added to a conical flask containing 5 ml of 3 mg aqueous $AgNO_3$ solution heated at 65°C with continuous stirring. The silver ions were reduced to silver nanoparticles within few minutes by plectranthus amboinicus leaves extract. The quick conversion of solution color showed the formation of silver nanoparticles (figure: 3) by observing color change from colorless to yellowish-brown color.

Figure 3: Synthezised Silver Nitrate Solution

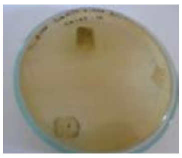
2.3 Antimicrobial activity

2.3.1. Agar Diffusion Test (AATCC 147)

Agar diffusion test is used for qualitative analysis. It is simply carried out and suitable for a large number of samples are to be screened for the presence of antimicrobial activity. In this test, first microorganisms were inoculated on agar plates.

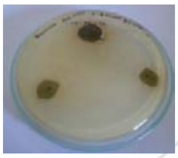
Polypropylene non woven were cut and placed above inoculated agar plates for close contact. The plates were then incubated at 37°C for 18–24 h and analyzed for zone of inhibition. No bacterial growth directly below the fabric sample indicates the presence of antimicrobial activity. A zone of inhibition becomes obvious when the antimicrobial agent can diffuse into the agar. Effectiveness of the antimicrobial activity or the release rate of the active agent can be checked by the size of zone of inhibition.

2.3.2. Antimicrobial activity against Bacillus subtilis

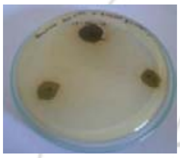


UP (Untreated polypropylene)

C-PME (Polypropylene with crude Methanolic extract)



C-PEE (Polypropylene with crude Ethanolic extract)



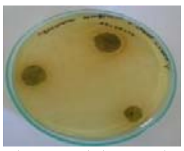
C-PEAE (Polypropylene with crude Ethyl acetate extract)

N-PSE (Polypropylene with Silver Nitrate herb Extract)

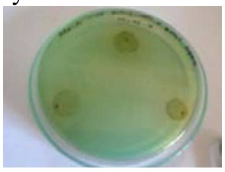
2.3.3. Antimicrobial activity against Pseudomonas aeruginosa

UP (Untreated polypropylene)

C- PME (Polypropylene with crude Methanolic extract)



C- PEE (Polypropylene with crude ethanolic extract)



C-PEAE (Polypropylene with crude Ethyl acetate extract)

N-PSE (Polypropylene with Silver Nitrate herb Extract)

2.4 Air permeability

Prolific Air Permeability Tester was used to find the air permeability of the mask using BS3321: 1960 test method at 10 mm water column. The experiment was repeated five times and the mean was calculated. The experiment was conducted for the control and herbal treated fabrics at different places where lengthwise and widthwise threads do not repeat. Air permeability was calculated by the following formula:

AP = k Rota meter reading

Where k is the conversion factor i.e. = 0.01667 (the 10cm2 area of fabric exposed for checking the air permeability in cubic m/m^2 /minute).

2.5 Bacterial filtration efficiency (BFE)

Bacterial filtration efficiency measures how well the mask filters out bacteria when challenged with a bacteria-containing aerosol. ASTM specifies testing with a droplet size of 3.0 microns containing Staphyloccus aureus (average size 0.6-0.8 microns).

2.6 Development of product

The typical material used to manufacture surgical face masks are polypropylene with 20 gsm made using spun bond technology and 25 gsm polypropylene non-woven treated fabric made using melt blown technology. The surgical face masks are made in 14.5 X 9.5 cm size are shown in figure: 4

Figure 4: Surgical Mask

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3. Results and Discussion

3.1 FTIR Analysis

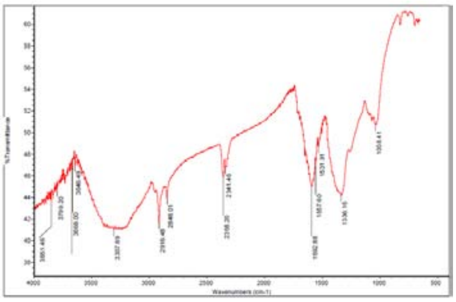

Figure 5: FTIR Spectrum of Ag

From the FTIR spectrum, various functional groups in the compounds were analyzed. In additional, an absorption band at 3307 cm^{-1} ,2916 cm^{-1} and 1592 cm^{-1} appears in the IR spectrum of pure Ag nano particles which attribute to the O-H, C-H and C=C confirming the presence of alkaline, amine and alcohol.

