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## Research Article

### Comparative evaluation antimicrobial and antitumor activities of natural colostrum peptide and its synthesized analogue

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#### Abstract

Antimicrobial and antitumor activities of bovine colostrum peptide and its synthesized analogue were carried out. The peptide was produced by standard solid phase synthesis followed by purification by high-performance liquid chromatography. Peptide purity and primary structure were confirmed by MALDI and ESI MS. It was found that the synthesized peptide with ACSAG amino acid sequence and a molecular weight of 430.2332 was an analogue of the natural one. The synthesized peptide had a high hydrophilic value and isoelectric point of 5.22. The obtained data theoretically confirm that the studied peptide belongs to a class of antimicrobial peptides. It was experimentally established that both natural and synthesized peptides had antimicrobial activity against *E. coli* ATCC 25922, *P. aeruginosa* 27/99 and *B. subtilis* ATCC 6633, antifungal and anti-tumor activity against C6 cells. After 48 h there was a significant 50% decrease in the tumor cells population at the concentration of the natural peptide of  $365.5 \pm 3.8$  mg.ml<sup>-1</sup>, and the synthesized peptide concentration of  $312.7 \pm 3.5$  mg.ml<sup>-1</sup> in the nutrient medium. This synthesized peptide has a greater biological activity and can be effective at lower concentrations, than the natural one.

#### Keywords

three-phase peptide synthesis, bovine colostrum, antimicrobial, antitumor, antifungal activities, isoelectric point, hydrophilicity

#### Abbreviations

AMPs – antimicrobial peptides; BMP – bone morphogenetic protein; DMF – dimethylformamide; FBS – Fetal Bovine Serum; 4HNE – 4-hydroxynonenal;  $\gamma$ -IFN – gamma-interferon; NC – negative control; PC – positive control; PRP – proline peptides; SD – standard deviation; SPPS – solid-phase peptide synthesis;  $\alpha$ -TNF –  $\alpha$ -tumor necrosis factor; TFA – trifluoroacetic;

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## Introduction

Antimicrobial peptides (AMPs) are small molecular weight peptides that provide innate immunity against a wide range of microorganisms, including bacteria, fungi, parasites and viruses (Wang et al. 2019; Mohammed et al. 2017; Kang et al. 2017; Bechinger et al. 2017; Kapustian et al. 2018).

The treatment of diseases caused by pathogenic bacteria has long relied on antibiotics. However, the emergence of bacterial resistance to long term and broad-spectrum antibiotics becomes a serious problem for clinical management of infections and for a wide range of consumers who have the potential risk of acquired antibiotic resistance (Hiltunen et al. 2017). The advantage of using AMPs is that they act on multiple targets of the pathogenic bacteria plasma membrane and have a potent activity against antibiotic resistant bacteria (Mwangi et al. 2019).

AMPs are classified into cationic and anionic. Cationic AMPs connect with the microbial membrane because of electrostatic interaction and interact with the anionic components of the plasma membrane, increase permeability by forming pores, lead to lysis and release of cellular contents (Rajagopal et al. 2017).

There're several base models of membrane pores. After AMPs enter the phospholipid membrane, their hydrophobic regions will combine with the inner hydrophobic regions of the phospholipid bilayer. Another bactericidal mechanism consists in AMPs penetrating the cytoplasm and inhibiting the synthesis of DNA, RNA, enzyme activity and cell wall synthesis, and also stimulating the release of lipases that destroy cell structures (Baek et al. 2016).

Unlike cationic AMPs, the mechanisms of action of anionic AMPs remain poorly understood. It is believed that the antibacterial mechanism of the peptide action (maximin H5) against *S. aureus* (killed *S. aureus*, minimum inhibitory concentration of 90  $\mu$ M) is associated with the dissolution of the membrane (Dennison et al. 2016).

There are other mechanisms of the peptide antimicrobial action. For example, buforin penetrates the bacterial membrane without disturbing its permeability, interacts with the genomic DNA and RNA of microorganisms and inhibits DNA replication or transcription (Zhang et

al. 2017). Consequently, AMPs represent a new alternative for antibiotics.

