## ANTIBACTERIAL FABRICS MODIFIED WITH BETULIN FOR MEDICAL AND GENERAL APPLICATIONS

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## Abstract

This study examines the potential use of betulin as an alternative to silver in enhancing vinyl-coated fabrics. Silver, commonly used to impart antimicrobial properties to polymers, raises environmental and cytotoxicity concerns. Betulin, known for its antibacterial, anti-inflammatory, antiviral, and antifungal characteristics, emerges as an eco-friendly alternative. The study highlights the possible applications of betulin in various sectors, such as medical, military, and public settings, where addressing the challenge of harmful biofilms is critical. The aim of this research was to assess the possible effectiveness of betulin as a modifier in polyvinyl chloride (PVC) coated textiles. The study involved extracting betulin, preparing plasticizer-based betulin premix, incorporating it into plastisol, and then coating fabrics to analyze its effect on surface bioactivity. Preliminary tribological studies were conducted to assess the durability of the coating. According to the ISO 22196:2007 standard, significant antibacterial effects were observed, with an activity rating (R) ranging between 1.55 and 2.0. In addition, tribological studies indicated an improvement in coating durability compared to conventional PVC coatings. The results suggest that betulin shows potential as a cost-efficient and environmentally friendly alternative, contributing to improved product functionality while minimizing environmental impact. Further research is planned to investigate the potential of betulin in polymer modification and to exploit its positive impact on human health and environmental sustainability.

Keywords: betulin, antimicrobial fabrics, biofilm prevention, polyvinyl chloride coating, activity rating, tribological studies

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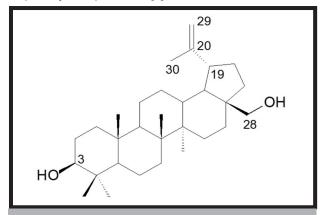
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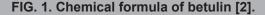
## Introduction

The modification of synthetic materials is an important aspect in the design of polymers with specific properties. The continuous exploration of new modifiers and methods is essential to achieve the desired material characteristics.

In order to enhance the antimicrobial properties of synthetic materials, a common practice is to make modifications by incorporating silver ions into the chemical composition [1]. The antibacterial activity of these modified polymers is dependent on the release of silver ions. However, it has been observed that these ions exhibit cytotoxic effects on living organisms, and their release can potentially lead to environmental sterilization [2].

An innovative approach to modifying synthetic materials appears to be the application of betulin, a natural and organic compound derived from the white bark of birch trees. Betulin is a substituted triterpene (FIG. 1) with highly valuable properties, demonstrating antibacterial, anti-inflammatory, antiviral, and even antifungal activities [2,3]. This compound is an economical alternative to silver ions, as its production cost is relatively lower. Betulin has a vide range of applications in various fields. In addition to its antibacterial, antiviral and anti-inflammatory effects, birch bark extract exhibits antioxidant and even anticancer properties [4]. It may also be useful in personal protection, serving as a modifier in the chemical composition of respiratory masks for upper respiratory tract protection [5].





In the context of current technological developments, fabrics containing betulin as a coating are not yet widely available on the market. Current practice is dominated by fabrics coated with PU (polyurethane), PA (polyamide) or PES (polyester), DWR (Durable Water Repellent), and microporous coatings, or Kevlar fabrics [6-8]. However, such materials do not fully meet the requirements for military and civilian applications because they lack the microbiological activity crucial for protection against harmful biofilm formation. It is important to note that each type of fabric has its own characteristic features that determine its suitability for specific implementations.

The aim of this study was to evaluate the potential utility of betulin as a modifier in textiles coated with polyvinyl chloride (PVC). This compound facilitates the integration of coated fabric structures with a natural extract, allowing the assessment of its impact on the final product. Such an enhancement of the product could improve its functional qualities and allow for the elimination of problematic, commonly used modifiers. The study also investigated the influence of betulin on the bioactivity of the surface. Preliminary tribological evaluation was performed as well.

### **Materials and Methods**

In this experiment, a 500D polyester fabric, with a density of 15 fibers/cm<sup>2</sup> (15x15) was used as the base material. Plastisol (PVC + plasticizer + modifiers) with a composition identical to the reference sample was used for modification, incorporating 1% betulin.

The first step was to extract betulin from the birch bark using the Soxhlet extraction method (FIG. 2a). In order to combine the obtained active substance with plastisol, it was necessary to process the extract appropriately. For this purpose, a plasticizer-based betulin premix was prepared to increase affinity and facilitate homogenization. The active ingredient was extracted from the alcoholbased extract (FIG. 2b), and then the dry mass was grated in a mortar together with the plasticizer (FIG. 3a). In the next stage, the prepared mixture could easily be introduced into the plastisol used in the experiment (FIG. 3b).

