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Kutsyk Roman Volodymyrovych
Doctor of Medicine, Professor,
head of a Chair of Microbiology,
Virology and Immunology,
Ivano-Frankivsk national
medical university, Ivano-
Frankivsk, Ukraine.

Kosenko Svitlana Valentynivna
Associate Professor of
Stomatology Department at the
Institute of Advanced Studies
(Professor Rozhko M.M. is head
of a chair), Ivano-Frankivsk
National Medical University,
Ivano-Frankivsk, Ukraine.

Haioshko Olena Bohdanivna
postgraduate Student of
Stomatology Department at the
Institute of Advanced Studies
(Professor Rozhko M.M. is head
of a chair), Ivano-Frankivsk
National Medical University,
Ivano-Frankivsk, Ukraine.

Correspondence

Haioshko Olena Bohdanivna
Postgraduate Student of
Stomatology Department at the
Institute of Advanced Studies
(Professor Rozhko M.M. is head
of a chair), Ivano-Frankivsk
National Medical University,
Ivano-Frankivsk, Ukraine.

Pilot research of antimicrobial characteristics of pectin-containing compositions for healing wounds after teeth extraction

Kutsyk Roman Volodymyrovych, Kosenko Svitlana Valentynivna, Haioshko Olena Bohdanivna

Abstract

To tackle the problem of keeping a look-out for the patients who are in need of teeth extracting it is important to start with the doctor's decision-making about it. It is a vital issue in Ukraine as it includes a lot of population. In our country the problem is not tackled positively because the predominant majority of scientists and doctors concentrate on the very procedure of extracting and medicine application after operating the patients on. There are the issues of medicine staying in the specific medium of oral cavity. The preliminary preoperative period of teeth extraction with following infection of oral cavity is out of note. The antiseptic medicines used five years ago considerably have reduced for different reasons [3]. At present it could be held only the preparation for the extracting of peccant teeth at the best but the medicines have been withdrawn from the sale. That is why considering the needs of the society it is still topical to develop the antimicrobial means for oral cavity to provide the market to the full extent with the necessary antiseptic and hygienic – prophylactic medicines for the doctors and patients to use aiming at the hygiene of oral cavity and preventing from the complications in postoperational period. The given article is sequels of investigation series of development the industrial samples of antimicrobial means without antiseptic medicines and antibiotics. It has been developed the stomatological bandage with prolonged staying in the extracted alveolar socket. The base of medicines being under development is apple pectin. The pectin substances are widely used in pharmacology, cosmetology and food industry. They have their own sense in every scientific branch being natural, non-toxic, high-molecular anion polysaccharides [3]. It should be noted that apple pectin is the only one in Ukraine validated as an additional pharmacopeial medical drug.

Keywords: apple pectin, antimicrobial activity, staphylococci, streptococci

Introduction

The scientific literature analysis proves that different groups of pectin substances are used in medical practice when curing the healing wounds. The clinical observations confirmed that the local administration of 2% solutions of apple and beet pectin provides with the healing of complicated burn wounds saving. Such patients had more effective wounds cleaning and the level of their microbial dissemination decreased. It helped to reduce the exudation phase of wound process and to accelerate the regenerative phase due to the stimulation of granulation tissue development and epithelization. Besides the patients with burns had less intoxication, less risk of bacteriemia and mortality, the quantitative and qualitative composition of gut organisms normalized after peroral pectin application [5].

The sorptographic pectin characteristics are well known. It has been proved experimentally their power to combine not only the radionuclides and ions of heavy metals but also the microbial toxins in particular the staphylococci enterotoxins [5]. In virtue of gel-forming and obducing characteristics the pectins effectively inhibit the adherence of pyogenetic, enteropathogenic and parodontopathogenic bacteria in different types of cells [4]. The medical value of pectins is ensured by their probiotic and immunomodulatory characteristics [6].