A growing body of research confirms the antitumor activity of AMPs (Zhang et al. 2019). Particularly, LL-37 activates bone morphogenetic protein (BMP) signaling through a proteasome-dependent mechanism to inhibit the proliferation of gastric cancer cells. KT2, BG-4 and KL15 induce apoptotic or necrotic death of cancer cells (Maraming et al. 2019; Maijaroen et al. 2018).

It should be noted that long-term chemotherapy in cancer patients not only leads to resistance to traditional methods of cancer treatment, but also weakens the immunity and susceptibility of pathogenic microorganisms to antibiotics. AMPs often have antitumor properties, but they don't weaken the body's immune responses to infection, so it's a new treatment option for cancer patients.

With the recent emergence of new synthetic approaches that allow the biophysical and biochemical properties of natural peptides to be altered, they are considered as effective drugs (Angelova et al. 2019; Lee et al. 2018).

It should be noted that with increasing demand for peptide drugs synthesized peptides obtained with a polymer carrier (by solid-phase synthesis) have been increasingly used (Apostolopoulos et al. 2021). At the same time, toxic solvents used in peptide synthesis can reduce their biological activity and have a negative impact on living organisms. In this regard, the new standards of "green chemistry" pose a huge task for chemists of improving and developing new schemes for the synthesis of peptides, selecting suitable, inexpensive, low toxic solvents, as well as creating new linkers. A sulfo fluorenyl oxy carbonyl (Smoc) strategy was developed based on the use of water-soluble protective groups that can reduce the amount of toxic solvents. Promising developments also include the N-carboxyanhydride method of activating amino acids with urethane protective groups, which completely eliminates the use of carbodiimides, nucleophilic toxic additives based on hydroxybenzotriazoles and solvents (Martin et al. 2020). However, many years may pass before new technologies are introduced and affect the peptide industry. Comparison of the biological activity of natural peptides and their synthesized analogues can help to develop new peptide drugs.

Therefore, comparative evaluation of effectiveness of natural and synthesized peptides remains topical today.

One of the promising sources of biologically active peptides is bovine colostrum. It contains a complex of proline peptides (PRP) which affect the differentiation and maturation of thymocytes in the synthesis of interleukin 6, gamma-interferon ( $\gamma$ -IFN),  $\alpha$ -tumor necrosis factor ( $\alpha$ -TNF) and some other types of cytokines. PRP peptides induce an increase in peripheral leukocytes in the blood, relieve stress in cells due to oxidation and cell damage, and suppress signaling mediated by (4HNE)-4-hydroxynonenal (Zablocka et al. 2020). The purpose of this research is to make a comparative assessment of antimicrobial and antitumor activity of bovine colostrum and its synthesized analogue.

## Materials and Methods

**Materials.** The following materials were used: synthesized ACSAG peptide (manufacturer PEPMIC CO. Ltd, China) (ACSAG amino acid sequence, sequence (from left to right) from the N-terminus to the C-terminus of the peptide) in the form of a lyophilized powder.

### Methods.

**Peptide synthesis.** The peptide was obtained at Pepmic Co., Ltd (Suzhou, China) by standard Fmoc solid-phase peptide synthesis (SPPS) followed by purification by high performance liquid chromatography on a SHIMADZU Inertsil ODS-SP chromatographic column (4.6x250mmx5 $\mu$ m). Confirmation of the purity and primary structure of the peptide was performed by MALDI and ESI mass spectrometry techniques. Trifluoroacetic acid (TFA) and triisopropylsilane (Sigma-Aldrich, St. Louis, USA), 1,3-diisopropylcarbodiimide (Fluka, Steinheim, Germany), 1-hydroxybenzotriazole (NovaBiochem-Merck, Darmstadt, Germany), N, N-dimethylformamide (DMF) and diisopropyl ether (Vetec, Duque de Caxias, Brazil), acetonitrile (HPLC grade) (JT Baker, Center Valley, USA). All solvents used in the HPLC system were produced in Tedia, Rio de Janeiro, Brazil.

