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Comparison of the Antimicrobial and Antioxidant Efficacy of Viscose Treated with Various Natural Compounds for Medical Use

DOI: 10.5604/01.3001.0014.3802

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Abstract
This research is based on the use of a variety of natural compounds and their mixtures with chitosan in order to create an efficient textile product for sanitary/medical use which shows antimicrobial and antioxidant effectiveness at the same time. It is assumed that natural compounds showing antimicrobial and antioxidant efficacy also do so even when applied on a non-woven viscose substrate intended for wound healing. A study of the effectiveness of the individual treatment was performed using antimicrobial (dynamic-stress test) and anti-oxidative (ABTS$^{\bullet+}$) testing. It was confirmed that the properties of functionalised viscose treated with different functionalisation formulations differ in dependence on the separate formulations. Results show that for a comprehensive insight into the antimicrobial and antioxidant activity of functionalised viscose, a very detailed study of the results of antimicrobial and antioxidant testing is needed in order for it to be possible to create a textile material with the necessary functionality.

Key words: *chitosan, natural compounds, viscose, antimicrobial, antioxidative.*

Introduction

Nature is a source of many antimicrobial and antioxidant-efficient plants, thus the possibility of exploiting these two properties for the creation of medical textile-based products also means a big challenge for researchers. Chitosan is very often used for this purpose because it is distinguished primarily by its good antimicrobial efficacy, biodegradability, etc. [1-7]. On the other hand, honey is popular as a natural compound with high antimicrobial/antioxidant effectiveness. It stimulates the release of oxygen from hemoglobin, thus slowing down the action of proteases that otherwise inhibit wound healing. Besides this, honey, due to its high content of sugars, has high osmolarity, which means that, as such, it promotes the secretion of fluids, resulting in faster wound healing [5]. In addition, horsetail, cloves and olive leaves could be of big interest as they possess many healing properties (e.g. antimicrobial, antioxidant, antibiotic, antiviral, antiseptic, etc.). Horsetail is rich in silica, cloves in eugenol, and olive leaves in oleuropein [8-12], therefore they represent possible sources to create textiles with an antimicrobial/antioxidant effect, as it is desirable for medical purposes that the substrate combines both antimicrobial and antioxidant properties at the same time.

Notwithstanding the fact that many plants have an antimicrobial and antioxidant effect, the question arises as to whether or not their effectiveness varies. Many publications discuss and conclude that individual natural compounds have an antimicrobial/antioxidant effect [1-12], but there is relatively little debate on the extent to which their efficacy varies. In order to develop sanitary and medical textile materials, it is certainly necessary to recognise the ability of a separate functionalisation coating (i.e. chitosan, honey, or plant extract) to reduce different types of bacteria (Gram-positive, Gram-negative), as well as their antioxidant efficiency in the worse to better direction. Therefore, the purpose of this research was to functionalise a textile non-woven substrate made of viscose with a separate treatment bath in order to determine its ability to reduce different types of microorganisms, as well as to evaluate its antioxidant effectiveness. In this respect, various natural compounds (chitosan, honey) and extracts of horsetail, cloves (eugenol), and olive leaves (oleuropein) were used, as well as their mixtures.

Within the research, antimicrobial testing using the dynamic-stress test to observe the reduction of selected pathogenic microorganisms (*Staphylococcus aureus, Escherichia coli*) and the spectrophotometric method of ABTS$^{\bullet+}$ (2,2'-azinobis-3-ethylbenzothiazolin-6-sulfonic acid) to determine the content of total phenolic compounds, i.e. to evaluate the antioxidant effectiveness of the separate functionalisation bath, were used as the most important test methods.

Experimental

Materials

Viscose: In the course of the test, a nonwoven substrate of viscose in the form of a strap (manufacturer: Tosama d.o.o., surface mass 165 g/m^2, width 45 mm) produced by carding, laying and needling was used. Honey: 100% Slovenian chestnut honey. Preparation of chitosan solution: 0.5% solution of chitosan (Kitozyme, Belgium, molar mass- ~ 82.000, 22.4% acetylated) was prepared by suspending chitosan in ultra-pure Milli-Q water (pH 3.6, HCl). Preparation of the chitosan: honey solution: A mixture was prepared of 0.5% (w/v) chitosan solution and honey at a mass ratio of 1:3. Horsetail extract: 20 g of dried horsetail plant was poured into 500 ml of ultra-pure Milli-Q water, then treated for 4 h at 100 °C. Horsetail in combination with the chitosan: 0.5% (w/v) chitosan solution was prepared using the extract of horsetail instead of ultra-pure Milli-Q water to dissolve the chitosan (pH 3.6 (HCl). Preparation of chitosan:eugenol (clove oil) and chitosan: oleuropein (olive leaf) functionalisation bath: 200 ml of 0.5% (w/v) chitosan was prepared. To maintain a pH of about 3.6, i.e. to achieve complete dissolution of the chitosan, HCl was added dropwise to the solution. Then, a pH of 5.5 (4 N sodium hydroxide (NaOH)) was established

