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# Evaluation of Antibacterial Activity of Flax Fibres Against the *Staphylococcus aureus* Bacteria Strain

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**Abstract**
*Flax fibres are known as favourable to human skin, and linen clothing positively influences some physiological parameters, guaranteeing optimal comfort for users. The advantages of linen are not only related to ensuring proper breathability for human skin and to a lack of allergic activity, but also to flax fibre antibacterial properties, whose are not precisely described in available literature. This paper describes the examination of flax fibres extracted from Polish flax varieties: Artemida, Modran, Sara, Nike and Luna in terms of their antibacterial activity. The fibres were extracted from stems with the application of two different methods: dew and water retting. The extraction method had an influence on the chemical composition of the fibres, resulting in different levels of fibre antibacterial properties. The biological activity of the flax fibres was estimated using clinical strains of Staphylococcus aureus. The flax fibres extracted by the dew retting method from the varieties MODRAN, SARA and NIKE showed the highest amount of lignin content in their chemical composition and the strongest antibacterial activity in comparison to other fibres tested.*

**Key words:** *flax fibres, variety of fibrous flax, bactericidal activity, dew retting, water retting.*

## Introduction

Flax fibres have been known for ages, have served to manufacture clothing, thereby ensuring human protection against weather conditions, and have been used for technical applications.

Clothing made of flax fibres is known as favorable in contact with human skin and has a positive influence on the parameters of human physiology [1 - 3], but there is no sufficient explanation of the antibacterial properties of flax fibres in available literature. The aim of the study was to assess the antibacterial activity of raw fibres extracted from Polish varieties of flax plants with the use of two methods: dew and water retting.

Nowadays the subject of the antibacterial activity of textile fibres is investigated by many researchers, because the textile market situation forces to develop new multifunctional products more attractive and competitive than others.

Several studies have been conducted to develop a new method of bast fibre chemical modification to improve their antimicrobial activity. Fillat *et al.* [4] conducted a study on the use of laccases in graft natural phenols (syringaldehyde, acetosyringone and p-coumaric acid) on unbleached flax to obtain paper products with antimicrobial properties. Chun *et al.* [5] investigated the antibacterial properties of nonwovens with different shares of flax in the cotton/flax blend. The authors tested the nonwovens after scouring and drying, but they did not confirm that increasing the flax share in the fabric increased antibacterial properties. The authors focused on the drying effect on antibacterial properties, but they did not evaluate the chemical composition of flax fibres used for nonwoven preparation and did not refer to the method of fibre extraction. There is no information about the antibacterial properties of raw unmodified flax fibres obtained from traditional varieties of flax plants.

Genetic engineering has devised new tools for obtaining other properties for bast fibres, e.g. genetically modified flax fibres containing strong antioxidants (phenolic acids, lignans) from transgenic plants have been developed and applied for medical application [6]. That research resulted in three different patent submissions linked with GM flax [7 - 9].

*Table 1. Conditions of the processes applied for fibre extraction.*

| Fibre extraction method | Time | Temperature | Equipment |
|---|---|---|---|
| Dew retting | 43 days | Ambient weather conditions | Field |
| Water retting | 74 h | Water temp. 32 – 33 °C | Lab-scale tank |

The results of research in respect of the genetic modification of flax fibres to improve their biological activity has generated a question about the biological activity of natural unmodified flax. The aim of the current study was the investigation of the antibacterial activity of natural flax fibres without any genetic modification from the point of view of the fibrous flax variety and extraction method, which can be a complement to the research cited.

The presence of phenolic substances in flax fibres has been studied by a few researchers [10, 11], but there is no analysis of the substance content in the fibres in terms of their antibacterial activity.

The bioactive potential of raw fibres coming from Polish varieties of flax has been investigated within multi-stage studies. The previous study described in [12] was focused on evaluation of the antioxidant activity of fibres. This research completes the previous study by investigation of flax antibacterial properties against the *Staphylococcus aureus* bacteria strain.

## Materials and methods

Five different Polish varieties of fibrous flax growing in the same vegetative season were used in the current study: Artemida, Modran, Sara, Nike and Luna. Flax fibres were extracted from the straw with the use of the dew retting and water retting methods.