3.2 SEM Analysis

Figure.6 shows the overall surface morphology of Ag nanoparticles. Differences in their morphology can be found from the analysis of the corresponding images obtained at different magnification. These results show that the specific surface area of sample depends strongly on the method of sample prepared.

The figure reveals that the pure Ag compound consists of small aggregates those are transformed to more fine aggregates on increasing the concentration of dopant. The image shows that they are nearly in spherical nano-crystals and uniformly formed. The SEM investigations of all samples reveal the crystallites nature of nanoparticles. In Fig. 6 Pure Ag particles are spherical in shape and the size is 15.36 nm.

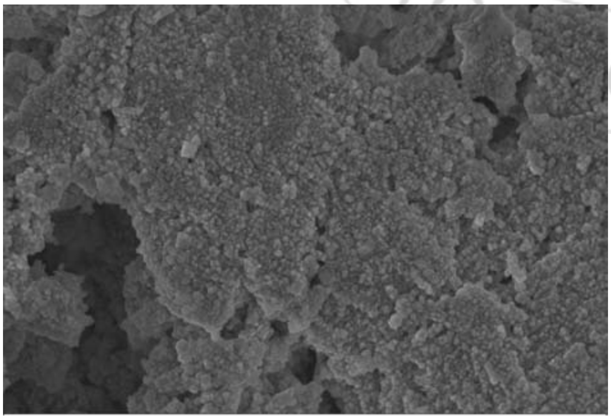

Figure 6: Morphological Analysis for Pure Ag

3.3. EDX analysis

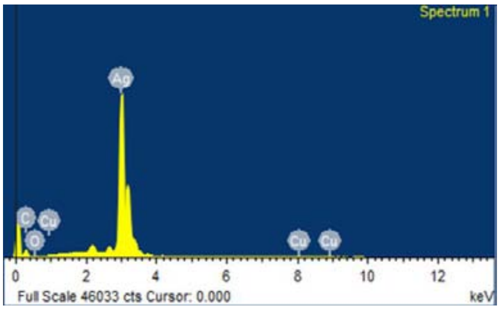

Figure 7: EDAX Analysis for Ag

Fig. 7 shows the EDX analysis of Ag nanoparticles. From the analysis, it can be observed that the peaks corresponding to the energies of 3.1keV indicate the presence of silver atom.

3.4 Antimicrobial activity

Antimicrobial activity of untreated and treated polypropylene nonwoven samples against *Bacillus subtilis* (AATCC test method147)

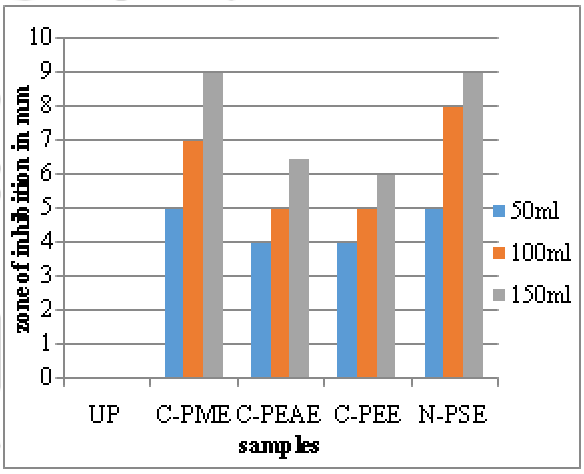

Figure 8

The highest antimicrobial activity against bacillus subtilis was found in N-PSE (polypropylene with silver nitrate herb extract) and C-PME (Polypropylene with crude Methanolic extract) with 9mm zone of inhibition when compared to other samples. Antimicrobial activity of untreated and treated polypropylene nonwoven samples against *Pseudomonas aeruginosa*