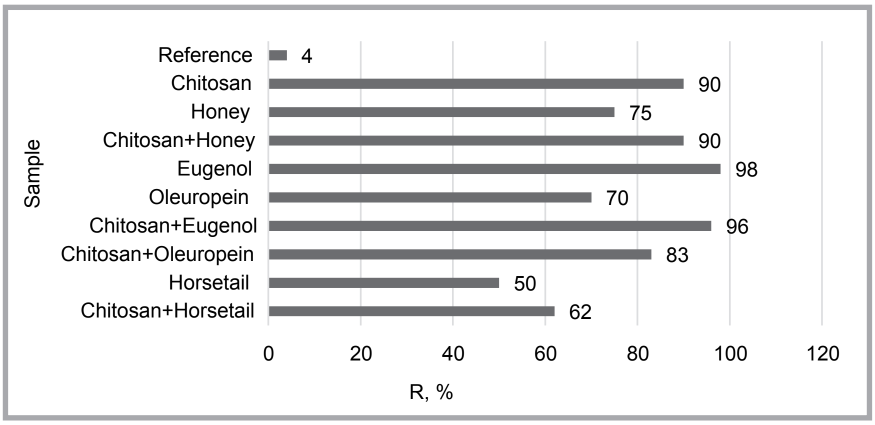

Figure 1. *Reduction (R, %) in Staphylococcus aureus as a representative of Gram-positive bacteria.*

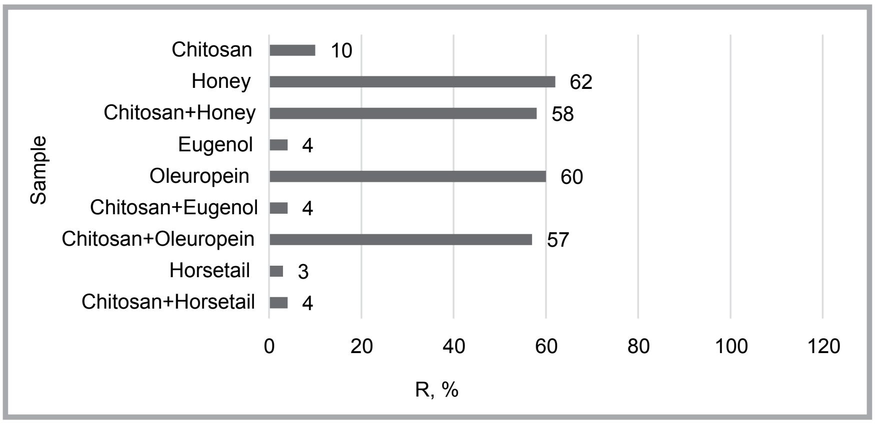

Figure 2. *Reduction (R, %) in Escherichia coli as a representative of Gram-negative bacteria.*

(this pH is required for successful enzyme coupling). This chitosan solution was transferred to a reaction flask, and 60 μL of 0.5 x 10^{-3} M of eugenol (Merck, Molecular weight (M) = 164.21 g/mol)/ oleuropein (Sigma Aldrich, Molecular weight (M) = 540.518 g/mol) was added. After 15 min, 500 mg of laccase enzyme (0.5 U/mg, Sigma-Aldrich) was added. With constant stirring and protection from light, the solution was treated with an air supply for 6 h. The reaction product was then precipitated in acetone, and the resulting precipitate was centrifuged for 10 min at 5.000 rpm. The supernatant was then removed and the precipitate washed several times with ethanol and finally with ultra-pure Milli-Q water. Viscose functionalisation: Viscose samples with dimensions of 100 mm (length) x 45 mm (width) were treated by impregnation (30 min, room temperature, wet pickup 80-100%; manufacturer: W. Mathis, Switzerland) and drying (T_{drying} = 30 °C, t_{drying} = 16 min; manufacturer: Kambič d.o.o, Slovenia). Due to the possible temperature sensitivity of the natural compound, a relatively low drying temperature was used; therefore, the samples were left overnight at room temperature to dry.

