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Antibacterial activity and cellulose acetate electrophoresis in monitoring collagen hydrogels modified with saccharides

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Antimicrobial compounds of plant origin (for example fucoidan, lignans, β-glucans, polyphenols), occurring in roots, leaves, flowers and fruits of plants, have demonstrated antitumor antioxidant, antibacterial or antifungal activities. The samples of collagen hydrogels or saccharide incorporated collagen hydrogels (for example, fucoidan from the bladderwrack or β-glucans from the oatmeal fibre) were examined. They were exposed to bacteria that can cause nosocomial infections, that is the Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. In electrophoretic analysis, the samples of oat, oatmeal, furoxin (dietary supplement), cranberry juice, reference β-glucan, baker's yeast, and the dried algae from *Fucus vesiculosus* L. were hydrolysed and monosaccharide derivatives were subjected to electrophoresis on a strip of cellulose acetate membrane. Good bacteriostatic properties were determined for samples of partially hydrolysed fucoidan against the pathogenic bacteria *E. coli* and *S. aureus* as well as *Candida albicans*. It was observed that partially hydrolysed fucoidan incorporated into collagen films can be used as therapeutically active biomaterials that speed up the process of wound healing and may increase the anticancer activity of fucoidan. The microbiological procedure of analysing hydrogels can serve as a kind of monitoring to find their antibacterial properties. Cellulose acetate electrophoresis is a useful method of analysing saccharide hydrolysates, solutions of collagen modifier.

Key words: Antimicrobial resistance, carbohydrates, glucan, collagen.

INTRODUCTION

Several traditional plant extracts have historically been known to have antimicrobial activity, to-date there has been relatively little reports examining the activity against several medically important bacterial and fungal pathogens. Plant-derived phytoalexin, isothiocynates, allicins, anthocyanins and essentials oils (Alzoreky and Nakahara, 2003; Smith-Palmeret al., 1998; Dorman and Deans, 2000), tannins, polyphenols (Caanadanovic-Brunet et al., 2008) and terpenoids have demonstrated antibacterial or antifungal activities. They are all useful in treating some forms of cancer and have styptic and antibacterial properties that can assist wound healing

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(Borchardt and Wyse, 2008). The *in vitro* antimicrobial activity of the marine green algae *Ulva lactuca* and brown seaweed *Phaeophyceae* were examined against Gram-positive bacteria, Gram-negative bacteria and a fungus. This result confirms the potential use of seaweed extracts as a source of antibacterial compounds and a health-promoting food (Chotigeat et al., 2004; Kim et al., 2007; Sandsdalenet et al., 2003). Cranberries are also distinguished for their antimicrobial properties. They are a particularly rich source of phenolic phytochemicals, including phenolic acids (benzoic, hydroxycinnamic and ellagic acids) and flavonoids (anthocyanins, flavonols and flavan-3-ols) (McKay and Blumberg, 2007). Cranberries are distinguished by a wide spectrum of bioactive substances. American cranberries contain ascorbic acid, phenolic compounds, titratable acids and sugars (Povilaityté et al., 1998; Úwieczkowska et al., 2004).

Polysaccharides may be a useful material as a skin scaffold with the capability of delivering molecules involved in wound healing; some polysaccharides even improve wound healing (Paulsen, 2002; Yang et al., 2009). It has been found that polysaccharides, crude or purified, are strong scavengers of free radicals as well as inhibitors of liposome peroxidation. Some of them exhibit various biological activities such as anti-inflammatory, anticancer, antiviral, spasmolytic, antibacterial, anti-oxidant or immunostimulatory (Paulsen, 2002; Yang et al., 2009). A number of studies have concentrated on the anticancer activity of fucoidan polymers (Yang et al., 2009). Until fairly recently, it was believed that only the low molecular weight compounds in plants could be of importance in pharmacy and medicine. Fucoidan and especially, partially hydrolysed fucoidan isolated from different species have been extensively studied on account of their varied biological properties, including anticoagulant and antitumor effects. It was especially thought that the polysaccharides might play a role in wound healing, both internally and externally, and also that they could play a role against inflammation.

Mostly natural polysaccharides have been investigated as biomaterials, such as chitosan, cellulose, collagen and hyaluronic acid. The anticancer properties of the lignans are well documented. Beta-glucan is a major component of the water-soluble dietary fibre of oats and barley. A glucan of cellular origin (Ishurd and Kennedy, 2005) has been isolated from Libyan dates (*Phoenix dactylifera* L). Glucans have been found to exhibit potent antitumor activity that could be correlated to their (1-3)-β-D-glucan linkages (Olafsdottir et al., 1999).