After incorporating the active substance into the mixture and seasoning the modified paste, the process of producing an experimental coated fabric was initiated using blade coating technology under laboratory conditions (FIG. 4a). Subsequently, a sample fabric was produced (FIG. 4b) and subjected to further examinations. The parameters and details of the fabric production process are proprietary knowledge of the Partner Systems Sp. z o.o. company.

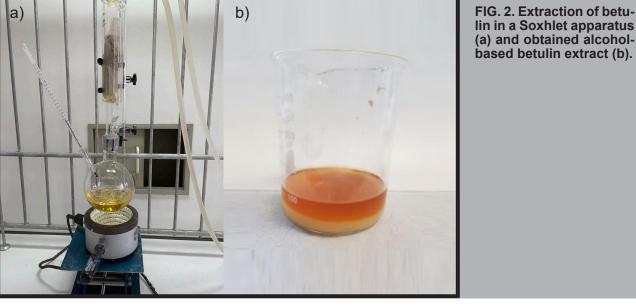




FIG. 3. Plasticizer-based betulin premix (a) and its addition to plastisol (b).

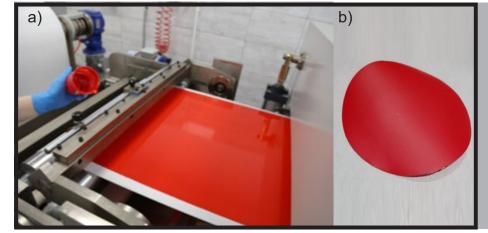


FIG. 4. The fabric coating process (a) and a cutout of the resulting coated fabric sample (b).

TABLE 1. Parameters of the microbiological activity tests.

Research standard	ISO 22196:2007(E) "Plastics - Measurement of antibacterial activity on plastics surfaces"	
Type of plastic used for control samples (size, shape, thickness)	50 mm x 50 mm x 1 mm	
Type of plastic used for test samples (size, shape, thickness)	50 mm x 50 mm x 1 mm	
Type of polymer used as "cover film" (size, shape, thickness)	PP film, 40 mm x 40 mm, 0.05 mm	
Bacterial species and type of strain used         Escherichia coli - DSM 1576; Staphylococcus aureus DSM 346		
Inoculum volume	0.4 ml	
Concentration of bacteria in inoculum	6 x 10 <sup>5</sup> cells/ml	
Volume, type of neutralizer	10 ml, SCDLP broth	

The assessment of the antibacterial activity of the obtained polymeric materials was carried out in accordance with the ISO 22196:2007 (E) standard "Plastics - Measurement of antibacterial activity on plastic surfaces" (TABLE 1). Escherichia coli DSM 1576 and Staphylococcus aureus DSM 346 were used as the reference strains. The bacterial inoculum had a volume of 0.4 ml with a concentration of 6 x 10<sup>5</sup> bacteria/ml. The samples were incubated at a temperature of 35°C with a humidity level of at least 90% for 24 h. Each sample was neutralized following the procedures described in the standards PN ISO 18593:2005 and PN ISO 14562:2006. After the samples were subjected to a sequence of 10-fold dilutions, they were placed in Petri dishes and incubated as specified in the standard. The N coefficient, which represents the number of live bacteria recovered per cm<sup>2</sup> of the sample, was calculated for both test and control samples. For antibacterial tests, the coated fabric without betulin served as the reference sample. Three reference samples for each strain, along with three samples of material containing 1% betulin, were used for the study.

The average antibacterial activity (R) for the assessed fabric was calculated based on:

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t$$

where:

 $U_0$  – the average decimal logarithm of the count of viable bacteria (cells/cm<sup>2</sup>), retrieved from untreated samples after inoculation;

 $U_t$  – the average decimal logarithm of the count of viable bacteria (cells/cm<sup>2</sup>), retrieved from untreated samples after 24 h;  $A_t$  – the average decimal logarithm of the count of viable bacteria (cells/cm<sup>2</sup>), retrieved from treated samples after 24 h.

Subsequently, tribological tests were conducted to assess the impact of the examined extract on the surface of the coated fabric, as well as to determine the average coefficient of friction ( $\mu$ ). All samples were tested on a standard tribometer (Anton-Paar, Corcelles-Cormondrèche, Switzerland) under identical conditions: temperature 21°C, air atmosphere, humidity 40%. The test parameters were as follows: sequence count 1; single-way mode; radius 5.99 mm; linear speed 10 cm/s; acquisition rate 20 Hz; normal load 5 N. The samples used for the tribological tests included the reference sample, three samples containing 1% betulin, and the coated fabric sample with betulin sprayed on the surface.