The parameters of processes applied for fibre extraction are presented in ***Table 1***.

The raw fibres obtained from all mentioned-above varieties of fibrous flax were the materials of the study. All types of flax fibres were evaluated in terms of the determination of their chemical composition as well as their antibacterial activity against *Staphylococcus aureus* bacteria.

The tests done within the current study were conducted in ambient conditions similar for all flax varieties and according to relevant standards described below. The fibre moisture content is almost similar for all varieties of pure flax fibres and depends on the humidity of ambient air, e.g. flax moisture content is 8.3% in conditions of 40% relative air humidity, 9.9% in 60% relative air humidity, 10.7% in 70% relative air humidity, and so on. Because the relative air humidity was similar in the tests of each fibre variety, the fibre moisture content did not have an effect on the diversity of fibre properties.

**Evaluation of flax chemical composition**

Flax fibres contain natural polymers in their chemical composition like cellulose, lignin, hemicelluloses, pectin as well as waxes, fats and others. The chemical composition of all the types of extracted fibres was evaluated with the use of relevant standards:

- Waxes and fats content in % was measured according to the Polish Standard no. BN-86/7501-10. The percentage content of wax and fat substances was determined by extracting them with an organic solvent (petroleum ether) in a Soxhlet extractor and weighing the residues after vaporization of the solvent.
- Lignin content in % was determined according to the Polish Standard BN-86/7501-11. The lignin content was measured by dissolving cellulose, hemicellulose and pectins with a mixture of concentrated sulfuric and ortho phosphoric acids, followed by draining off the remaining insoluble lignin.
- Pectin content in % tests were conducted using the gravimetric method according to a method developed at INF&MP. The percent share of pectins was determined by dissolving them in ammonium citrate and then precipitated from the solution with calcium chloride and by measuring the weight of the calcium pectinate precipitated from the solution.
- Cellulose content in % was measured according to the Polish Standard no. PN-92/50092. The cellulose content was measured by dissolving lignins and other substances conteined in fibre with a mixture of acetylacetone and dioxane, acidified with hydrochloric acid.
- Hemicellulose content in % was determined according to the Polish Standard BN-77/7529-02. The percentage of hemicellulose content was determine by dissolving hemicellulose contained in fiber with 1% sodium hydroxide, draining, drying and calculation of hemicellulose content based on weight loss [26].

Additionally the content of phenolic acids, e.g. coumaric and ferulic acid, in flax fibres was assessed by the HPLC method.

## Evaluation of antibacterial activity manifested by flax fibres

Tests of the antibacterial properties of flax fibres were conducted in cooperation with the Department of Medical Microbiology at the University of Medical Sciences in Poznan. In the studies, a standard strain of *S. aureus* - ATCC 6538 was used (in accordance with PN-EN-ISO 20645:2006) [13]. *Staphylococcus aureus* belongs to the most common etiological factors of inflammation in surgical laces. Moreover five clinical strains of *S. aureus* were used, originating from patients with dermal lesions. The strains from patients were isolated on sheep blood agar for 20 - 24 h at a temperature of 37 °C in aerobic conditions. The colonies developed were identified on grounds of morphological and biochemical traits. Final identification of *S. aureus* was conducted using the automated system ATB with the application of ID 32 Staph strips (bioMérieux). All the strains were sensitive to oxacillin (MSSA). The bacteria were cultured on Trypticase Soy Agar (TSA). Tests were conducted as specified by the PN-EN-ISO 20645:2006 standard. Every Petri dish contained 10 ml of TSA, providing a lower (free of bacteria) layer overlayed with 5 ml of TSA with the strains studied, at $1.5\times10^8$ cfu/ml (0.5 McF) of *Staphylococcus aureus*. The agar plates prepared were overlaid with control samples (sterile discs of filter paper) and samples of the fibres studied, 25 mm in diameter. The media with implanted samples were incubated for 18 to 24 hours at a temperature of 37 °C. Following the incubation, bacterial growth was appraised within the zone of contact between the medium and the sample studied. Then the material samples and control discs were removed and bacterial growth within the contact zone was evaluated under a microscope. The analysis was conducted in triplicates. The results obtained were evaluated as:

- a good effect: absence of bacterial growth under material studied,
- restricted efficacy: the growth almost fully inhibited under material studied,
- average effect: growth reduced by 50% under material studied, as compared to the control,
- insufficient effect: pronounced growth of bacteria under material studied.