Analytical methods:

Antimicrobial activity

Microbiological testing (antimicrobial activity) was performed at the National Laboratory for Health, Environment and Food (NLZOH) in Maribor, Slovenia. The antimicrobial activity was tested for Gram-positive bacteria *Staphylococcus aureus* (*SA*) and for Gram-negative bacteria *Escherichia coli* (*E. coli*), respectively. Antimicrobial testing was performed according to Standard ASTM E 2149-01 (i.e. the dynamic-stress test). The effectiveness of antimicrobial functionalisation was evaluated based on the % of bacterial reduction R ***Equation (1)***.

$$R = (A - B)/A \times 100\% \qquad (1)$$

Where, R – bacterial reduction/%, A – the number of colonial units (CFU) after one minute of shaking (time 0), B – the number of colonial units (CFUs) after one hour of shaking (time 1 h).

Antioxidant activity

The antioxidant activity of the functionalised non-woven viscose was evaluated using the $ABTS^{\bullet+}$ method (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, Sigma Aldrich). The radical $ABTS^{\bullet+}$ occurs during the oxidation of ABTS and potassium persulfate absorption in the visible region at a wavelength of 734 nm. This is determined spectrophotometrically. Antioxidants are hydrogen donours; therefore a free radical is reduced in contact with potassium persulfate, which is visible as discolouration i.e. with a change in the solution absorbance ***Equation (2)***.

$$Inhibition = \frac{A_{starting} - A_{sample}}{A_{starting}} \cdot 100/\% \qquad (2)$$

Where, $A_{starting}$ – the absorbance measured at a starting concentration of $ABTS^{\bullet+}$, A_{sample} – the absorbance measured at the rest concentration of $ABTS^{\bullet+}$ [13].

Results and discussion

Antimicrobial activity

The antimicrobial activity of the viscose functionalised with different treatment baths (Chitosan, Honey, Chitosan + Honey, Eugenol, Oleuropein, Chitosan + Eugenol, Chitosan + Oleuropein, Horsetail, Horsetail + Chitosan) is seen in ***Figures 1*** and ***2***.

Although antimicrobial activity is attributed to all the compounds discussed, the results show considerable differences in their antimicrobial activity in terms of *Staphylococcus aureus* (*SA*) inhibition. Eugenol (clove oil) with 98% inhibition proved to be the most effective in inhibiting *SA*, followed by chitosan in combination with eugenol (96%), and chitosan itself (90%), as well as with chitosan in combination with honey (90%). Good inhibition of *SA* was also seen with viscose treated using chitosan in combination with oleuropein (83%). Viscose treated with honey (75%) and oleuropein (70%) also showed an exemplary level of *SA* inhibition, while in the case of horsetail in combination with chitosan (62%), a moderate *SA* inhibition ability was seen. Viscose treated with horsetail (50%) is considered as insufficient in *SA* inhibition; following Standard ASTM E 2149-01, inhibition was successful only if the reduction (R/%) was above 60%. Results show that the addition of chitosan to other natural compounds generally improved *SA* inhibition (***Figure 1***). The exception was viscose functionalisation using chitosan in combination with eugenol, since eugenol itself is more efficient in *SA* inhibition if compared to chitosan. Microbiological testing also showed a reduction in the selected pathogenic microorganisms in the case of the reference sample (non-functionalised viscose). The fact is that microorganisms could be adsorbed relatively easily into the non-woven substrate. Since the test method covers taking the sample from the solution, it is possible for the result to be influenced by the method of sampling (taking the sample near or away from the surface) [6].