Natural polymers like collagen possess good cytocompatibility which makes them a popular choice for tissue engineering scaffolding applications. Scaffolding materials for cell proliferation and differentiation is one of the key technologies for tissue engineering. Three-dimensional engineered collagen matrixes are fabricated with or without peptide modification and chemical cross-linking with glutaraldehyde mimicking *in vivo* like conditions (Hosseinkhani et al., 2013a; Hosseinkhani et al., 2013b). A collagen sponge and a 3D hybrid scaffold (Hosseinkhani et al., 2006a) are highly porous with interconnecting openings, good for cell infiltration and for supplying oxygen and nutrients to cells. However, its usage has been limited by poor mechanical properties. To improve these, a novel porous scaffold for bone tissue engineering was prepared with collagen sponge reinforced by polypropylene/polyethylene terephthalate (PP/PET) fibres (Mohajeri et al., 2010).

Incorporation of PP/PET fibres not only improves the mechanical properties of collagen sponge, but also enables mesenchymal stem cells to positively improve their proliferation and differentiation. To improve the mechanical properties of collagen, it is also necessary to fabricate composites of collagen with electrospun poly (glycolic acid) PGA fibres (Hosseinkhani et al., 2010a). PGA/collagen nanofibres fabricated through electro-spinning significantly enhance cell adhesion compared with PGA/collagen microfibres (Hosseinkhani et al., 2010a). The incorporation of nanoparticles into nanofibre sheets is a very promising strategy to genetically engineer mesenchymal stem cells, and it can be used for further applications in regenerative medicine therapy (Hosseinkhani et al., 2012, 2005).

Collagen sponge reinforced by incorporation of poly(glycolic acid) (PGA) fibres was selected as the cell scaffold and was used to evaluate effect of the 3D culture system and cell scaffold type on the transfection efficiency of DNA nanoparticles on mesenchymal stem cells (MSC). Positively charged DNA nanoparticles can interact electrostatically with the cell membrane for internalization (Hosseinkhani et al., 2006b, 2010b). These findings provide an attractive combined strategy of tissue engineering principles with gene therapy for tissue regeneration. With their ability to serve as gene carriers, the nanoparticles are poised to play an important role in the field of regenerative medicine. Collagen also has plenty of applications in the field of wound healing due to its biodegradability and biocompatibility. Implanted collagen is degraded through native enzymatic pathways without any toxic response. There is a growing interest in collagen as a drug delivery vehicle. It seems that the near feature may be a renaissance period for collagen, an excellent natural, fully biocompatible and biodegradable drug carrier material. These technological possibilities open a new perspective, especially in creation of product utilizing antibacterial drug for either prophylactic or therapeutic use (Ruszczak and Friess, 2003).

The aim of this research is to find biologically active, exhibiting antibacterial activities, forms of polysaccharides such as fucoidan from the bladderwrack and β-glucans from the oatmeal fibre. Finally, the goal is to obtain hydrogels that can be used as biologically and therapeutically active biomaterials.

MATERIALS AND METHODS

Reagents

The following materials and reagents were used in the examination: reference crude fucoidan from *F. vesiculosus* L (CAS Number 9072-19-9, Sigma–Aldrich, Poland); Flos algae from *F. vesiculosus* L. packed and distributed by Zakład Zielarski, Piotrków Trybunalski, Poland; Furoxin dietary supplement produced by pharmaceutical company LEK-AM, Zakroczyn, Poland; reference β-glucan produced by Walmark, a.s. Oldrichovice, Třinec, Czech Republic; oat, oatmeal and cranberry juice.

Collagen film formation

Fresh carp fish scales were collected, washed thoroughly and stored at -25°C until used. First, they were washed twice in 10 wt% of NaCl solutions to remove unnecessary proteins on the surface by stirring the solution for 24 h. Demineralization was achieved with 0.4 M HCl solution (dry scales: solution = 1:15) for 90 min. The demineralized scales were then washed three times with distilled water for collagen extraction. All the preparative procedures were performed at 4°C. Carp fish scales were extracted with 0.5 M acetic acid for two days, and the extracts were centrifuged for 30 min. The residues were re-extracted with the same solution for one day and these extracts were centrifuged under the same conditions. The supernatants were combined and salted out by adding NaCl to a final concentration of 0.7 M. The precipitated collagens were separated by centrifugation for 30 min and redissolved in 0.5 M acetic acid to precipitate with NaCl again. The samples (0.5 g) of oat, oatmeal, cranberry juice and the dried algae from *F. vesiculosus* L. were extracted with 0.1 M H_2SO_4 and maintained at 80°C or in boiling water with constant mechanical stirring for 30 min. The extract was centrifuged for 30 min and the supernatant was collected. All reagents used were of analytical grade. The samples of collagen hydrogels or saccharide incorporated collagen hydrogels (for example, fucoidan from the bladderwrack and β-glucans from the oatmeal fibre) were examined.