At the same time the researchers' ideas of pectins antimicrobial activity are polysemantic and even discrepant. The statements of different scientists about bactericidal and bacteriostatic activity of pectins differ [5-7]. Purpose-oriented course of our research was the development of the base of water rinses for oral cavity not having in its composition the synthetic antiseptic means and antibiotics affected the reduction of microbial medium of oral cavity in pre-term or short-term perspective.

The purpose of this research is the study of antimicrobial characteristics of apple pectin and its composition with lincomycin with respect to the main exponents of odontogenic abscessing processes.

Materials and Methods

For pilot research the apple pectin and its composition with lincomycin (50 mg/g) have been employed.

The staphylococci and streptococci strains are employed as test-strains being the main agents of abscessed complications after teeth extractions: collection strain *Staphylococcus aureus* 209-P as well as clinical isolates *S. aureus*, *S. epidermidis*, *S. haemolyticus*, β -hemolytic streptococcus of G group, β - hemolytic streptococcus *Streptococcus constellatus*, α -hemolytic streptococcus *Streptococcus salivarius* and *Streptococcus mitis*. Clinical strains of microorganisms were distinguished from abscessed exudation of patients with complicated course of postoperational period after teeth extraction. They were identified on the basis of morphological, culturological characteristics and biochemical microtests with the help of sets «STAPHYtest 16» and «STREPTOtest 16» (Lachema, Czech). The staphylococci cultivation was carried out at the customary agar and the streptococci – at the blood agar.

The bactericidal characteristics of pectin wound healing medicines were observed when cultivating test strains on the nutrient agar with addition of 20 mg/ml (2%), 10 mg/ml (1%), 5 mg/ml (5%) and 2,5 mg/ml (0,25%) apple pectin and its composition with lincomycin. On the surface of the Petri dishes it was brought on every of them 100 microliter of suspension for the diurnal test-strains cultures standardized by the optical turbidity. The check-study was performed on the Petri dishes with nutrient agar without pectin. After diurnal incubation in thermostat at 37 °C it was compared the intensity of cultures growth at the control and experimental dishes. It was received the digital images of cultures on the dishes the processing of which (colonies computation) was carried out with the help of computer program TotalLab TL120 v 2008 (Nonlinear Dynamics Ltd.).

In the second series of experiments it was studied the impact of staying with pectin medicines on the viability of golden staphylococcus in the suspension. The collection strain *S. aureus* 209-P was added to the solutions of tested medicines in concentration of 5mg/ml (0,5 %) and 2,5 mg/ml (0,25%) in the final quantity 10^5 /ml. The received microbial suspensions were placed on shaker at room temperature for 1 day directly after their producing and also after 2, 6, and 24 hours it was taken 100 microliter of samples each for inoculation with agar and computation of colonies number. The control study was performed with the sterile physiological solution.

The growth dynamics of microbial cultures on the liquid medium with pectin addition was analyzed through their optical density rise. In the sockets of 96 flat-bottomed polystyrene dishes were brought in 200 microliter of nutrient medium each prior planted with test-cultures. Pectin was added to the nutrient medium prior to the cultures inoculation in concentrations of 20 mg/ml (2%), 10 mg/mml (1%) and 5 mg/ml (0,5%). For control it was simultaneously carried out the cultures inoculation on the medium without pectin. The study with every strain in all above-mentioned variants of culture medium composition was performed concurrently in 4 sockets of a dish. Optical density of medium (OD₄₉₅) was recorded by spectrophotometer at 495 nm directly after the

cultures were brought in the sockets and after 18 hours of incubation in thermostat at 37 °C in the hermetic chamber with enough level of density. With every variant of experiment it was fixed the mean value of the medium optical density growth.

For studying the ratio between the plankton phase and biofilm the broth cultures containing plankton phase of microorganisms were brought with the micropipette in the corresponding sockets of a new dish then determining their optical density^[8]. The dish sockets with biofilms were singly rinsed out with the phosphate buffer (pH 7, 2). After addition of a new dose of buffer they were thoroughly reslurried with the micropipette and fixed the optical density of received suspensions.

