

## **Propolis Extract-PVA Nanocomposites of Textile Design: Antimicrobial Effect on Gram Positive and Negative Bacterias**

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**Abstract:** Potential antimicrobial efficiency of propolis extract (bee glue) was experimentally studied on gram positive and negative bacterias by manufacturing propolis extract-based textiles. Pre-samples were prepared by varying percentage concentration of propolis extract in PVA polymer solution and homojenous solutions were electrospun onto polypropylene nonwoven fabric. In-vitro experiments showed that antimicrobial efficiency of extract-containing nanocomposite samples were better than those of not including. According to investigations nanocomposite fabrics with propolis extract sol. were provided antimicrobial effect against to gram positive bacteria (*S. aureus*) but not to gram negative bacteria (*A. baumannii* and *P. aeruginosa*). The results indicated that the electrospun PVA/propolis extract nanocomposites provided a good means for healing of wounds or decreasing infection proliferation caused by gram positive bacteria.

**Keywords:** nanocomposite, PVA polymer, propolis, hospital infections

### **1. INTRODUCTION**

Medical textiles contribute to increase on quality of health service. Improving functionality of textiles used in hospitals can limit some problems due to infection spreading. Bed coverings, bedspreads and some products for patients' personel care are commonly used for supplying sublevel hygienic demands. In order to prevent from hospital infections, these products can be manufactured with pharماسotical materials by considering controversial effects of bacterias causing these problems. By means of bacteria types, it is seen that commonly-known hospital infections are results of gram positive (*Staphylococcus aureus*) and gram negative (*Klebsiella pneumoniae*, *Enterobacter* spp. and *E. Coli* from *Enterobacteriaceae*; *Pseudomonas aeruginosa*, *A. Baumannii* and *Stenotrophomonas maltophilia* from nonfermentative gram-negatives and less frequently *Burkholderia cepacia*) bacterias. Due to increased drug resistance of these bacterias, preventing of hospital infections take great attention for both patience and personnel health [1].

As considering natural-based pharماسotics, propolis has become a popular material due to its abundant phenolic compounds, mainly with potential antioxidant namely "flavonoids" and aromatic acids. It is a strongly adhesive resinous substance used in traditional medicine and

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been reported to have a broad spectrum of biological activities, such as anticancer, antioxidant, antiinflammatory, antibiotic, and antifungal activities [2-7].

It has recently become popular as a health drink and it has been claimed to prevent diseases such as dermatological wounds, inflammation, heart disease, diabetes, cancer, etc. These facts encourage researchers to draw interest in the extraction of flavonoids from propolis esters and adapt these flavonoids to care products [2, 8-10]

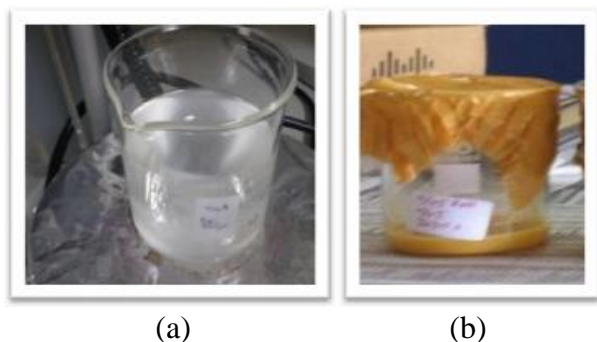
By means of adapting pharmasotics or drug delivery systems onto textile materials, electrospinning is the most preferable method due to producing compatible nanofibers from a large variety of bulk starting materials. Electrospun bicomponent nanofibrous fabrics with high specific surface area, aspect ratio and porosity as a result of random deposition of fibers, could have a great potential in biomedical applications such as tissue engineering scaffolds, drug delivery carriers, wound dressings etc. [11, 12]. By handling of electrospun pharmasotic material, healing period of wounds can be limited and their high surface area of electrospun surfaces can provide optimum healing dosage by using low pharmasotic material extense.

In this study, aqueous poly (vinyl alcohol) (PVA) and propolis extract solutions were blended in proper volume ratio and were electrospun under constant conditions. Electrospun PVA/Propolis extract nanofibrous structure were manufactured and their wound healing performances and bacterial proliferation rates were macroscopically compared by counting colony occured on both subject and control agars.