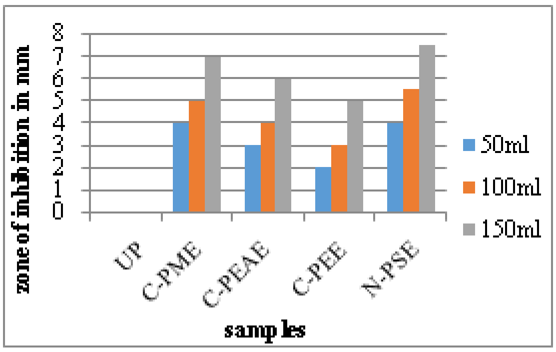

Figure 9

The highest antimicrobial activity against pseudomonas aeruginosa was found in N-PSE (Polypropylene with Silver Nitrate herb Extract)) with 7.5mm zone of inhibition when compared to other samples.

3.5 Air permeability

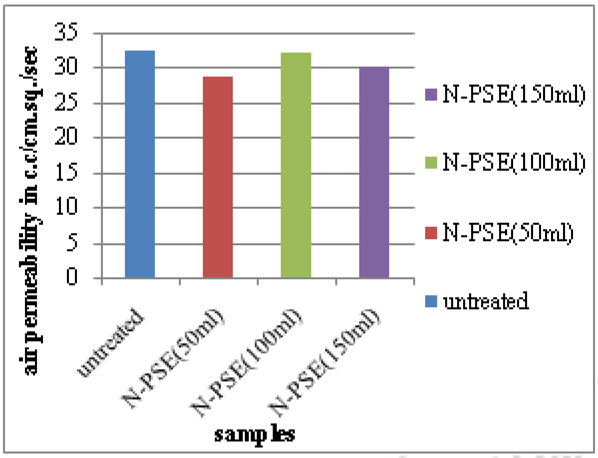

Figure 10

The air permeability of the treated masks (28.6) was higher than the untreated mask (32.4).

3.6 Bacterial filtration efficiency

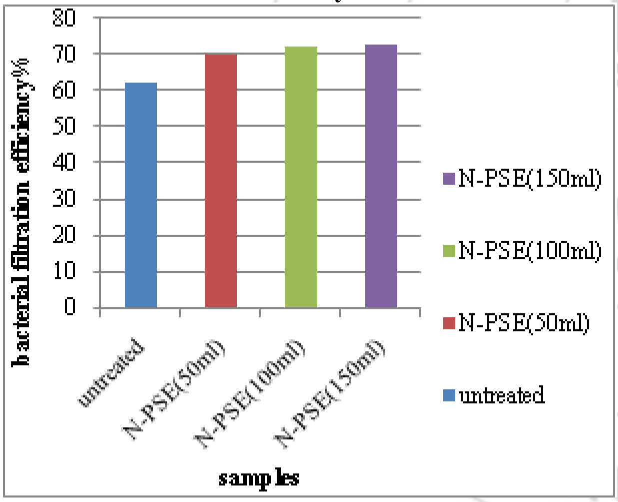

Figure 11

The bacterial filtration efficiency of untreated mask was found to be 62%, where as the treated mask efficiency was found to be 73%. It's clear that the bacterial filtration efficiency is found to be maximum for the treated fabric with 150ml concentration.

4. Conclusion

The non woven polypropylene fabric treated with plectranthus amboinicus herbal extracts showed very good antimicrobial activity, air permeability and bacterial filtration efficiency. This study will help the manufacturers of healthcare products to adopt better herbal finishing treatments for the products used in hospitals and apparels.

5. Acknowledgement

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