#### **Results and Discussions**

TABLE 2 presents the results of the microbiological activity tests conducted on the surface of the coated fabric. The parameters  $U_0$ ,  $U_t$ , and  $A_t$ , together with the calculated antibacterial activity denoted as R (1.85), provide insight into the antibacterial properties of the fabric.

The derived antibacterial activity R (1.85) obtained from the tests, indicates a moderate level of antibacterial efficacy for the betulin-modified fabric. This value falls within the range of 1.5 to 2.0, as shown in TABLE 3, corresponding to an acceptable level of bacteriostatic activity according to the ISO 22196:2007 standard. These findings underscore the potential of betulin as a promising alternative to silverbased modifiers in fabric modification processes, suggesting its capacity to enhance microbial resistance in polyvinyl chloride-coated textiles.

# TABLE 2. Results of the microbiologicalactivity tests.

Parameter	Measured value
U <sub>o</sub>	4.21
Ut	4.91
A <sub>t</sub>	3.46
Antibacterial activity - R	1.85

# TABLE 3. Assessment of antibacterialeffectiveness.

Antibacterial efficacy R according to ISO 22196:2007	Number of killed bacteria [%]	Assessment
< 1.5	< 96.8	Poor
1.5 - 2.0	96.8 - 99.0	Acceptable
2.0 - 3.0	99.0 - 99.9	Good
> 3.0	> 99.9	Excellent

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TABLE 4 shows the test results of the reference materials using *Staphylococcus aureus* and *Escherichia coli* bacteria. The presence of bacterial growth observed in both cases underscores the imperative for antimicrobial intervention in fabric modification processes to mitigate pathogenic proliferation effectively.

TABLE 5 presents preliminary results of the tribological tests of coated fabric samples, elucidating the influence of betulin content on the coating strength and the average coefficient of friction ( $\mu$ ) to damage the coating. The results indicate a noticeable enhancement in coating strength in the betulin-modified samples compared to the reference sample, suggesting the potential of betulin in reinforcing coating durability. These findings substantiate the effectiveness of betulin as a modifier in improving both product functionality and durability.

Betulin-modified fabrics possess significant potential for use in many sectors, including military and civilian applications, due to their inherent ability to provide robust protection against harmful biofilms and pathogens. Additionally, these fabrics offer a sustainable solution by minimizing the environmental impact, attributed to the ecofriendly nature of the betulin derivatives used in their modification.

## TABLE 4. Growth of *Staphylococcus aureus DSM* 346, Escherichia coli DSM 1576.

Validation conditions	Staphylo- coccus aureus DSM 346	<i>Escherichia col</i> i DSM 1576
(Lmax - Lmin) / Lmean ≤ 0.2	0.17	0.17
Average number of bacterial colonies covering the control sample immediately after inoculation 6.2 x 10 <sup>3</sup> CFU/cm <sup>2</sup> : 2.5 x 10 <sup>4</sup> CFU/cm <sup>2</sup>	1.0 x 10 <sup>4</sup> CFU/cm <sup>2</sup>	9.2 x 10 <sup>3</sup> CFU/cm <sup>2</sup>
The average number of bacterial colonies covering the control sample after 24 h $\ge 6.2 \times 10^1 \text{ CFU/cm}^2$	1.2 x 10⁵ CFU/cm²	3.1 x 10⁵ CFU/cm²
Validation conditions:	Fulfilled	Fulfilled

# TABLE 5. Preliminary results of the tribological tests.

	Average coating strength [m]	Average coefficient of friction μ to damage the coating
Reference sample	4.52	0.579
Betulin contents 1% by mass sample (1,2,3)	26.17	0.645
Coated fabric sample with betulin (sprayed on the surface)	13.10	0.651

## Conclusions

Birch bark-derived betulin is a promising eco-friendly alternative to silver in modifying vinyl-coated fabrics, offering antibacterial properties without environmental or cytotoxicity concerns.

The microbiological study demonstrates the effectiveness of betulin-modified polyvinyl chloride (PVC) coated textiles in inhibiting bacterial growth, as indicated by an activity rating (R) ranging from 1.55 to 2.0, meeting acceptable antibacterial efficacy standards.

Tribological evaluations demonstrate increased durability of coatings compared to conventional silver-based counterparts, thus explaining betulin's latent ability to enhance both functional effectiveness and product durability.

Betulin-modified fabrics have potential applications in military, medical, and public settings, providing protection against harmful biofilms and pathogens while minimizing environmental impact.

As a result, it has been concluded that further research on combining the beneficial properties of betulin with the practical aspect of coated fabrics is warranted.

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