Although the results of *SA* inhibition are, in many cases, 90% or more, this trend was not seen with *Escherichia coli* (*E. coli*) reduction, since the highest inhibition achieved was 62% (viscose functionalised with honey). Viscose functionalised with oleuropein (60%), chitosan in combination with honey (58%), and chitosan in combination with oleuropein (57%) showed approximately the same level of *E. coli* reduction. In all other cases, viscose treated with chitosan (10%), chitosan + eugenol (4%), eugenol (4%), chitosan + horsetail (4%) and horsetail (3%) was insufficient for the inhibition of *E. coli*. As already mentioned, following Standard ASTM E 2149-01, inhibition was successful only if the reduction (R/%) was above 60%, which means that, except for honey, with respect to *E. coli* inhibition, no compound proved to be successful enough. Results show that the relatively high inhibition of Gram-positive bacteria (*SA*) does not necessarily mean successful reduction of Gram-negative (*E. coli*) bacteria. A gram-negative bacteria cell membrane is thin, but difficult to penetrate. Because of this nearly "bulletproof" membrane, they are often resistant to antibiotics and other antibacterial interventions [14-15]. In all cases, the reduction in *SA* was much higher if compared to *E. coli*. As shown by the results, viscose functionalided by treatment baths that inhibit *SA* significantly was, in general, very poor at inhibiting *E. coli*. Compared to SA, chitosan-treated viscose shows 80% less inhibition of *E. coli*, honey-treated viscose 13%, chitosan in combination with honey-treated viscose 32%, eugenol-treated viscose 94%, oleuropein-treated viscose 10%, chitosan in combination with eugenol-treated viscose 92% and horsetail-treated viscose 47%. Viscose functionalised with honey showed exclusively sufficient inhibition of both *SA* and *E. coli*; all other functionalisation baths were not successful in inhibiting gram positive (*SA*) and gram-negative (*E. coli*) bacteria at the same level. As is shown by the results, there is no general rule, thus the inhibitory efficiency of viscose functionalised with antimicrobial compounds must be determined for each type of micro-organism and treatment bath separately. Only in this way would it be possible to conclude on the efficiency of the functionalisation bath used for viscose functionalisation as objectively as possible.

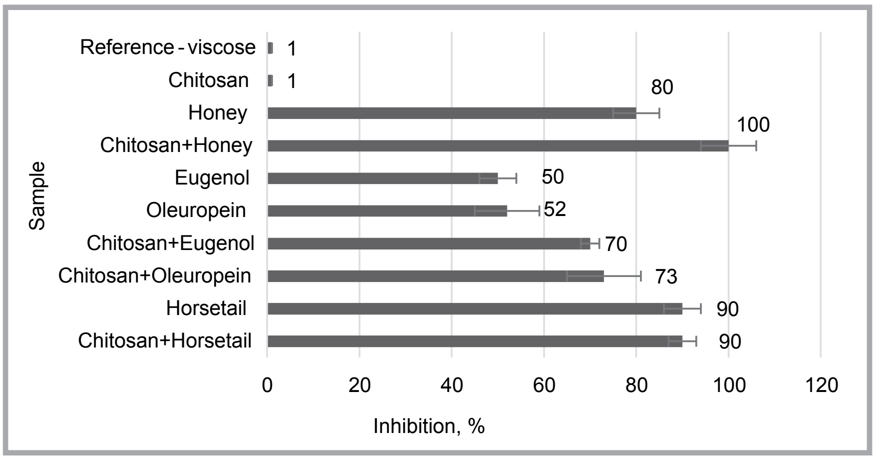

Figure 3. *Antioxidant activity of viscose functionalised with different treatment baths.*