Natural ACSAG peptide was isolated from trypsin hydrolyzate of bovine colostrum at the Department of Food Engineering of Agricultural Production at

the Ural State Agrarian University (Ekaterinburg, Russia) in the form of a lyophilized powder; Colostrum (from cows of black-and-white breed) was collected 4 h after calving (Averino farm, Sverdlovsk region, Russia). The content of protein, fat and ash in the colostrum was as follows: 19.2 $\pm$ 1.6, 5.9 $\pm$ 0.2 and 1.20 $\pm$ 0.01%, respectively. Fat-free colostrum with a residual fat content of 0.56 $\pm$ 0.02% was used for hydrolysis. The colostrum hydrolysis regime was as follows: hydrolysis duration - 6 hours, amount of enzyme (trypsin) – 1.8%, pH 7.8 and t 39°C. At the end of fermentolysis, the temperature was increased to 75°C for 7 min to inactivate the enzyme. The hydrolyzate was purified from salts and inorganic impurities using an Amberlit XAD2column, eluent: Buffer A: 10 mM CH<sub>3</sub>COONa pH = 6, 10 mM CH<sub>3</sub>COONa pH = 4, 10 mM KCl/HCl pH = 1.5 with a salt gradient, buffer A + 0.2%, 0.4%, 1% NaCl.

The peptide was isolated by preparative chromatography on silica gel, eluent PBS and EtOH in an isocratic ratio of 9:1, respectively. The resulting peptides were freeze-dried Martin Christ Alpha 1-4 LSCplus (Martin Christ, Germany).

The molecular mass of peptides was determined in the laboratory of the Department of Food Engineering of Agricultural Production at the Ural State Agrarian University. The molecular weight of the synthesized peptide was determined on a Bruker Amazon mass spectrometer in the ESI mode (AmaZon SL, Bruker Daltonik (Bruker, Germany)). A sample was preliminarily prepared: For this, 3 mg of the studied peptide was dissolved in 1 ml of phosphate buffer with pH of 8.6.

RP-HPLC-DMD method, was carried out in the laboratory of the Department of Food Engineering of Agricultural Production of the Ural State Agrarian University.

Qualitative analysis of the native and synthesized peptide was carried out by the RP-HPLC-DMD method without sample dilution and additional sample preparation at the Ural State Agrarian University.

The analysis of the peptide was carried out on a Shimadzu LC-20AB chromatograph with a binary pump, autosampler, and diode array detectors. A column with nonpolar C18 phase (Supelco, Discovery, 4.6x150mm, 5 $\mu$ m) was used. Gradient

step mode, eluent rate 1.4 ml/min, 1% to 65% B in 45 min. Eluents: A - 0.02 M sodium acetate, B - methanol. Temperature 37°C, analytical wavelength 338 nm.

**Spatial structure.** The spatial structure of the synthesized peptide was modeled using the Schrodinger Maestro program (USA). Schrodinger Maestro is a streamlined portal providing access to state-of-the-art predictive computational modeling and machine learning workflows for molecular discovery

(<https://newsite.schrodinger.com/platform/products/maestro/>).

**Study of antimicrobial activity.** The study of the peptide antimicrobial activity was carried out by the disk-diffusion method presented in MUK 4.2.1890-04. To study antimicrobial activity, test strains of *Escherichia coli* ATCC 25922 (*E. coli*), *Pseudomonas aeruginosa* 27/99 (*P. aeruginosa*) and *Bacillus subtilis* ATCC 6633 (*B. Subtilis*) were used. *B. subtilis* and *E. coli* strains were cultivated on solid LB nutrient medium (agar 1.5%, trypton 1%, yeast extract 0.5%, NaCl 1%) at 37°C. Cultivation of *P. aeruginosa* 27/99 test strain was carried out on meat peptone agar with addition of glucose at a temperature of 37°C.