## Results

**Evaluation of the chemical composition of flax fibres**

The analysis of the chemical composition of fibres obtained from Polish varieties of flax was discussed in [12] in the context of fibre antioxidant activity. Results of the test of the chemical composition of flax fibres extracted from different varieties of fibrous flax are presented in ***Table 2*** (see page 122).

The flax fibres extracted from straw with the use of the dew retting method contained a higher amount of lignin and hemicellulose as well as a lower amount of cellulose and waxes in comparison to relevant water retted fibres. Two varieties: Modran and Sara are characterised with the highest level of lignin. Such differences in the fibres' chemical composition influence their biological activity.

The evaluation of flax in terms of the phenolic acid content was possible only for dew retted fibres. In the case of water retted fibres the traces of acids were extremely weak and their detection by the HPLC method applied was impossible. Ferulic acid is extremely soluble in water and can be easily removed from fibres during the water retting process. Coumaric acid is poorly soluble in water and its removal could be partial.

The phenolic acid content in dew retted flax fibres is presented on ***Figure 1***.

Apart from the fact that all varieties of dew retted flax fibres contained a higher amount of lignin and hemicellulose

*Table 2. Chemical composition of fibres extracted from fibrous flax [12].*

| Fibre | Flax variety | Lignin content, % | Lignin SD, % | Cellulose content, % | Cellulose SD, % | Waxes and fats content, % | Waxes and fats SD, % | Hemicelluloses content, % | Hemicelluloses SD, % | Pectin content, % | Pectin SD, % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dew retted | Artemida | 6.33 | 0.41 | 64.86 | 0.64 | 1.30 | 0.02 | 19.31 | 0.14 | 1.32 | 0.17 |
| | Modran | 7.96 | 0.11 | 64.16 | 1.22 | 1.72 | 0.03 | 21.64 | 0.13 | 1.44 | 0.09 |
| | Sara | 7.42 | 0.22 | 62.21 | 0.22 | 1.63 | 0.02 | 22.30 | 0.14 | 1.20 | 0.13 |
| | Nike | 6.40 | 0.41 | 64.31 | 0.87 | 1.19 | 0.03 | 20.91 | 0.02 | 1.72 | 0.16 |
| | Luna | 5.18 | 0.17 | 69.61 | 0.14 | 1.06 | 0.13 | 16.86 | 0.04 | 2.12 | 0.19 |
| Water retted | Artemida | 3.51 | 0.04 | 74.01 | 0.49 | 4.40 | 0.00 | 14.57 | 0.05 | 0.58 | 0.15 |
| | Modran | 3.52 | 0.02 | 72.42 | 1.00 | 4.25 | 0.02 | 15.30 | 0.31 | 1.43 | 0.23 |
| | Sara | 3.18 | 0.13 | 70.76 | 0.14 | 4.43 | 0.11 | 17.31 | 0.17 | 2.15 | 0.08 |
| | Nike | 3.05 | 0.13 | 71.31 | 0.91 | 4.41 | 0.12 | 15.55 | 0.19 | 1.35 | 0.07 |
| | Luna | 2.56 | 0.21 | 72.77 | 0.35 | 4.49 | 0.02 | 13.91 | 0.08 | 0.65 | 0.02 |