The pectin impact on microbial cultures adhesion to the polymer surface and their power to create the biofilms were studied by A. Nostro and coauthors' approach. In the sockets of flat-bottomed polystyrene dishes were brought in 200 microliter of nutrient medium each (with various concentrations of pectin and without it) prior planted with test-cultures. After 24 hours of incubation at 37° C the medium with plankton phase of microorganisms was removed. The microorganisms without adhesion were removed from the sockets in the process of threefold rinsing with phosphate buffer (pH 7, 2). Adhesive microorganisms that formed biofilms at the bottom and walls of the sockets were fixed within an hour with Bouin fixator: picric acid - formalin (40%) - acetic acid (73:25:2).

After removing the socket fixator for the second time it was rinsed out thrice with the phosphate buffer. The biofilms of adhesive bacteria had been coloured for 10 minutes with 0,2% solution of violet crystal. The surplus of a colourant was removed and the sockets were rinsed out with distilled water. In the rinsed out sockets it was brought in 250 microliter of ethanol each for colourant liberation and it was measured the optical density at 495 nm. The experiment with every strain with various variants of culture medium composition was performed simultaneously in 4 sockets of a dish and the mean value of biofilms colouring intensity was determined. For statistical treatment of results it was employed the methods of variation statistics, one-way and two-way variance analyses (program ANOVA).

Results and Discussions

For studying the bactericidal impact of pectin medicines on the microorganisms was chosen the method of cultivation on the nutrient agar that provides with the enough contact duration of microbial cells with components being the ones of nutrient medium. The experiments' results presented in table 1 prove the lack of bactericidal characteristics in apple pectin. The number of colonies *S. aureus* and β -hemolytic streptococcus *S. Constellatus* on the medium with apple pectin was less than without it (control) (Fig. 1A and 1B). Moreover, on the agar with comparatively low pectin concentrations (0,5% and 0,25%) it was observed the proved increased amount of colonies both staphylococci (26% and 72%, $p < 0,05$) and streptococci (15% and 29%, $p < 0,05$).

The growth lack of staphylococcus and streptococcus on the medium with composition pectin+lincomycin (Table 1, fig. 1C) is explained exceptionally by antibiotic spectrum as both test-cultures were characterized with good sensitivity to macrolides and lincosamides.

Table 1: Pyogenetic cocci growth on the agar with pectin

	Number of colonies on the dish	
	<i>Staphylococcus aureus</i>	β -hemolytic <i>Streptococcus constellatus</i>
Control	1708±39	1407±37
Pectin		
20 mg/ml (2%)	2011±23*	1544±49
10 mg/ml (1%)	1967±77	1587±61
5 mg/ml (0,5%)	2152±65*	1621±26*
2,5 mg/ml (0,25%)	2949±57*	1819±74*
Pectin + lincomycin		
20 mg/ml (2%)	0	0
10 mg/ml (1%)	0	0
5 mg/ml (0,5%)	0	0
2,5 mg/ml (0,25%)	0	0

Comment: $p < 0,05$ compared with control.