To obtain the inoculum of microorganism cultures, the same type of colonies was taken from a daily culture, which was inoculated by the thinning stroke method, in 5 ml of a liquid medium and cultivated in a shaker thermostat until OD 450 equal to 0.10–0.11, which corresponds to a cell content of  $1.5 \times 10^8$  cfu.ml<sup>-1</sup>. Further, 1 ml of the resulting inoculums were applied to Petri dishes with solid nutrient medium LB and MPA, respectively, and distributed by shaking, after which the excess was removed with an automatic pipette. Petri dishes with inoculum were dried at room temperature for ten min; pre-preparing sterile paper discs soaked in the studied peptides were then applied. A disk soaked in distilled water was used as a negative control, and an antibiotic, kanamycin (Sigma-Aldrich, USA), was used as a positive control. After 15 min required for the pre-diffusion of antibacterial agents into

**Statistical analysis.** Statistical data analysis was carried out using GraphPad Prism 8.1 (GraphPad Software, USA).

agar, the test strains were incubated for 24 h at a temperature corresponding to the cultivation of the test strains of *E. coli* ATCC 25922, *P. aeruginosa* 27/99 and *B. subtilis* ATCC6633. The experiment was repeated 5 times.

**Study of antitumor properties.** The study of the antitumor properties of the natural and synthesized peptide was carried out on C6 rat glioblastoma cell line.

**Cell culture.** C6 cell lines (ATCC CCL-107™) were used as a model object, the passage of which didn't raise 15 during the time of an experimental work. For cultivation, we used DMEM medium (Gibco, USA) supplemented (to the final volume) with 10% Fetal Bovine Serum (FBS) (Capricorn, USA), 1% Sodium Pyruvate (Gibco, USA), 1% GlutaMAX (Gibco, USA), 1% Penicillin/Streptomycin (Gibco, USA).

Cells were stored in a CO<sub>2</sub> incubator under conditions of 5% CO<sub>2</sub> and 95% humidity for testing. Two weeks before the start of the experiment, the cells were checked for the presence of mycoplasma using the MycoReport kit (Evrogen, Russia).

**Determination of cell viability.** The following values of the studied peptide samples were used as standard concentrations: 500, 400, 300, 200, 100 and 50 mg.ml<sup>-1</sup>. Negative control suggested cells without addition of peptides. As a positive control, PBS (pH 7.4) was added to a volume of 50% of the total volume of the well medium (100 µl).

During the experiment, C6 cells were seeded in a 96-well plate (Eppendorf, Germany) in the amount of 10.000 cells per well. The experiment was carried out in five replications; therefore, only two samples (natural and synthesized peptide) were in the same 96-well plate.

After 48 h of cultivation, PrestoBlue reagent (Merck, USA) (final concentration 1 µg.ml<sup>-1</sup>) was added to the wells for *in vivo* evaluation of cell viability. The plate was placed then in a CO<sub>2</sub> incubator for 20 min. After that, analysis was carried out on a ClarioStar (BMG Labtech, Russia).

This section can be divided into subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.



**Results and Discussion**

The peptide with ACSAG amino acid sequence was obtained by three - phase synthesis. A synthetic raw product was cleaned with VEZH and evaluated using Maldi-TOF-MS analysis. The results are presented in Table 1. Fig. 1 shows a chromatogram and a mass spectrum of the natural and synthesized peptides (the tests were carried out at the Ural State Agrarian University).

The chromatogram and mass spectrum of the peptide showed that a peptide with ACSAG amino acid sequence was obtained, which is an analogue of the natural one. The resulting peptide had a molecular weight of 430.2332 Da, corresponding to the molecular weight of the natural peptide.

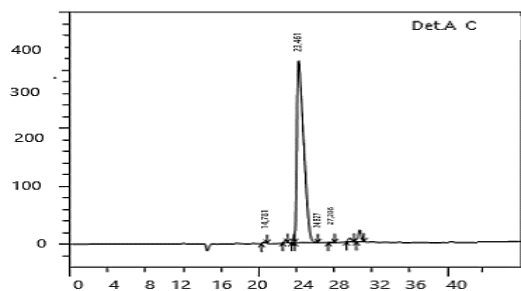
It is known that the chemical structure, biological activity and functions of the active substance interrelate. In this regard, we have carried out modeling of the spatial structure of the peptide, namely the Structural Activity Quantification Ratio (QSAR), because thanks to this tool one can try to predict the activity of a molecule based on its molecular features.