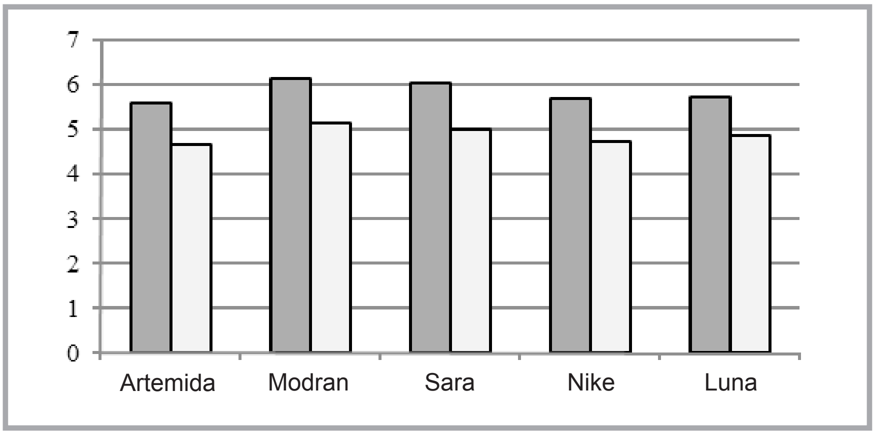



***Figure 1.** Phenolic acid content in different varieties of dew retted flax fibres [12]; ■ - content of coumaric acid in mg/100 g, □ - content of ferulic acid in mg/100 g.*

in comparison to water retted fibres, analysis of the fibre chemical composition proved that the dew retted fibres contained some amount of phenolic acid, while higher amounts of phenolic acid occurred in the Modran and Sara varieties. It is known that ferulic acid is a component of the primary cell wall and is bonded with lignin and hemicellulose in plants [14, 15]. To confirm this, the relationship between the phenolic acid,lignin and hemicellulose contents in the dew retted flax fibres tested was analysed and presented in ***Figures 2*** and ***3***.

The relationship between the lignin and phenolic acid content was high (***Figure 2***), confirmed by correlation analysis - the Pearson correlation coefficient was 0.76. The relationship between the hemicellulose and phenolic acid content was weaker (***Figure 3***) – the coefficient was 0.58. Analysis of the correlation was conducted, regardless of the low number of observations, to have preliminary information about the relationship between the main fibre components and bioactivity.

## Evaluation of the antibacterial activity of flax fibres

Microscopic images of *Staphylococcus aureus* bacteria growing on a medium of flax fibres extracted from different varieties of flax plants with the use of the dew and water retting methods are presented in ***Figure 4***.

Comparison of microscopic images of *Staphylococcus aureus* bacteria growth on different fibrous media allows to see differences between the size of bacterial colonies. The size of bacterial colonies growing on the medium of fibres extracted with the use of the dew retting method is smaller in comparison to bacteria growing on water retted fibres. However, it must be emphasised that each type of flax fibre used as a culture medium in this study caused a reduction in the colonies of *Staphylococcus aureus* bacteria. Bacteria growth reduction with dew retted fibres Sara, Modran and Nike was the most efficient in comparison to the others and should be classified at a level between restricted efficacy and average effect. Water retted fibres Artemida and Luna were characterised by the lowest capacity for bacteria elimination and should be classified at a level between average and insufficient effect.

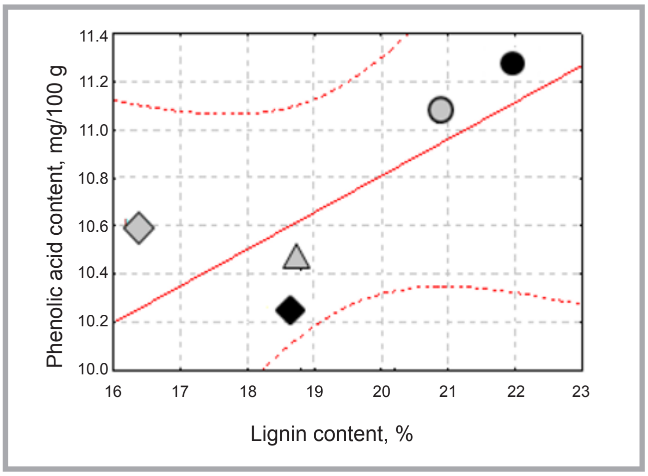



***Figure 2.** Relationship between lignin and phenolic acid content in dew retted flax fibres [12]; ◆ - Artemida, ● - Modran, ○ - Sara, ◇ - Luna, △ - Nike.*