## **2. MATERIAL and METHODS**

### **2.1. Electrospinnability of Materials**

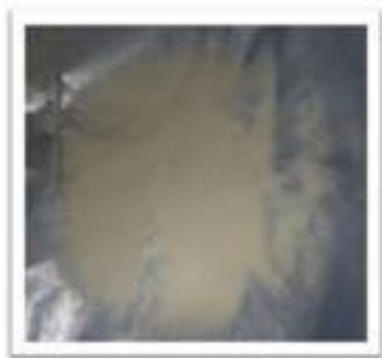
PVA polymer (Mw:80.000 g/mol) was dissolved in distilled water at 60 °C during 8 hours. Propolis extract was diluted with ethanol by concentration of 3% in weight. 3% propolis/ethanol solutions were added into 5%, 7%, 9% and 11% PVA/water solutions drop by drop and mixtures were stirred during 15 hours at room temperature.



**Figure 1.** Prepared solutions (a) 7% PVA polymeric solution, (b) mixture of 5% PVA polymeric solution and 3% propolis extract in weight of 5% ratio of PVA solution.

Homogenized solutions were electrospun onto PP nonwoven surface (17 g/m<sup>2</sup>). Potential differences between the tip and the counter electrode (collector) used to electrospin the polymer /propolis solutions were 37 kV. Stationary collector covered with aluminum foil, placed 10 cm above the capillary tip, was used to collect the electrospun fiber material. Feeding rate was undercontrolled during manufacturing and it was adjusted to 2.5 ml/h. This study was carried out by following two stages: (a) electrospinnability of propolis by keeping constant propolis amount in polymer solutions to observe fiber formation; (b) efficiency of propolis/PVA nanostructures electrospun from solutions containing different amounts of propolis extract. Samples were characterised by SEM method and their antimicrobial efficiency were tested

against to gram positive bacteria (*S. aureus*) and gram negative bacteria (*A baumannii* and *P. aeruginosa*) either in serum physiologic and human blood.



**Figure 2.** Appearance of sample electrospun from PVA/propolis extract mixture

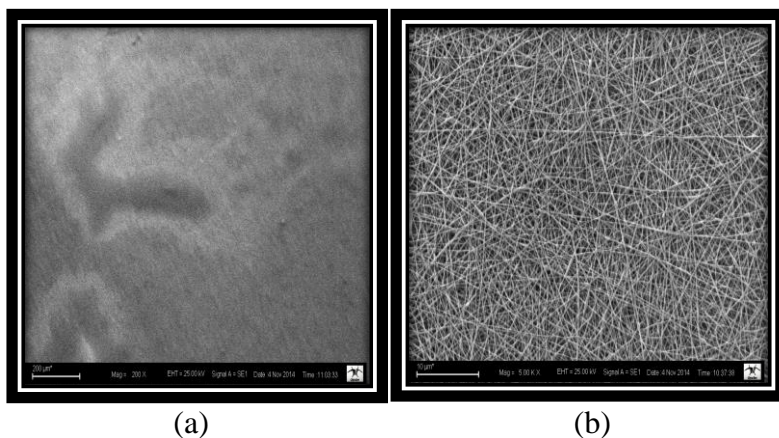
## 2.2. Efficiency of Samples on Gram-Negative/-Positive Bacterias in Different Medias

0.5 McFarland (0.33 ml) bacterial solutions of gram-positive (*S. Aureus*) and gram-negative (*A. Baumannii* and *P. Aeruginosa*) are prepared by 2 ml serum physiologic. Samples, which are electrospun from *various amounts of propolis extract* in PVA polymer solution, are penetrated into bacterias and serum physiologic containing tubes. Electrospun samples containing tubes are observed before subculturation process and it is seen that samples are completely dissolved. These solutions are subcultured on blood agars and it is repeated 3 times by every other day for each sample. Efficiency of propolis containing nanostructures on human blood is also experienced. 0.5 McFarland (0.33 ml) bacterial solutions of gram-positive (*S. Aureus*) are prepared by 0.1 ml centrifuged human blood and 2 ml serum physiologic.