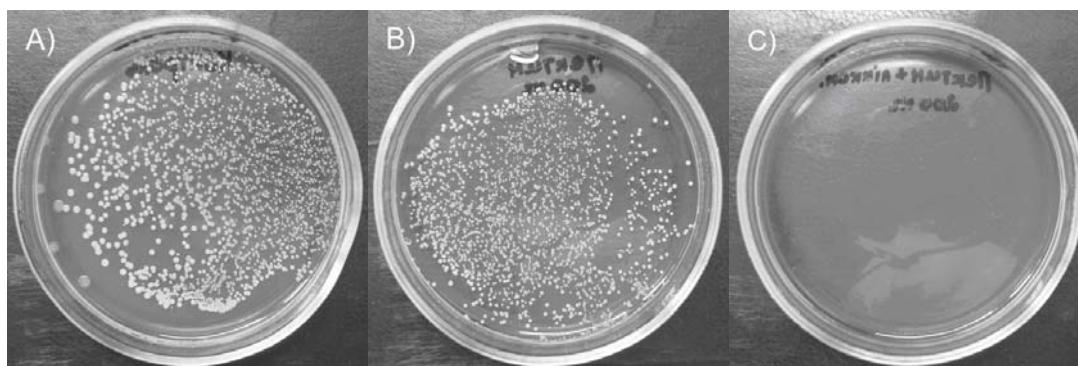


Fig 1: Culture growth of *S. aureus* 209-P in control (A) and on the medium with pectin (B) and composition of pectin with lincomycin (C) in concentration of 20 mg/ml (2%).

In the second series of experiments it was studied the impact of various periods of staying with pectin medicines on the amount of viable cells of golden staphylococcus *S. aureus* 209-P in suspension provided its constant mixing in the shaker (Fig. 2).

The composition pectin+licomycin displayed high bactericidal activity which directly depended on concentration of antibiotic ($F=247,88$; $F > F_{critic.max.}=4,26$) and less on time of staying $F=0,0207$; $F < F_{critic.min.}=0,9956$). The culture staying of *S. Aureus* with pectin without antibiotics also was attended by fewer amounts of microbial colonies in all samples, taken out in different time periods (Fig. 2). When analyzing the research findings with two-way variance analysis (ANOVA) it was proved that the time of staying impacts on the amount of viable cells of golden staphylococcus more ($F=89,63$; $F > F_{critic.max.}=5,14$) than tested pectin concentrations of 5 mg/ml and 2,5 mg/ml ($F=3,7060$; $F < F_{critic.max.}=4,7571$). It should be mentioned that enormous decrease of viable cells of *S. aureus* ($F=7,5627$; $F \approx F_{critic.max.}=7,7086$; $p=0,0514$) was observed in the samples taken out directly after mixing microbial suspension with pectin solutions. It proves that the decrease of the colonies amount up to 54-69% after planting the corresponding samples can't be the reason of microbial cells loss (it is time consuming to have such an effect) but sooner it is the result of their coaggregation under pectin interaction.

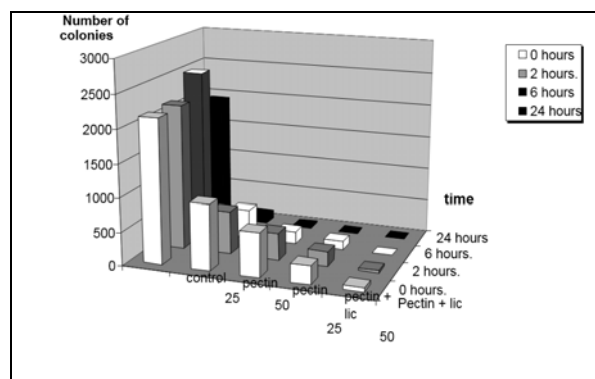


Fig 2: Impact of various staying periods with pectin medicines on the amount of viable cells *S. aureus* 209-P in suspension.

For this hypothesis testing it was analyzed the optical density of microbial cultures in dynamics of their growth on the liquid medium with pectin addition. The results given in Fig. 3 prove that in 5 out of 6 test-cultures of group G) the apple pectin had weak bacteriostatic action. It was significant ($p < 0,05$) with pectin concentration of 20 mg/ml (2%) and 10 mg/ml (1%). With pectin concentration of 5 mg/ml (0,5%) bacteriostatic activity was just with culture *S. aureus*. Relative inhibition of the cultures growth was 12,7-32,7% and 23,6-38,1% for 2% and 1% of pectin solutions respectively.