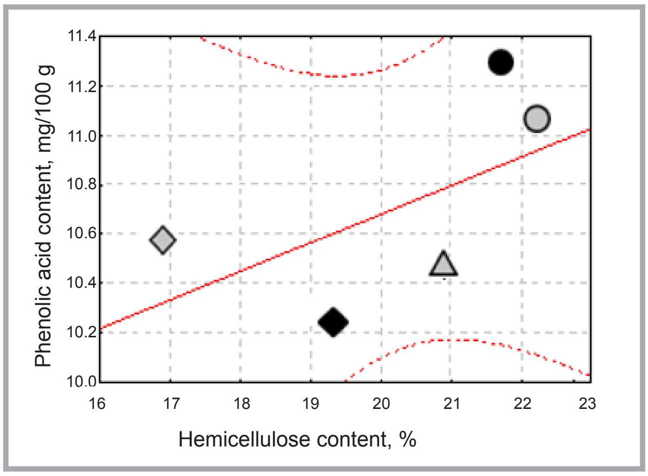



***Figure 3.** Relationship between hemicellulose and phenolic acid content in dew retted flax fibres; ◆ - Artemida, ● - Modran, ○ - Sara, ◇ - Luna, △ - Nike.*

Percentage differences in the antibacterial capacity of all types of flax fibres are shown in ***Figure 5***.

The ***Figure 5*** confirmed the observation from microscopic images of bacterial colonies growing on fibrous medium (***Figure 4***) that dew retted fibres extracted from all the varieties of fibrous flax tested showed higher antibacterial activity in comparison to equivalent fibres obtained by the water retting method.

Additionally comparison of the results of the antibacterial test of fibres extracted from different varieties of flax plants indicated a strong relationship between the amount of reduced bacterial colonies and the chemical composition of fibres. ***Figure 6*** (see page 124) show that the fibre ability to reduce the bacterial colonies decreased with the increasing of the cellulose share, but increased with growing lignin and hemicellulose content in the flax fibres (***Figures 7, 8***, see page 124). The lines of trend drawn in ***Figures 7.b*** and ***8.b*** confirmed the strong relationship between lignin and hemicellulose content and fibre antibacterial activity, especially in the case of dew retted flax fibres.

The dew retted flax fibres contained a higher amount of lignin, and it is known that lignin is associated with phenolic acids in flax plants, in the crosslinking between lignin and hemicellulose in the cell wall carbohydrates [16 - 19]. Phenolic compounds in plants have been listed as well as antibacterial agents [20, 21], which means that a larger amount of lignin and phenolic acids are responsible for the antibacterial activity of dew retted flax fibres.

Additionally it should be noted that in the dew retting process, naturally occurring fungi affected by moisture and ambient temperature conditions are responsible for the disintegration of the flax stem, with the subsequent release of fibres [22, 23]. The enzymes secreted by fungi can support the antibacterial activity of dew retted fibres, which is very limited in the case of the water-retting process because here the activity of anaerobic bacteria is mainly responsible for fibre release [16]. A quality and quantity analysis of fungi identified on all the varieties of fibres extracted from straw of dew and water retted flax is presented in ***Table 3***. The tests were conducted according to relevant standard: PN ISO 21527 – 1:2009.