Fig. 2 shows the 2D structure of the synthesized peptide.

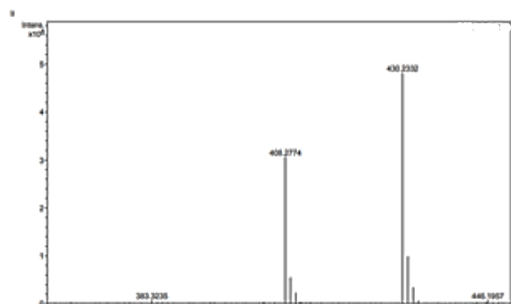
The structural model of the peptide allows to determine the level of hydrophilicity, by the number of amino acid residues. The larger the amino acid sequence, the lower the solubility of the peptides.

**Table 1.** Technological parameters of HPLC purification of the synthesized product (provided by Pepmic Co., Ltd (Suzhou, China))

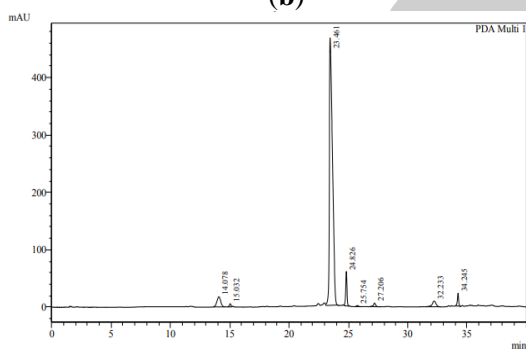
Amino acid sequence		ACSAG		
Series		PCM15527-3-0815		
Pump A		0.1% trifluoroacetic acid in 100% water		
Pump B		0.1% trifluoroacetic acid in 100% acetonitrile		
General flow		1 ml.min <sup>-1</sup>		
Wavelength		220 nm		
Type of analytical column		SHIMADZU Inertsil ODS-SP (4.6xmmx5µm)		
Method of dissolution		15% ACN+85% H <sub>2</sub> O		
Injection volume		12 µl		
Amino acid sequence		ACSAG		
Time, min	Module	Action	Significance	
0.01	Pumps		15	
20.00			55	
23.00			100	
38.00			B. Concentrate	100
40.00				15
50.00	Controller	Stop		



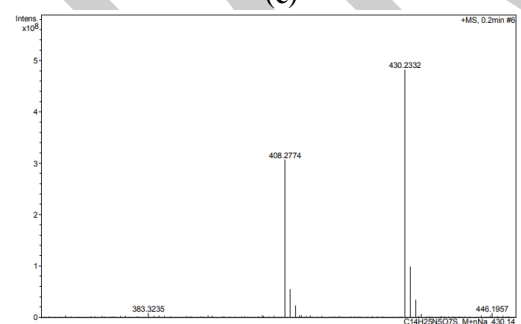
(a)



(b)

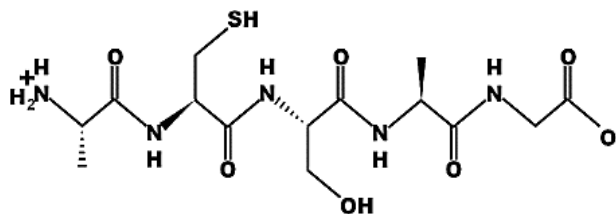


(c)



(d)

**Figure 1.** The chromatogram (a) and mass spectrum (b) of the natural peptide and the chromatogram (c) and mass spectrum (d) of the synthesized peptide



**Figure 2.** 2D structure of the synthesized peptide with ACSAG amino acid sequence

The ACSAG peptide (alanine-glycine-serine-alanine-glycine) is characterized by a high hydrophilic value of  $+10.49 \text{ kcal.mol}^{-1}$ , which indicates its significant biological activity, according to studies showing that peptides with increased hydrophilicity have a high biological activity. The obtained data are confirmed by the results of our study of the antitumor activity of the peptide (Lorenz et al. 2009).

