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Investigation of Antibacterial Activity of Juglone-Poly(ϵ -caprolactone) - Alumina Composite Films

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Abstract

The aim of this study was to produce the Poly(ϵ -caprolactone)(PCL)-Alumina (Al_2O_3) composite films impregnated with Juglone and determine their antibacterial properties. PCL- Al_2O_3 composite films containing 1% and 5% Juglone were prepared using the roll mill method. Juglone was chosen as the antibacterial agent in this study for the production of composite films due to its effective antimicrobial activity. The Juglone-Poly(ϵ -caprolactone)(PCL)-Alumina (Al_2O_3) composite films exhibited 100% antibacterial activity against both *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922. It was demonstrated that the Juglone-PCL- Al_2O_3 composite films may have various potential applications in food packaging and personal care products in order to ensure the microbial safety and extended shelf life of the foods and the personal care products.

Key words: Juglone, Poly(ϵ -caprolactone), Composite, Antibacterial

1. Introduction

Microbial contamination is one of the most serious problems in various fields such as medical devices, medicines, healthcare services, hygienic applications, water treatment systems, hospital and dental surgery equipment, textile, food packaging and storage. Therefore, the use of antimicrobials provides quality and safety to many materials and they draw the attention of the academic researchers and the industry [1]. It is well known that the human beings are frequently infected by the microorganisms such as bacteria, molds, yeasts and viruses in their environment. This has led to an intensive research of

antibacterial materials containing various natural and inorganic substances [2]. Recently, plant based antimicrobial agents are being developed in combination with the polymeric materials because of their antibacterial, eco-friendly and body-friendly properties [3].

Phenolic compounds, which are secondary metabolites found in plants, are responsible for the defense mechanisms of plants. Naphthoquinones and flavonoids are regarded as major phenolic compounds in walnut [4]. Naphthoquinones have been preferred in the treatment of fungal, bacterial, and viral infections for centuries in folk medicines [5]. Juglone (5-

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Hydroxy 1,4-naphthoquinone) draws attention among naftokinones due to its chemical reactivity [4]. It is known to have toxic effects, causes death of plants when injected into the petiole of many plants such as tomatoes and alfalfa. Another function of Juglone is that it is a seed germination inhibitor [6]. It has dark orange-brown dyeing properties and has important uses in the food and cosmetic industry as a dyeing agent [7].

Juglone is known to inhibit a broad spectrum of microorganisms including bacteria, algae, and fungi [8]. The mechanism of its action responsible for these effects are peptidyl-prolyl isomerase Pin-1 inhibition, transcription inhibition, DNA topoisomerase-II stimulation, elimination of cell membrane potential and/or formation of hydrogen peroxide [9]. Other positive properties of Juglone are the biodegradability with non-toxic degradation products, the enhancement of degradation in sunlight, and the short half-live of it ranging from a few hours to less than 2 days in natural saline waters [8]. Also, Juglone, a natural Quinone, is an electroactive molecule and has been used as a redox molecule bound to the surface in recent studies [10]. Juglone was chosen as antibacterial agent in this study for production of composite films because of its many interesting properties.

In this study, the antibacterial activities of Juglone and Alumina incorporated PCL films have been evaluated. In the first stage of this study, Juglone-Poly(ϵ -caprolactone) (PCL) - Alumina (Al_2O_3) composite films were produced. In the second stage, the antibacterial activities of the produced composite films were determined.

2. Material and Method

2.1. Material

The following commercially available products were used:

1. Poly(ϵ -caprolactone) (Mn:42500, M_w :65000, Sigma-Aldrich)
2. Juglone (5 - Hydroxy 1,4 Naphthoquinone $\text{C}_{10}\text{H}_6\text{O}_3$, 97%, Sigma-Aldrich)
3. Tryptic Soy Broth dehydrated medium (Merck), Nutrient Agar dehydrated medium

(Acumedia) and ready-to-use Sheep Blood Agar medium (Besimik)

4. *Staphylococcus aureus* ATCC 29213 (Microbiologics) and *Escherichia coli* ATCC 25922 (Microbiologics), which were used as test microorganisms.