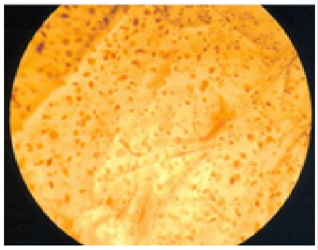
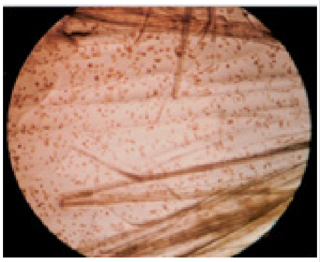
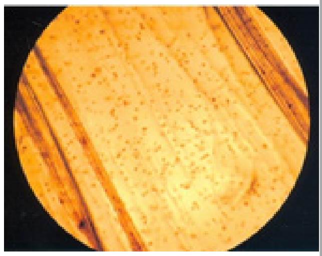
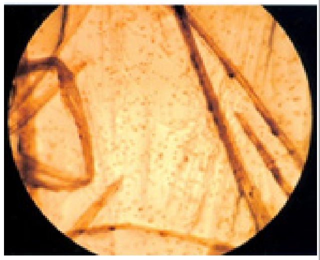
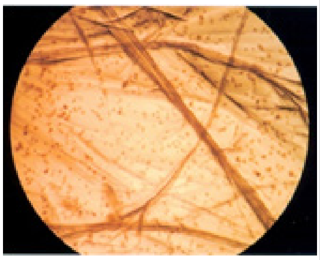
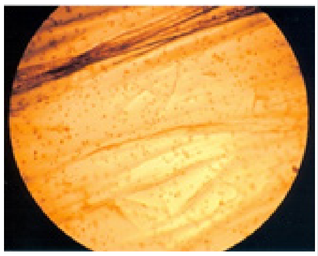
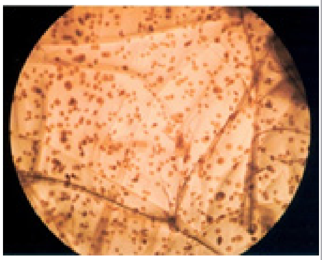
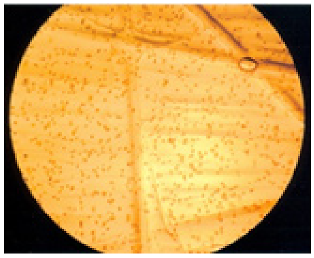
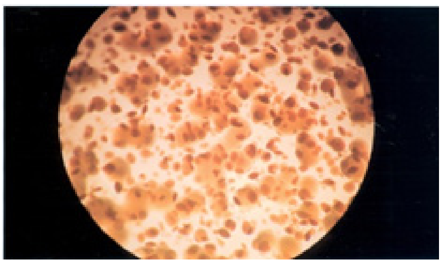
| | **Water retted fibers** | **Dew retted fibers** |
|---|---|---|
| *Staphylococcus aureus* growth with Artemida fibers |  |  |
| *Staphylococcus aureus* growth with Modran fibers |  |  |
| *Staphylococcus aureus* growth with Sara fibers |  |  |
| *Staphylococcus aureus* growth with Nike fibers |  |  |
| *Staphylococcus aureus* growth with Luna fibers |  |  |
| Control Test *Staphylococcus aureus* without fibers medium |  | |

***Figure 4.*** *Microscopic images of Staphylococcus aureus bacteria growing in contact with flax fibres.*

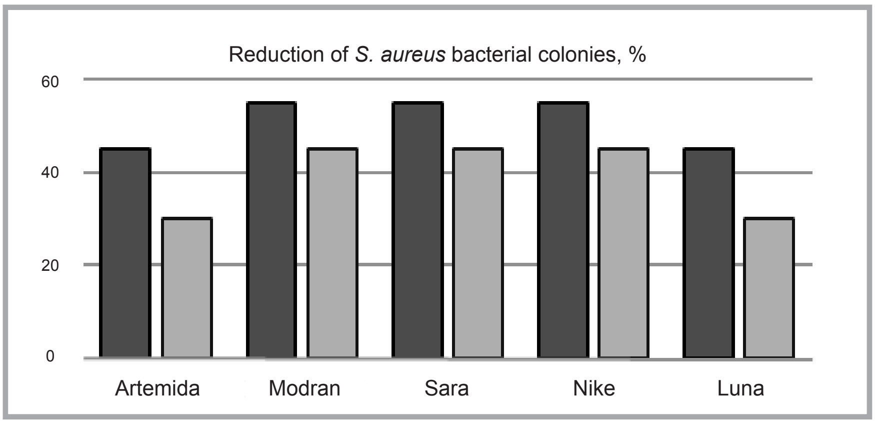



*Figure 5. Comparison of antibacterial capacity of flax fibres.* ■ *- dew retted flax fibres,* ■ *- water retted flax fibres.*

Dew retted flax fibres contained about 10,000 times more fungal colonies in comparison to relevant water retted fibres.