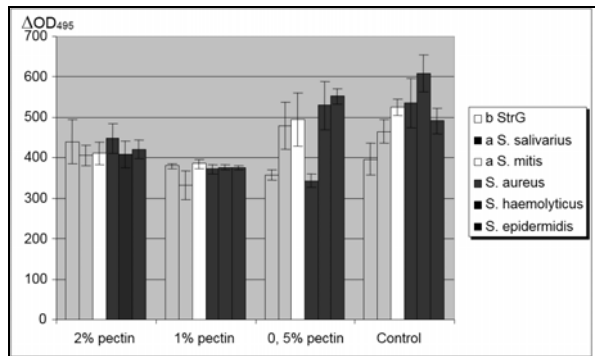


Fig 3: Impact of various pectin concentrations on the growth of broth cultures of staphylococci and streptococci.

The research findings got in this experiment coincide with the standard ones. If the agent of enteric infections shigells and salmonellas perish after 2-hour staying with 3% pectin solution [8], then staphylococci can be viable in the pectin solutions up to 48 hours [5]. The growth inhibition of *S. aureus* under influence of 2% apple pectin was insignificant – just one division [5].

When cultivating the microorganisms on the liquid nutrient mediums their growth is irregular. The part of microbial cells is in the free drifting position (plankton phase). Some microbial cells adhere to the bottom and wall of the dish and keeping dividing create on the surface the solid layer – biofilm. The intensity of biofilm creating can be determined not only by the microorganisms’ adhesive characteristics. It can be an important specific indication of bacteria and depend on the degree of culture virulence. Opportunistic microorganisms that are the exponents of true microflora of various organism biotopes especially their strains associated with opportunistic and hospital infections are characterized with high colonizational power and ability to create biofilms both *in vivo* and *in vitro*.

Therefore it was studied the pectin impact on the ratio between interplankton phase and biofilm *S. Epidermidis* in the broth culture.

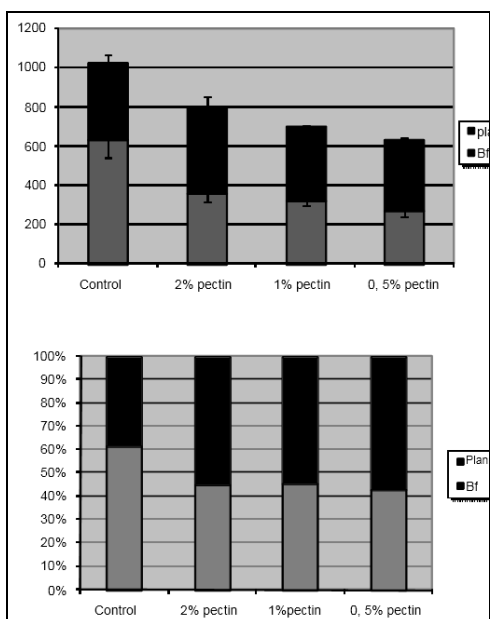


Fig 4: Impact of various pectin concentrations on the ratio of plankton phase and biofilm in broth culture *S. epidermidis*.

The given in Fig. 4 findings prove that in the concentration range of 0,5-2% apple pectin effectively inhibits the biofilm creation of *S. epidermidis* ($p < 0,05$). The reduction of total data of the culture growth originates due to the biofilm lessening. At the same time the intensity of the culture growth in the plankton phase (in absolute standard value of the light absorption) did not change significantly. That is why with pectin the interest division of culture changed between plankton phase and biofilm. The similar regularities occur when analyzing the growth of broth cultures of the other types of staphylococci, β - and α -hemolytic streptococci.