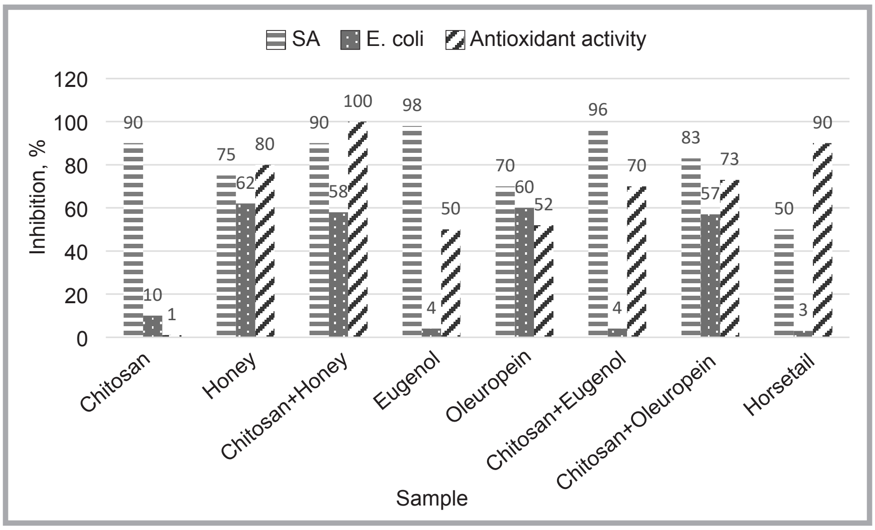

Figure 4. *Comparison of the results of antimicrobial: antioxidant activity.*

Results (average values of three replicates of measurement) of the antioxidant activity of viscose functionalised with different treatment baths (chitosan, honey, chitosan + honey, eugenol, oleuropein, chitosan + eugenol, chitosan + oleuropein, horsetail, horsetail + chitosan) are shown in ***Figure 3***. It can be seen that the reference (non-woven viscose) had no antioxidant activity; the same was valid for chitosan. Medical textile materials, however, are often expected to have both antimicrobial and antioxidant activity at the same time, which seems to be a lack of chitosan itself. The antioxidant effectiveness of viscose functionalised with the following treatment baths: chitosan + honey (100%), horsetail (90%), horsetail + chitosan (90%) and honey (80%), was at a very high level. These results are followed by viscose treated with chitosan + oleuropein (73%) and chitosan + eugenol (70%), and by viscose treated with oleuropein (52%) and with eugenol (50%). There is an interesting situation i.e. the addition of chitosan to individual natural compounds improved their antioxidant activity. Obviously, chitosan offers multiple binding sites, resulting in improved the antioxidant activity of viscose functionalised with the combination of chitosan and another natural compound. Therefore, to increase the antioxidant activity of a separate (already antioxidant-active) compound, the addition of chitosan to the individual functionalisation bath seems to be reasonable.

A comparison of the results of the antimicrobial: antioxidant activity of viscose functionalised with the individual treatment baths is shown in ***Figure 4***.

Comparison of the results (***Figure 4***) shows that of all the treatment baths used for viscose functionalisation, the most successful was the treatment bath where honey was part of it (combined with chitosan, or solely honey). Results show that in this case the inhibition of Gram-positive (*SA*) and Gram-negative bacteria (*E. coli*), as well as the antioxidant effectiveness of such functionalised viscose, was at a very high level. In all other cases, this trend was not seen; the individual functionality was emphasised, which did not cover the complete inhibition of both Gram-positive and Gram-negative bacteria supported by good antioxidant activity at the same time.

Therefore, for a comprehensive insight into the antimicrobial and antioxidant activity of functionalised viscose, a very detailed study of the results of antimicrobial and antioxidant testing is needed in order for it to be possible to create a textile material with the necessary functionality. It is seen from the results that there is no general rule that natural compounds that are otherwise antimicrobial and antioxidant active are also effective in a way that medical textiles containing them will meet harsh conditions with excellent antimicrobial/antioxidative activity at the same time. As already mentioned, the actual antimicrobial and antioxidant efficacy of the compounds after being applied onto the desired substrate should be validated carefully for each individual case separately. Only in this way would the final product meet expectations regarding its effectiveness in the field of sanitation/medicine, where microbiological and antioxidative properties play an important role.

Conclusions

Although antimicrobial activity is attributed to many natural compounds, the results of viscose functionalisation using chitosan, honey, chitosan + honey, eugenol, oleuropein, chitosan + eugenol, chitosan + oleuropein, horsetail, and horsetail + chitosan treatment baths show considerable differences in their antimicrobial activity in terms of Gram-positive (*SA*) and Gram-negative (*E. coli*) bacteria reduction, as well as antioxidant efficiency. Eugenol proved to be the most effective in inhibiting *SA*. The addition of chitosan to other natural compounds generally improved *SA* inhibition, which in many cases was 90% or more; however, this trend was not seen with *E. coli* reduction, since the highest inhibition achieved was 62%. Among all the treatment baths used for viscose functionalisation, the most successful was the bath where honey was part of it.

The combination of chitosan with other natural compounds to achieve a simultaneous antimicrobial-antioxidant system of textile materials for medical use seems reasonable, since chitosan is not antioxidant-active. To increase the antioxidant activity of a separate (already antioxidant-active) compound, the addition of chitosan is needed for the individual functionalisation bath. Obviously, chitosan offers certain binding sites to the natural compounds, hence the antioxidant efficiency was greatly improved.

For a comprehensive insight into the antimicrobial and antioxidant activity of functionalised viscose intended for sanitary/medical use, a very detailed study of the compounds used and their influence on separate types of bacteria is needed, in order to create a textile material with as much antimicrobial activity as possible.

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Received 21.02.2020 Reviewed 30.04.2020