Some of the fungi residing on the flax fibres are able to support antibacterial properties of the fibres, e.g. *Epicoccum nigrum* is capable of producing secondary metabolites with antibiotic activity [24], and Xiao *et al* [25] proved that *A. alternata* has the capacity to produce feruloyl esterase in addition to some glycoside hydrolases, which enabled the efficient release of a phenolic antioxidant.

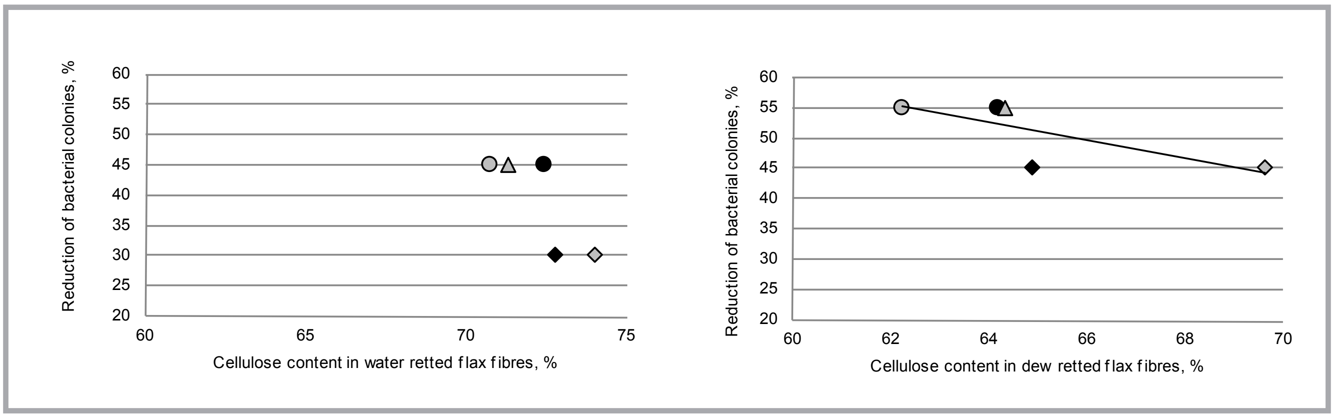



***Figure 6.*** *Relationship between cellulosic content in: a) water, and b) dew retted flax fibre and reduction of bacterial colonies.*

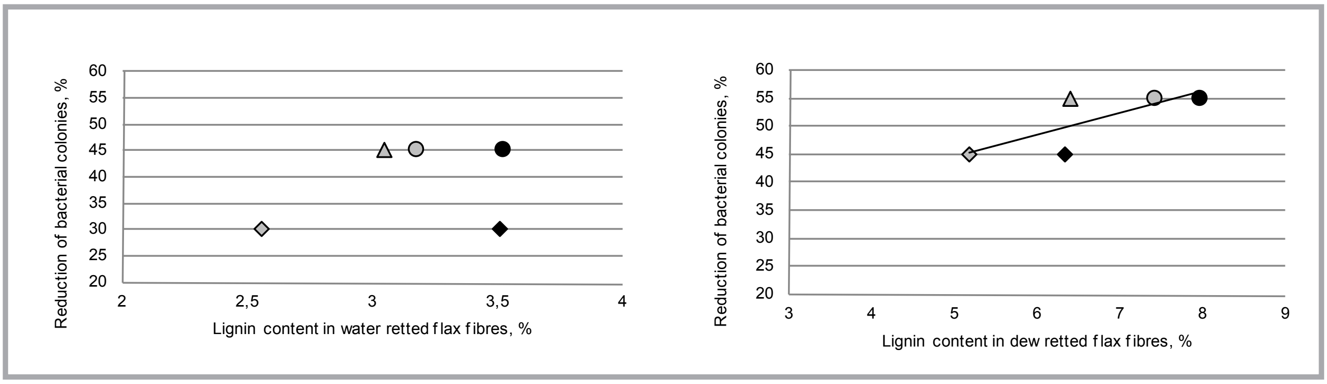



***Figure 7.*** *Relationship between lignin content in: a) water, and b) dew retted flax fibre and reduction of bacterial colonies*