More accurate assessment method of biofilm creating intensity with broth cultures of microorganisms provides for their fixation with the following crystal coloring in violet. The employed in the given experiment cultures of staphylococci and streptococci differ from one another in biofilm creation. This characteristic was most evident in α -hemolytic oral streptococci *S. salivarius* and *S. mitis*. The staphylococci strains and β -hemolytic streptococcus of group G were characterized with moderate biofilm creation power. The availability in the nutrient medium of 20 mg/ml (2%) and 10 mg/ml (1%) apple pectin inhibited the intensity of biofilms creation with all tested strains ($p < 0,05$) (Fig. 5). The pectin activity in concentration of 5 mg/ml (0,5%) was less significant. This pectin concentration didn't impact on the biofilm creation with the cultures of α -hemolytic *S. mitis* and coagulatively negative staphylococci (*S. epidermidis*, *S. haemolyticus*) at all. The established growth inhibition of biofilms with pectin 0,5% occurred in the cultures of β -hemolytic streptococcus of group G and α -hemolytic *S. salivarius*. At large it should be mentioned more inhibiting pectin impact on the biofilms creation by the strains with high biofilm creation power. The starins with the moderate biofilm creation power demonstrated the weak sensitivity to the pectin with 5 mg/ml (0,5%) concentration.

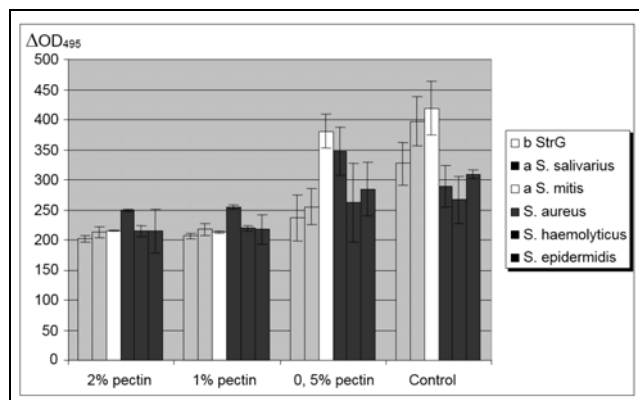


Fig 5: Impact of various pectin concentrations on the biofilm creation with cultures of staphylococci and streptococci.

The research findings proved that the impact of various pectin concentrations on the broth cultures growth of staphylococci and streptococci and on the biofilms creation with the cultures of staphylococci and streptococci is the most effective in 1% solutions.

Results and Discussions

Pectin compositions in agar (Fig. 1 B) provide with the microorganisms the consourse of nutriments especially when knowing their high bioavailability with significant spreading in the microbial world of metabolizing enzymes [3]. It can be

admitted that in comparatively high concentrations of pectin compositions this stimulation is leveled with its sorbing characteristics. We consider that the experiment result must be interpreted as follows: immobilized in the nutrient agar pectin molecules are characterized with limited spatial contact with applied on the medium surface microbial cells and display their sorbing characteristics as a minimum. Therefore when making the base for the industrial samples it should be taken into account the lack of bactericidal activity at short-term staying with microorganisms of oral cavity. It can be applied clinically just in scheduled teeth extraction as a hygienic-prophylactic mean when there are a number of contacts with oral cavity. The long-term staying provides with therapeutic effect. In short-term staying it is better to apply the medicines invented by us with antibiotic (Table 1, Fig. 1 C) the clinical application of which can be in urgent teeth extraction. Pectin composition with antibiotic had significant bactericidal activity (Fig. 2) which directly depended on the antibiotic concentration ($F=247,88$; $F>F_{critic. max.}=4,26$) and to a lesser extent on the time of staying ($F=0,0207$; $F<F_{critic. min.}=0,9956$). The received data proved our viewpoint. It is essential when making the medicines base for the oral cavity: the medicines for the scheduled and urgent teeth extraction. Studying the standard monoculture *S. aureus* with pectin without antibiotic (Fig. 2) proved that decrease of the colonies amount up to 54-69% after planting the corresponding samples can't be the reason of microbial cells loss (it is time consuming to have such an effect) but sooner it is the result of their coaggregation under pectin interaction. When analyzing the research findings with two-way disperse analysis (ANOVA) it was proved that the time of staying impacts on the amount of viable cells of golden staphylococcus more ($F=89,63$; $F>F_{critic. max.}=5,14$) than tested pectin concentrations of 5 mg/ml and 2,5 mg/ml ($F=3,7060$; $F<F_{critic. max.}=4,7571$). It means that when applying clinically it can be expected beyond all doubts the prolonged effect from the other medicines based on pectin substances.