Agar dilution method

They were exposed to bacteria that can cause nosocomial infections, that is the Gram-positive *S. aureus* and Gram-negative *E. coli*. Physiological salt (2 cm^3) was poured into two sterile test tubes. Using a sterile (red hot) inoculation loop, *E. coli* sample was taken from its culture on enriched agar (used for growing particularly demanding bacteria strains), inserted into one of the test tubes and diluted in the salt. Using a pipette, 3 drops of the suspension were transferred onto enriched agar; then, using a cooled sterile bacteria spreader, they were spread all over the agar surface. After that, the spreader was sterilized again and 3 drops of collagen hydrogels or saccharide incorporated collagen hydrogels were placed in the centre of the Petri plate, using a dropper. The same procedure was repeated for *S. aureus*, which was placed on mannitol salt agar (containing 7.5% NaCl for inhibiting the growth of other bacteria). The Petri plates were subsequently placed in a tube and then kept in a laboratory heater at 37°C for 24 and 48 h. Collagen hydrogels or saccharide incorporated collagen hydrogels were exposed to the same bacteria: *E. coli* on MacConkey agar (containing salts of bile acids and crystal violet inhibiting the growth of Gram-positive bacteria) and *S. aureus* on mannitol salt agar (with high concentration of NaCl inhibiting the growth of other bacteria). The samples were kept in a laboratory heater at 37°C for 24 h, and then photos were taken (Tables 1 to 3 and Figure 1).

Partially hydrolysed fucoidan acid extracts produced from the common bladder wrack were examined (Figures 2 to 4): collagen hydrogels-pure collagen (PC); partially hydrolysed fucoidan (from algae) incorporated into collagen films; 15 m incubation (H1s), 30 min incubation (H2s). They were exposed to bacteria that can cause nosocomial infections, that is the Grampositive *S. aureus* and Gram-negative *E. coli* and *C. albicans*. Chosen species of microorganisms, *E. coli, S. aureus* and *C. albicans*, were being bred on Mac Conkey, Chapman and Candida agars. Grown cultures were washed out with 1 ml of physiological salt solution, and added to the sterile broth nutrient sample. This was sterilized for 30 min in an autoclave at a temperature of 121°C, and a pressure of 150 kPa. The samples were cultivated in a thermostat at 37°C and tested for antibacterial activity against the sensitive strain of *S. aureus, E. coli* and *C. albicans* at one-day intervals up to 1 month (Figures 2 to 4).

Statistical analysis

The results presented here were performed in 10 stages, and arithmetic average and standard deviation were carried out. Standard deviation was determined according to estimator of highest credibility in STATISTICA 6.0.

Electrophoretic analysis

In electrophoretic analysis, the samples (0.2 g) of oat, oatmeal, furoxin dietary supplement, cranberry juice, reference β-glucan, baker's yeast and the dried algae from *F. vesiculosus* L. were hydrolysed into component monosaccharides with 80% H_2SO_4 at 0°C for 24 h, and monosaccharide derivatives were subjected to electrophoresis on a strip of cellulose acetate membrane. The samples (1 µl non-precipitated extract) and references were subjected to electrophoresis on a strip of cellulose acetate membrane (CA-SYS-MINI Cellulose Acetate Systems) in 0.2 M $Ca(OAc)_2$ (pH 7.5) at 7 mA, max. 240 V for 1.5 h. The strips were stained with 0.5% toluidine blue in 3% HOAc solution and then rinsed in distilled water and air-dried. Semi-quantitative analysis of monosaccharides content in the samples was also conducted using GelScan v.1.45 software (Kucharczyk T.E., Poland) (Figures 5 to 7).

RESULTS

Partially hydrolysed fucoidan incorporated into collagen films can be used as therapeutically active biomaterials that speed up the process of wound healing and may increase the anticancer activity of fucoidan. This thesis, present in many ongoing studies worldwide, was the basis of this investigation.

Three series of semi-quantitative studies of hydrogels placed directly on agars or on paper discs are discussed here (Tables 1 to 3). Antibacterial properties of the modified hydrogels were compared with a reference sample of hydrogels. For collagen gels modified with bladderwrack (*F. vesiculosus* L.) extracts in 0.1 and 0.01 m H_2SO_4, in distilled water and in 0.1 m NaOH, the inhibition zones are within 6 to 10 mm against Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria (Table 1). In the investigated group of gels, the best bactericidal properties, with a 10 mm inhibition zone, can be found in collagen gels

Table 1. Antimicrobial activity caused by flos extract (water or acid fraction) through agar diffusion method.

Parameter	Microorganisms	
	Escherichia coli	***Staphylococcus aureus***
Sample 1: Collagen hydrogels in 0.1 m NaOH	-	-
Sample 2: Collagen hydrogels in 0.1 m H_2SO_4	-	+
Sample 3: Flos incorporated collagen hydrogels in 0.1 m H_2SO_4	+	+
Sample 4: Flos incorporated collagen hydrogels in 0.01 m H_2SO_4	+	+
Sample 5: Flos incorporated collagen hydrogels in H_2O	+	+
Sample 6: Flos incorporated collagen hydrogels in 0.1 m NaOH	+	+

(+) susceptibility (inhibition zone > 6 mm), (-) absence of susceptibility.

Table 2. Antimicrobial activity caused by plant extracts: Fucoidan and Furoxin (water or acid fraction) through agar diffusion method.