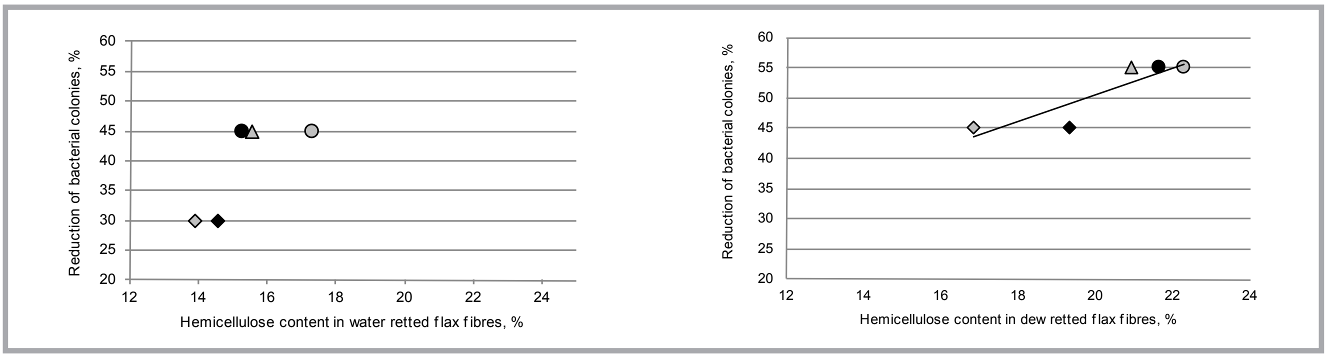



***Figure 8.*** *Relationship between hemicellulose content in: a) water and b) dew retted flax fibre and reduction of bacterial colonies. Artemida Modran Sara Luna Nike*

## Conclusions

Flax fibres extracted from Artemida, Modran, Sara Nike and Luna varieties with the use of the dew retting or water retting methods are characterised by the ability to reduce colonies of *Staphylococcus aureus* bacteria. The fibres' antibacterial capacity is strongly related to their chemical composition, mainly lignin content, which is cross-linked with phenolic compounds. The fibres' chemical composition depends on the fibre extraction method and flax plant variety. Dew retted Modran, Sara and Nike contained the biggest amount of lignin and were characterised by the highest ability for the reduction of bacteria colonies. Dew retted flax fibres obtained from all the varieties tested were characterised by better antibacterial properties in comparison with respective water retted fibres. It was confirmed that the dew retted fibres contained a higher amount of lignin and phenolic acids related with it.

Additionally the antibacterial properties of dew retted flax fibres are improved by the activity of fungi residing on the fibres.

**Table 3.** *Fungi identified on the different varieties of fibres extracted from straw of dew and water retted flax; *CFU/g - colony forming units per gram.*

| | Fibres | Number of fungi, CFU/g* | Species of fungi | Share, % |
|---|---|---|---|---|
| Dew retted flax | Artemida | $1.3 \times 10^4$ | *Alternaria alternata* | 100 |
| | Modran | $7.0 \times 10^4$ | *Alternaria alternata*<br>*Mucor circinelloides*<br>*Fusarium poae* | 80<br>10<br>10 |
| | Sara | $7.3 \times 10^4$ | *Alternaria alternata* | 100 |
| | Nike | $1.5 \times 10^5$ | *Alternaria alternata*<br>*Fusarium poae*<br>*Epicoccum nigrum* | 70<br>15<br>15 |
| | Luna | $3.7 \times 10^4$ | *Alternaria alternata*<br>*Epicoccum nigrum* | 90<br>10 |
| Water retted flax | Artemida | 40 | *Alternaria alternata*<br>*Epicoccum nigrum*<br>*Aspergillus flavus* | 50<br>25<br>25 |
| | Modran | 10 | *Alternaria alternata* | 100 |
| | Sara | 50 | *Eurotium chevalieri* | 60 |
| | | | *Cladosporium cladosporioides* | 20 |
| | | | *Rhizopus nigricans* | 10 |
| | | | *Chaetomium funicola* | 10 |
| | Nike | 20 | *Aspergillus niger* | 100 |
| | Luna | < 20 (below of limit of detection) | | |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