The results of determination in the microbial cultures of optical density with the dynamics of their growth on the liquid medium with pectin addition proved that exclusive of β -hemolytic streptococci of group G, for all cultures the apple pectin had weak bacteriostatic activity (Fig. 3). Relative inhibition of the cultures growth was 12,7-32,7% and 23,6-38,1% for 2% and 1% of pectin solutions respectively. In other words when applying clinically 1% and 2% of apple pectin water solutions it must be expected the bacteriostatic activity to more exponents of flora, being the main agents of abscessed complications.

With pectin the interest-bearing culture division between plankton phase and biofilm changed. In the concentration range of 0,5-2% apple pectin (Fig. 4) effectively inhibits the biofilm creation of *S. epidermidis*. In other words when making industrial samples like rinses for oral cavity in the clinics may be expected the inhibition of plankton sedimentation on the wounds surface that will help to heal postoperational wounds. It is considered that soft dental deposit creates the most dangerous pathologic situations in the oral cavity as it contains the biggest number of pathogenic microorganisms.

The biofilm creation intensity with broth cultures (Fig. 5) of microorganisms in the studies was compared between most evident in α -hemolytic oral streptococci *S. salivarius* and *S. mitis* and moderate biofilm creation power in β -hemolytic streptococci of group G. The findings proved that the

availability in the nutriment of 20 mg/ml (2%) and 10 mg/ml (1%) apple pectin inhibited the intensity of biofilms creation with all tested strains. It should be taken into account that for industrial samples development more applicable proved to be the samples with 1% concentration of apple pectin as the base for oral cavity rinses, the solutions for bathing of wounds applied intraoperatively. The intensity of biofilm creation is determined not only by the adhesive characteristics of microorganisms it also can be a specific evidence of bacteria and depends on the culture virulence extent. The opportunistic microorganisms that are the exponents of standard microflora of various biotypes of organism especially their strains associated with opportunistic and hospital infections are characterized with high colonizational and biofilm creation power both *in vivo* and *in vitro*. Consequently the application of apple pectin as a liquid water pharmacopeial drug is completely reasonable as in combination with other medical medicines can present synergistic result.

Conclusion

1. The above-mentioned study has the importance for the development of the industrial samples of medicines as the water base for the rinses, solutions for the bathing of wounds applied presurgically, intraoperatively in the oral cavity and upon rehabilitation.
2. The microbiological research proved that when cultivating the microorganisms [standard strains of *Staphylococcus aureus* 209-P, microorganisms, clinical isolates *S. aureus*, *S. epidermidis*, *S. haemolyticus*, β -hemolytic streptococcus of group G, β -hemolytic streptococcus *Streptococcus constellatus*, α -hemolytic streptococci *Streptococcus salivarius* i *Streptococcus mitis*, as well as clinical strains of microorganisms were distinguished from abscessed exudation of patients with complicated course of postoperational period after teeth extraction] on the liquid nutriments with apple pectin their growth is irregular. The part of microbial cells is in the free drifting position (plankton phase). Some microbial cells adhere to the bottom and wall of the dish and keeping dividing create on the surface the solid layer – biofilm.
3. Bacteriostatic activity of various pectin concentrations on the growth of broth cultures of staphylococci and streptococci and on the biofilms creation with the cultures of staphylococci and streptococci more efficient proved to be in 1% solutions where the inhibition of the cultures growth was 23,6-38,1%.

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