Parameter	Microorganism	
	Escherichia coli	***Staphylococcus aureus***
Sample 1: Collagen hydrogels in 0.1m H_2SO_4	-	-
Sample 2: Fucoidan incorporated collagen hydrogels in H_2O	-	+
Sample 3: Fucoidan incorporated collagen hydrogels in 0.1m H_2SO_4	+	+
Sample 4: Fucoidan incorporated collagen hydrogels in 0.01m NaOH	+	+
Sample 5: Furoxin incorporated collagen hydrogels in 0.1m H_2SO_4	+	+

(+) susceptibility (inhibition zone > 3 mm), (-) absence of susceptibility.

Table 3. Antimicrobial activity caused by plant extracts: β-glucan and cranberry juice (water or acid fraction) through agar diffusion method.

Parameter	Microorganism	
	Escherichia coli	***Staphylococcus aureus***
Sample 1: Collagen hydrogels in H_2O	+	+
Sample 2: Reference β-glucan incorporated collagen hydrogels in H_2O	-	+
Sample 3: β-glucan (from oat) incorporated collagen hydrogels in H_2O	+	+
Sample 4: β-glucan (from oat) incorporated collagen hydrogels in 0.1 m H_2SO_4	+	+
Sample 5: Cranberry juice incorporated collagen hydrogels in H_2O	-	+
Sample 6: β-glucan (from oatmeal) incorporated collagen hydrogels in 0.1 m H_2SO_4	+	+

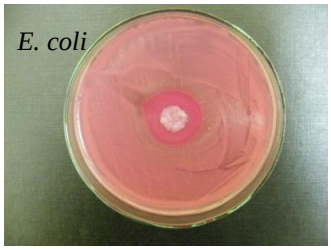
(+) susceptibility (inhibition zone > 2mm), (-) absence of susceptibility

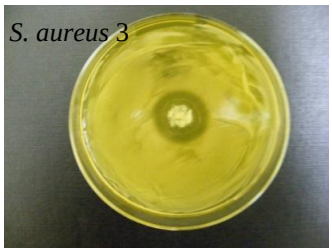
modified with bladderwrack extracts in 0.1 and 0.01 m sulfuric acid (VI).

Do the antibacterial properties of the hydrogels obtained depend on the type of modifier? To answer this question, the properties of gels obtained using *F. vesiculosus* L. extracts (Table 1) were compared with those obtained using reference fucoidan extracts (Table 2). When analysing the results presented in Tables 1 and 2, it should be said that fucoidan solutions as the modifier of collagen hydrogels are responsible for the bactericidal properties of these gels. Examples of increasing inhibition zones are shown in Figure 1, while Tables 1 and 2 provide complete information on the antimicrobial properties of the modified hydrogels against Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria.

Do the antibacterial properties of the hydrogels obtained depend on the type of modifier? To verify this, the properties of gels obtained using extracts of oats and oatmeal as the source of β-glucan were compared with those obtained using β-glucan extracts from a dietary supplement (Table 3) as well as using cranberry extracts from Furoxin dietary supplement (Table 2) and cranberry juice (Table 3). For collagen gels modified with β-glucan extracts in 0.1 m H_2SO_4 and in distilled water, the inhibition zones are within 1 to 3 mm against Gram- negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria (Table 1).

Sample 3. Flos incorporated collagen hydrogels in 0.1m H_2SO_4





Sample 3. Fucoidan incorporated collagen hydrogels in 0.1m H_2SO_4

Figure 1. Antimicrobial activity caused by flos and fucoidan extracts (acid fraction) through agar diffusion method.

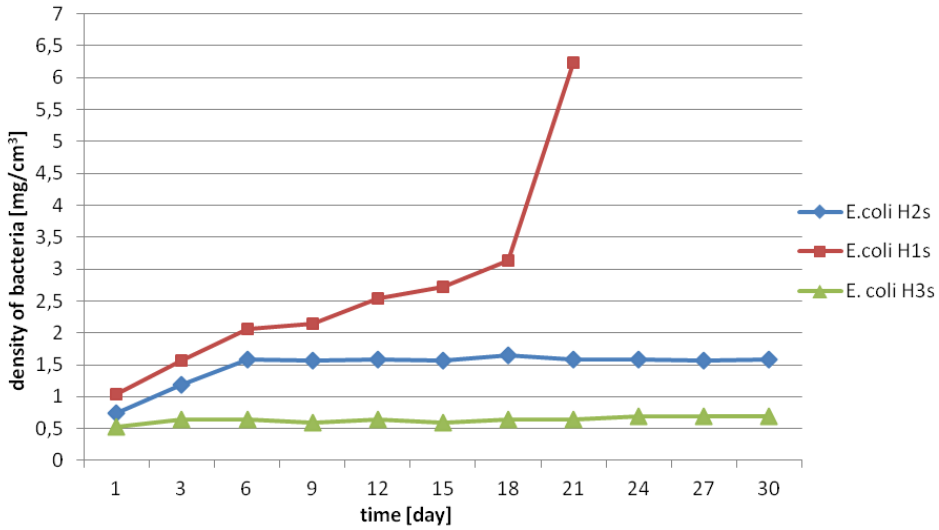


Figure 2. Change of density *E. coli* biomass in time.