## 3. RESULTS and DISCUSSIONS

### 3.1. SEM Micrographs of Electrospun Samples

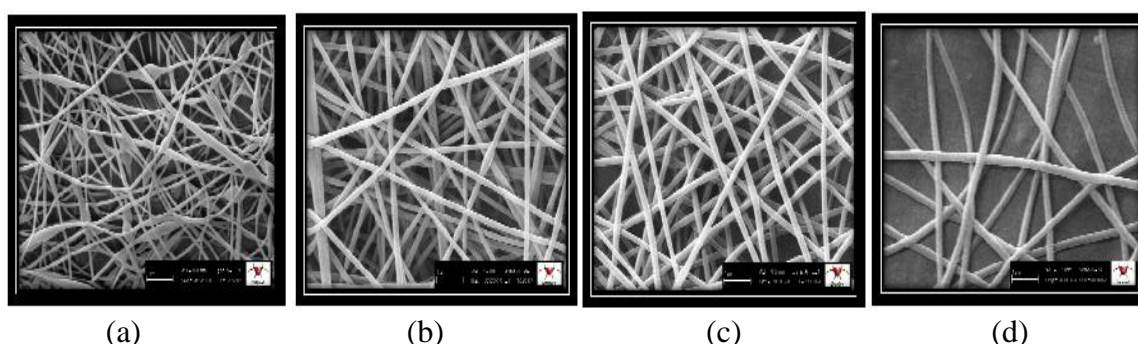
Figure 3 (a) shows that there is no fiber formation on electrospinning of 3% propolis extract solution and electrospun surface of propolis extract is composed of nearly particular substances. Electrospinning of PVA polymer solution successfully provides nanoweb formation in various concentration rates, mainly in Figure 3 (b). According to SEM micrographs, it is seen that propolis extract can be electrospun by adding into PVA polymer solution.



**Figure 3.** SEM images of electrospun reference samples (5000x ve 200x)a) 3% propolis extract, b) 7% PVA

Easily electrospinnability of PVA polymer solution contribute to adaptable of propolis by getting further away from electro spraying. Electrospinnability of propolis by help of polymer solutions has been investigated with other types of polymers in recent studies [13, 14].

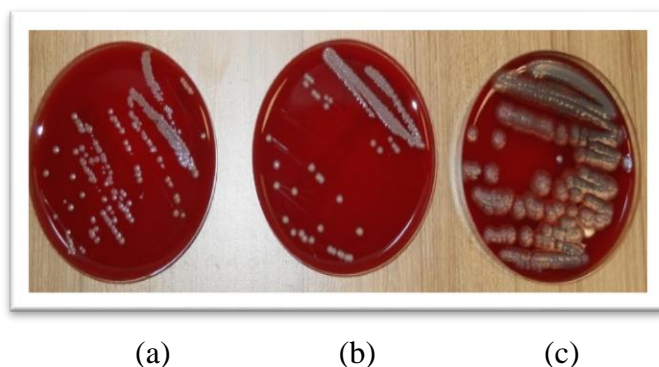
By means of fiber fineness, it is manufactured finer nanofibers from 100% PVA polymer solution than those from propolis-containing polymer solutions. Addition of propolis into PVA polymer solution cause nano/micro-sized fiber manufacturing with thicker diameter and homogeneous structural properties. Figure 4 shows the compatibility of propolis extract on PVA polymer. At decreasing concentration of PVA polymer in polymer/propolis mixture, beading formation and clustering are seen on electrospun structure. There is no seen any beading formation on higher concentrations of PVA polymer. From Figure 4, it is observed that network clusters are occurred in propolis containing nanofibers. But electrospun fibers from 100% PVA polymer solution or high PVA-containing mixtures exhibits more regular, uniform fiber formation and less network clustering.



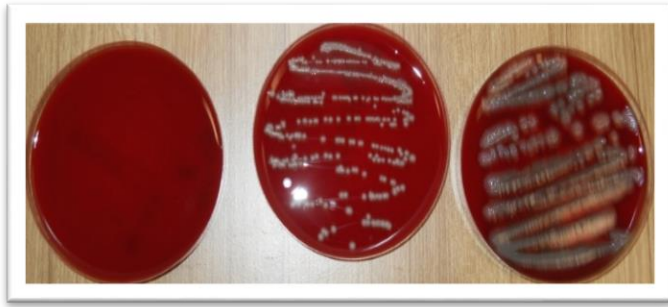
**Figure 4.** SEM micrographs of nanostructures electrospun from PVA/propolis extract solutions, a) 5% PVA-3% propolis b) 7% PVA-3% propolis, c) 9% PVA-3% propolis, d) 11% PVA-3% propolis

### 3.2. Efficiency of Samples on Gram-Negative/-Positive Bacterias in Serum Physiologic Solutions

Subculturation of samples of 0.5 McFarland bacteria, serum physiologic solution and with/without propolis nanostructure is illustrated in Figure 5 and Figure 6. Figure 5 shows subculturation of bacteria and serum physiologic mixture on blood agar and colony proliferation are available for each bacteria types. 100 colonies are proliferated in sample shown in Figure 5 (a). However, there are 4 colonies in Figure 6 (a). It is seen that propolis nanostructure containing solutions prevents proliferation of *Staphylococcus aureus* colonies effectively but not of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* [15]. This proves that propolis extract containing nanostructure is effective on gram positive bacteria, *Staphylococcus aureus* [16-19].