2.2. Preparation of Juglone-PCL- Al_2O_3 composite films

Juglone-PCL- Al_2O_3 composite films were prepared by adding the Juglone and Al_2O_3 into the PCL polymer melt at about 100°C . Then, the melted polymer composite mixtures turned into the thin film forms using the roll mill method [11]. The composite films were cut to a certain size (2 cm \times 5 cm) and used in experimental studies (Figure 1).

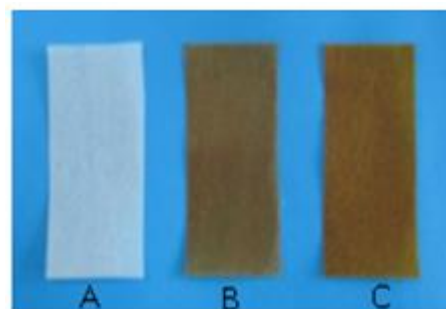


Figure 1 A) Control film (PCL- Al_2O_3), B) Juglone (1%) - PCL- Al_2O_3 composite film, C) Juglone (5%) - PCL- Al_2O_3 composite film

During the melting and stirring process, Juglone was added to PCL- Al_2O_3 at a percentage of 1% and 5%. The different amounts of Juglone and the PCL- Al_2O_3 used in this study were given in Table 1.

Table 1 Contents of Juglone-PCL- Al_2O_3 composite films

Composite Film	PCL - Al_2O_3 (30%)	Juglone
PCL- Al_2O_3 (control film)	0.8746 g	–
Juglone (1%) - PCL- Al_2O_3	0.8748 g	0.0088 g
Juglone (5%) - PCL- Al_2O_3	0.8747 g	0.0435 g

2.3. Determination of antibacterial activity of Juglone-PCL- Al_2O_3 composite films

The antibacterial activity of Juglone-PCL- Al_2O_3 composite films against *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 was determined by the contact plate method [12]. Each sides of the composite films were sterilized by UV light (Philips Ultra Violet TUV 30W) for 5 minutes. Test microorganisms were cultivated in Tryptic Soy Broth medium at $37 \pm 1^\circ\text{C}$ for 24 hours. Next, they were inoculated to Sheep Blood Agar and were incubated at $37 \pm 1^\circ\text{C}$ for 24 hours. A bacterial suspension of 1×10^6 CFU/mL was obtained from the 24-hour bacterial culture by using the densitometer (Biosan).

30 μL of the bacterial suspension was sprayed on the surfaces of the control film and composite films. The films were placed in such a way that their sprayed surfaces contact with the Nutrient Agar medium. All microbiological studies were performed under aseptic conditions. At the end of the incubation, bacterial colonies growing on the composite film surfaces were counted and the antibacterial activity was determined using the antimicrobial activity formula given below:

$$R = [(B-C)/B] \times 100$$

R: Antimicrobial activity (%)

B: Number of bacteria in the control sample (CFU/sample)

C: Number of bacteria in the modified sample (CFU/sample)

3. Results

Juglone-PCL- Al_2O_3 composite films with 30% added alumina containing different percentages of Juglone (1% and 5%) were prepared using the roll-mill technique. The alumina and Juglone additives in the specified amounts were added into the PCL polymer melted at 100°C and mixed. In the next step, the melted mixture was transformed into thin films using the roll-mill. The images of composite films and the chemical structure of Juglone and PCL were given in Figure 2.



Figure 2 The images of composite films and chemical structures of Juglone and poly(ϵ -caprolactone)

The antibacterial activities of Juglone-PCL- Al_2O_3 composite films were determined by using the contact plate method. At the end of the incubation period, the bacterial growth was observed as expected in the composite films used as the control. However, in Juglone-PCL- Al_2O_3 composite films, no bacterial growth was observed. The results of antibacterial activity (R%) calculated for the control film and the Juglone-PCL- Al_2O_3 composite films were given in Table 2.