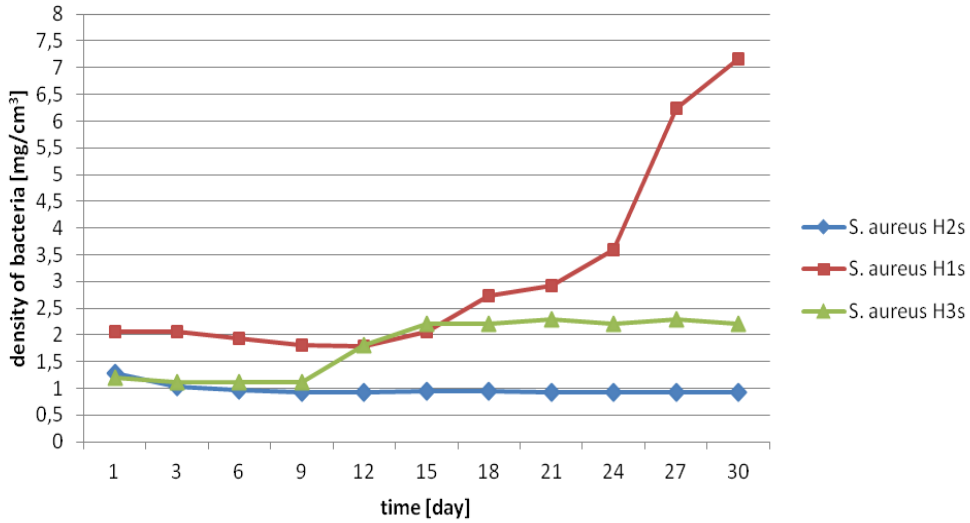


Figure 3. Change of density *Staphylococcus aureus* biomass in time.

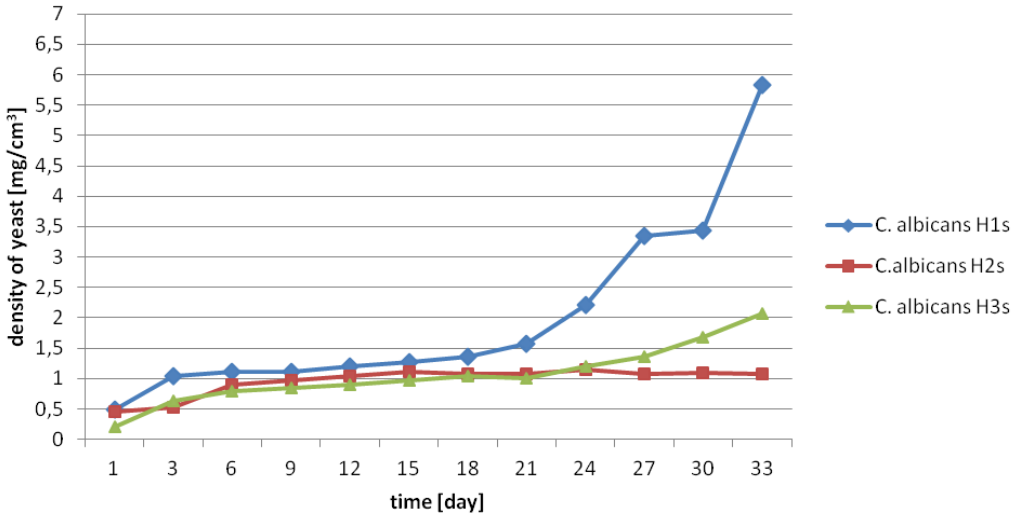


Figure 4. Change of density *Candida albicans* in time.

It should be noted that in this study of β-glucan extracts the method of placing diluted hydrogels on paper discs was adopted. Also in this case, the gels reveal good antibacterial properties (Table 3).

A variable sensitivity of *S. aureus*, *Enterococcus faecalis* and *Micrococcus luteus* to proanthocyanidin-rich fractions was also observed (Leitão et al., 2005). It was found that the extracts from common Finnish berries (blueberry, raspberry, lingonberry, blackcurrant, cloudberry, cranberry, sea buckthorn berry and strawberry) inhibited the growth of Gram-negative bacteria, while Gram-positive bacteria were quite resistant (Puupponen-Pimiä et al., 2001). However, other researchers reported that there was no correlation between Gram-positive or Gram-negative bacterial status and their sensitivity to the raspberry, blackberry, cranberry and black currant fruits (Cavanagh et al., 2003). For collagen gels modified with cranberry extracts in 0.1 m H_2SO_4 and in distilled water, the inhibition zone is 5 mm against Gram-negative (*E. coli*) bacteria (Table 2; Furoxin) while for collagen gels modified with cranberry juice extracts, the inhibition zone is 3 mm against Gram-positive (*S. aureus*) bacteria (Table 3; cranberry juice; on paper discs). Generally, the antibacterial activity of all samples is better against *S. aureus* than against *E. coli*.