**Figure 5.** Bacterial subculturation of control samples from tubes containing serum physiologic and bacteria a) *S. Aureus*, b) *A. baumannii*, c) *P. Aeruginosa*



(a) (b) (c)

**Figure 6.** Bacterial subculturation of experimental samples from tubes containing serum physiologic, bacteria and electrospun surface (7% PVA solution / 9% propolis extract) a) *S. Aureus*, b) *A. baumannii*, c) *P. Aeruginosa*

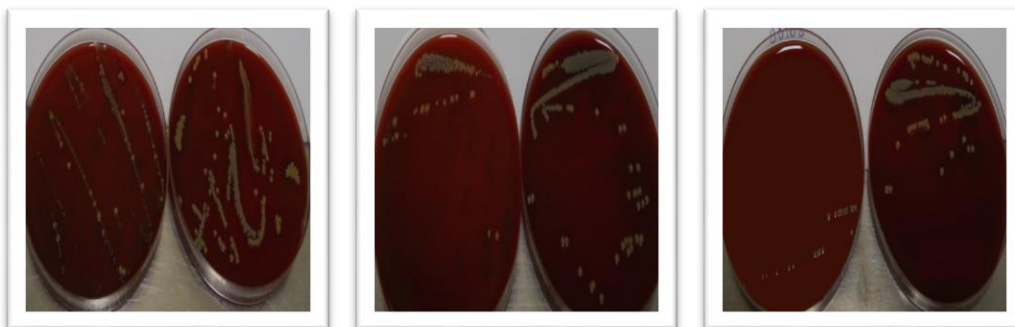


(a) (b) (c)

**Figure 7.** Colony proliferation of *S. Aureus* bacteria for solutions containing electrospun samples and control samples without electrospun surface in serum physiologic (a) electrospun 7%PVA / 5% propolis extract (4 colonies) (b) 7%PVA / 7% propolis extract (no colony proliferation) (c) 7%PVA / 9% propolis extract (no colony proliferation)

### 3.3. Efficiency of Samples on Gram-Negative/-Positive Bacterias in Human Blood

Subculturation of controls (solution in which propolis containing electrospun is not penetrated) result to occurrence of 100 colonies proliferation. The least proliferation among control solution subculturation is observed in Figure 8c as 60 colonies. But all subculturations from propolis containing solutions, namely illustrated as “sample”, lead to decrease on proliferation of *S. Aureus* bacterias although different concentration amount in polymer solutions. This case declares the effectiveness of propolis extract on preventing proliferation of *S. Aureus* bacterias in human blood [20].



(a: sample/control) (b: sample/control) (c: sample/control)

**Figure 8.** Colony proliferation of *S. Aureus* bacteria for solutions containing electrospun samples and control samples without electrospun surface in human blood (a) electrospun 11%PVA / 7% propolis extract (50-60 colonies) (b) 11%PVA / 9% propolis extract (30-40 colonies) (c) 11%PVA / 11% propolis extract (20 colonies).

#### 4. CONCLUSION

Electrospinnability of propolis is possible by addition of this material into easily electrospun biocompatible polymeric solution. Micro/nanocomposite structure is obtained by mixing propolis extract into PVA polymer solution. According to experiments electrospun fabrics with propolis sol., especially 11%, were provided antimicrobial effect against to gram positive bacteria (*S. aureus*) and not provided antimicrobial effect against to gram negative bacteria (*A. baumannii* and *P. aeruginosa*). According to studies, *S. aureus* bacteria is the most initiative and encountered bacteria type among hospital bacterias. By adapting propolis onto hospital textiles or patients' personal care products, hospital infections caused by *S. aureus* bacteria can be limited by these resistive natural pharomasotic, propolis.

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#### Conflict of Interests

Authors declare that there is no conflict of interests.

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