Table 2 Antibacterial activity (R%) for Juglone-PCL- Al_2O_3 composite films

Test microorganism	Antibacterial activity (R%)		
	PCL- Al_2O_3 (control)	Juglone (1%)-PCL- Al_2O_3	Juglone (5%)-PCL- Al_2O_3
<i>E. coli</i>	0	100	100
<i>S. aureus</i>	0	100	100

4. Discussion and Conclusion

The use of antimicrobials as an additive in polymers provides great benefit in terms of both quality and microbial safety. Microorganisms such as bacteria, fungi and algae affect the aesthetic and physical properties of plastics. Antimicrobial additives are used in many areas, including food production equipment, medical devices in hospitals (catheters), external instruments (stethoscope, medical gloves), flooring and wall coverings [13].

Today, new types of biocidal materials need to be investigated because of the acquired resistance of the pathogenic microorganisms to the known antimicrobial agents. Recent studies in this area have focused on obtaining natural compounds with bactericidal and fungicidal properties from plants and using them as the antimicrobial additives. Novel materials intended to have antimicrobial effects are also expected to be harmless to the environment. In our study, we have used Juglone as a natural compound, and poly(ϵ -caprolactone) as a biodegradable polymer. These are suitable materials in that respect. In a study by Mirjalili *et al.*, the antibacterial activity of walnut, turmeric and henna-dyed viscose fabrics were compared with viscose fabrics treated with Ag nanoparticles. Natural dyes were demonstrated to have strong antibacterial activity against *E. coli*, similar to Ag nanoparticles [14]. In our study, Juglone-PCL- Al_2O_3 composite films showed high antibacterial effects against both *E. coli* and *S. aureus*.

The use of antibacterial composite materials in packaging, personal care products and food industry products may increase the quality of these products and extend their shelf life. Recently, the increase in the use of packages made from conventional polymers that are not biodegradable, causes serious ecological problems. Solano and Gante reported that they developed antimicrobial packaging materials by incorporating known concentrations (w/w) of essential oils of oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) into low-density polyethylene (LDPE). They determined the antimicrobial films developed by them showed antimicrobial activity against food pathogens such as *S. typhimurium*, *L. monocytogenes*, and *E. coli* O157:H7 [15]. Many more studies about the incorporation of antimicrobial agent into the polymeric matrix performed by the researchers [16,17]. Examples can be listed as follows: Sodium benzoate incorporated into Poly (butylene adipate-co-terephthalate) / Organoclay nanocomposite [18]; Plantaricin BM-1 incorporated into PE, LDPE and HDPE [19]; Potassium sorbate and Vanillin incorporated into Chitosan films [20]; Potassium sorbate and Oregano essential oil incorporated into

Thermoplastic starch and Poly(butylene adipate-coterephthalate) [21], *Thymus kotschyanus* essential oil incorporated into starch-chitosan composite film [22].

In our literature search, no study was found about the preparation of Juglone-Poly(ϵ -caprolactone) (PCL)-Alumina (Al_2O_3) composite films. Also, no previous work can be found about the antibacterial activity of these PCL polymer composites. Therefore, in this present work, we have reported the first method for the preparation of and tested their antibacterial surface properties.

As shown in our study, the use of naturel antibacterial agents in polymer composites may have many advantages. For instance, the reduced toxic material properties, the decrease in production costs and no environmental contaminations can be achieved. These biodegradable polymer composites may have potential utilizations in packaging of personal care products, toys and foods due to their effective antibacterial properties. Furthermore, the medical uses of antibacterial biopolymer composite products are also well known. We are currently working on the wound healing and anti-aging properties of these composite films. These Juglone - Poly(ϵ -caprolactone) (PCL) - Alumina (Al_2O_3) composite films can be used also in implants and different tissue parts.

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The Declaration of Conflict of Interest/ Common Interest

No potential conflict of interest was reported by the authors.

The Declaration of Ethics Committee Approval

Ethics Committee Approval is not required.

The Declaration of Research and Publication Ethics

In the writing process of this study, international scientific, ethical and citation rules have been followed.

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