As stated earlier, the object of this study was partially hydrolysed fucoidan incorporated into collagen films that can be used as biologically active biomaterials. Partially hydrolysed fucoidan incorporated into collagen films was investigated in wound dressings against the pathogenic bacteria *E. coli* and *S. aureus* and also *Candida albicans*. Figures 2 to 4 show partially hydrolysed fucoidan acting against tested bacteria and yeast. This antibacterial and fungicidal activity depends on the conditions of the hydrolysis. Microbiological analysis revealed, in particular, density changes of the suspensions of *E. coli*, *S. aureus* and *C. albicans* (Figures 2 to 4) in contact with partially hydrolysed fucoidan (H2s and H3s) obtained by 30- or 60 min hydrolysis of 0.1 m H_2SO_4. H2s and H3s samples were demonstrated to exhibit better bacterio-static properties compared with the partially hydrolysed fucoidan (H1s) obtained by 15 min hydrolysis of 0.1 m H_2SO_4. For H1s samples, increased density of bacteria and yeast was found, starting on the 18th day of incubation, while samples H2s and H3s essentially retained constant density of *E. coli* and *S. aureus.*

The microbiological procedure of analysing hydrogels, as discussed above, can serve as a kind of monitoring to find their antibacterial properties. Key to this study is also selecting a therapeutic concentration of the modifier solution, promoting antibacterial properties of hydrogels. In this investigation, cellulose acetate electrophoresis is a useful method of analysing saccharide hydrolysates, solutions of collagen modifier. In electrophoretic analysis, a polysaccharide is hydrolysed into component monosaccharides with 80% H_2SO_4 at 0°C for 24 h, and monosaccharide derivatives are subjected to electrophoresis on a strip of cellulose acetate membrane. Representative examples of this analysis are presented in Figures 5 and 6.

Based on the analysis of these electrophoregrams, it can be said that CAE is a convenient analytic medium for monitoring the presence of saccharides in samples of herbs, algae, food products and dietary supplements. In

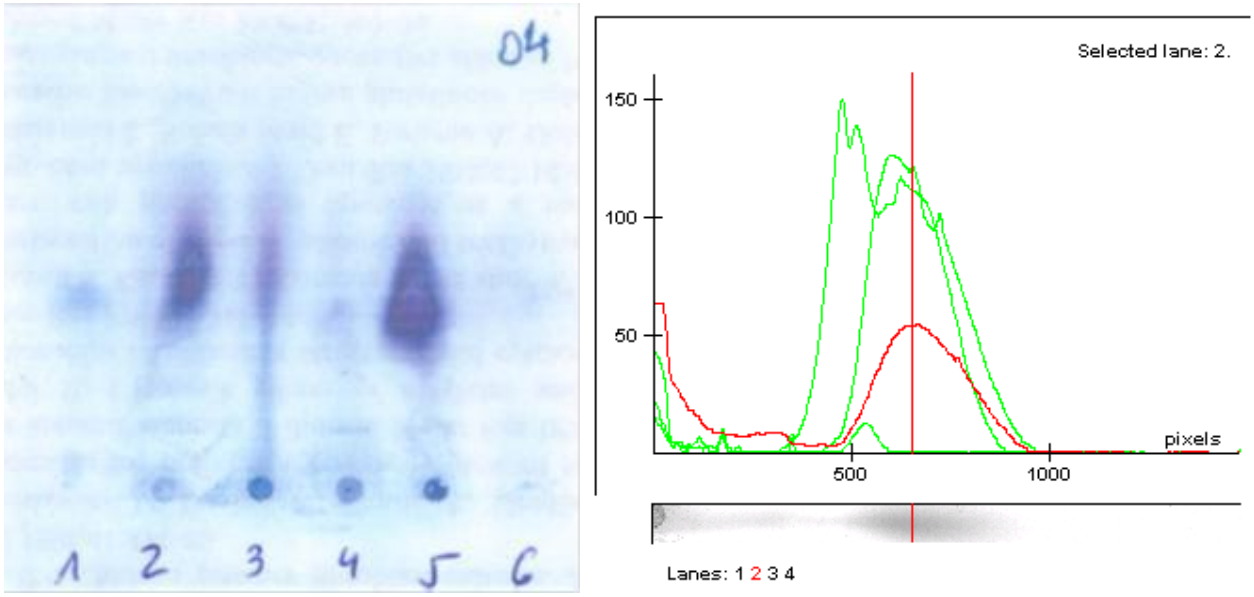


Figure 5. Cellulose acetate membrane electrophoresis and semi-quantitative analysis of hydrolysates (0.2 g of substance, 1 ml 80% H_2SO_4): from left to right: oatmeal, oat, baker's yeast, reference β - glucan.

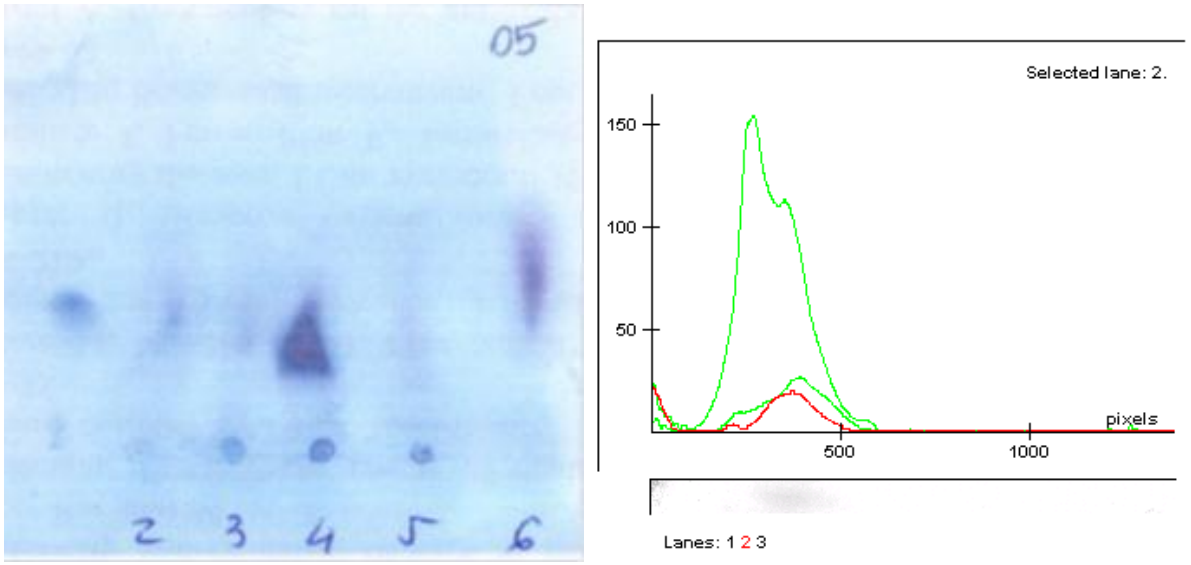


Figure 6. Cellulose acetate membrane electrophoresis and semi-quantitative analysis of hydrolysates (0.2 g of substance, 1 ml 80% H_2SO_4): from left to right: oatmeal, oat, reference β – glucan, baker's yeast.

the electrophoretic tests shown in Figure 7, the same concentrations of modifiers were selected as for the microbiological tests (that is, extraction at 80°C for 30 min in an aqueous medium or in 0.1 m H_2SO_4). CAE was performed on hydrolysate solutions in 80% H_2SO_4. Two distinct bands were observed: from an extract of *F. vesiculosus* L. (fucoidan) and from an extract of Furoxin, a dietary supplement (source of phenolic phytochemicals). No electrophoretic bands were found for the extracts of β-glucan, cranberry juice and oatmeal (Figure 7). The identification of electrophoretic bands for fucoidan and Furoxin can be explained by a weaker binding of sugars with the cell wall in herbs and algae, while the extraction and release of β-glucans is more

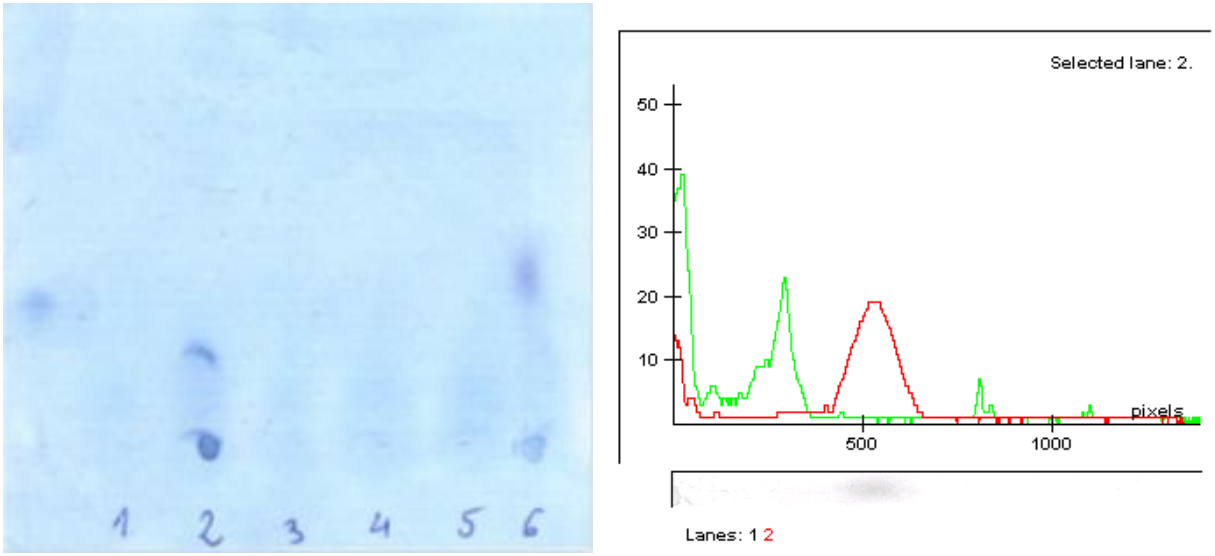


Figure 7. Cellulose acetate membrane electrophoresis and semi-quantitative analysis of hydrolysates : from left to right: reference β – glucan, furoxin, cranberry juice, oatmeal, *F. vesiculosus* L.

complex and requires a more aggressive environment.

DISCUSSION

The aim of this research is to find biologically active and therapeutically desirable chemical forms of polysaccharides such as fucoidan from the bladderwrack and β-glucans from the oatmeal fibre. The aim is also to develop a series of solutions and gels exhibiting anti-bacterial and potentially anticancer activities. Finally, the goal is to obtain hydrogels that can be used as biologically and therapeutically active biomaterials. For marking fucoidan in the bladderwrack and monosaccha-rides herbs and the fibre from oat, CAE electrophoresis has been selected with a system of computer image ana-lysis. Research will be complemented by microbiological analyses to prove the influence of hydrolysates and gels on pathogenic Gram-negative bacteria *E. coli* and Gram-positive *S. aures*.

As stated earlier, this study concentrated on partially hydrolysed fucoidan incorporated into collagen films that can be used as biologically active biomaterials. The reason for this choice is as follows: Fucoidans isolated from different species have been extensively studied because of their varied biological properties, including anticoagulant and antitumor effects. Their anticancer activity can be significantly enhanced by lowering their molecular weight only when they are depolymerized under mild conditions. Many modern wound dressings have a variety of properties that are designed to create an environment to encourage conditions that support wound healing.

Since bacteria are often present in high numbers in wound fluid, it is also important that dressings with high fluid retention levels be able to absorb and retain bacteria. Once the skin barrier is broken, there is a much greater risk for infection as the majority of wounds provide a favourable environment for both aerobic and anaerobic bacteria. In a bacteria-free wound, infection obviously cannot occur. The prevention of wound infection and a reduction in cross-infection of wound pathogens are primary concerns in infection control. It is crucial to investigate the possibility of producing antimicrobial films or biomaterials for modern wound dressings by incorporation of $AgNO_3$ and H_4SiO_4 or fucoidan and partially hydrolysed fucoidan (Pielesz et al., 2011b).

Do the antibacterial properties of the hydrogels obtained depend on the type of modifier? Modifying collagen with polysaccharide solutions was meant to obtain not only an antibacterial dressing, but one that would also reveal antitumor activity. Until fairly recently, it was believed that only the low molecular weight compounds in plants (Yang et al., 2008) could be of importance in pharmacy and medicine. Fucoidan and especially, partially hydrolysed fucoidan isolated from different species have been extensively studied on account of their varied biological properties, including anticoagulant and antitumor effects. The objective was to analyse, as reported in the literature, the anticancer activity of partially hydrolysed fucoidan polymers (Koyanagi et al., 2003; Matou et al., 2002; Yang et al., 2008; Zemaniet al., 2005) and to investigate the effects of molecular weights, also known from the literature (Matou

et al., 2002; Zemani al., 2005) and different hydrolysis conditions on potential inhibition of cancer-cell growth. This study continues earlier analyses of structural changes in fucoidan from *F. vesiculosus* L., examined by means of cellulose acetate membrane electrophoresis and Fourier transform infrared spectroscopy spectroscopy (Pielesz et al., 2011a).

In the investigated group of gels, the best bactericidal properties, with a 10 mm inhibition zone, can be found in collagen gels modified with bladderwrack extracts in 0.1 and 0.01 m sulfuric acid (VI). Without any doubt, methodology based on mild acid hydrolysis can be used as an efficient tool for studying the relationship of biological activities of partially hydrolysed fucoidan. In an earlier study (Pielesz, 2011a), it was determined that the formation of well-defined molecular-size oligosaccharides is notably dependent on the particular structure of the sulfated polysaccharides. Therefore, the specific cleavage of the polysaccharides by mild acid hydrolysis was influenced specifically by their pattern of sulfation. The band shifts observed in electrophoretograms and Raman wavenumber shifts (Pielesz et al., 2011a) may indicate increased anticancer activity of fucoidan, which remains to be determined in subsequent investigations. This study confirms the suggestions made in (Pielesz et al., 2011a).

Generally, the antibacterial activity of all the samples is better against *S. aureus* than against *E. coli*. This contrasting response towards the Gram-positive and Gram-negative bacteria could be attributed to the morphological differences between these bacteria. Indeed, the structure of the cell wall of the Gram-negative strain (*E. coli*) is much more complicated than that of the Gram-positive strain (*S. aureus*) because there is another layer outside the pepetidoglycan layer, called “the outer membrane,” which is mainly constructed from tightly packed lipopolysaccharide molecules, resulting in the effective resistive barrier against foreign compounds attack (Liu et al., 2006).

Conclusions

The microbiological procedure of analysing hydrogels can serve as a kind of monitoring to find their antibacterial properties. There is no doubt that methodology based on acid hydrolysis can be used as an efficient tool for studying monosaccharides and their biological activities. The advantage of this study was that a simple, repeatable analytical procedure was developed, using modern but inexpensive apparatus such as cellulose acetate membrane electrophoresis.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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