An important indicator of the peptide biological activity is the isoelectric point pI - the pH value at which the total charge of the molecule is zero, and therefore, its electrophoretic mobility stops (Kozłowski 2017). The pI of a peptide is primarily dependent on its native amino acid sequence and it's critical to understanding the biochemical function. Therefore, the determination of pI is an important aspect of proteomic research. During electrophoresis, the direction of a peptide movement in a gel or other matrix depends on its pI, hence multiple proteins can be differentiated based on their pI (Kumar et al. 2017).

However, given the influence of the peptide synthesis process and other biochemical changes (phosphorylation, methylation, alkylation), pI of the synthesized peptides, may differ from the pI of the natural ones, depending on the composition and amino acids in the peptide (Anderson et al. 2008). The theoretical isoelectric point of the studied synthesized peptide was at the level of 5.22. The obtained data on the isoelectric point of the synthesized peptide are consistent with the data showing that pI depends mainly on the acid dissociation constants of ionizable groups of charged amino acids: alanine-glycine-serine. In addition, the charge of the amine and carboxyl end groups contributes to pI and can strongly influence the pI of short peptides. The studied peptide belongs to the short ones, since it consists of 5 amino acids,

which is consistent with the pI value (Pace et al. 2009).

The isoelectric point of the peptide at the level of 5.22 theoretically confirms, that the studied peptide belongs to the class of anionic antimicrobial peptides (Rajagopal et al. 2017). The antimicrobial properties of the synthesized peptide are also

theoretically substantiated by the fact that bacterial membranes are anionic and undergo peptide-induced rupture and lysis, which increase with an increase in the positive charge of the peptide (Ringstad et al. 2007).

Table 2 shows the antimicrobial activity of natural and synthesized peptides.

**Table 2.** Antimicrobial activity of natural (PP) and synthesized (SP) peptides

Sample name	Lysis zone diameter, mm		
	<i>E. coli</i> ATCC 25922	<i>B. subtilis</i> ATCC 6633	<i>P. aeruginosa</i> 27/99
PP	17	21	20
SP	19	24	23
Control	0	0	0
Antibiotic "Kanamycin"	25	26	24
Sample name	Lysis zone diameter, mm		
	<i>E. coli</i> ATCC 25922	<i>B. subtilis</i> ATCC6633	<i>P. aeruginosa</i> 27/99

Synthesized and natural peptides have antimicrobial activity against *E. coli* ATCC 25922 and *B. subtilis* ATCC6633 (<https://www.atcc.org/products/6633>). It should be noted, that the synthesized peptide has a higher antimicrobial activity. Thus, the lysis zone during the cultivation of *E. coli* ATCC 25922 and *B. subtilis* ATCC6633 is 19 and 24 mm in diameter, when using the synthesized peptide, against 17 and 21 mm for the natural one.

The data obtained consistent with studies, in which it was found, that low molecular weight synthesized peptides have an antimicrobial activity (Wang et al. 2019).

According to our research, the ACSAG peptide is hydrophobic and consists of 5 amino acid residues, that matches the results (Miller et al. 2021), in which hydrophobic short peptides, with 5-7 amino acids, have a detrimental effect on *E. coli*, *P. aeruginosa*, *Klebsiella pneumoniae* (*K. Pneumonia*) and *Staphylococcus aureus* (*S. aureus*), *Mannheimia haemolytica* (*M. haemolytica*). The data, obtained correspond with studies (Nguyen 2011), which have established, that the cationic charge and hydrophobic residues in the peptide are considered the two main physical characteristics of an antimicrobial type peptide. A systematic study of gramicidin S has shown, that hydrophobicity is a

key factor, determining the antimicrobial ability of peptides (Prenner et al. 2005). The penetration of biofilm by a peptide occurs because of three driving forces: the total positive charge, the hydrophobic group and the selective permeability of a membrane, which allows peptides penetrate the cell. The mechanism of peptide penetration into the bacterial cell is explained by several hypothetical models, such as the "barrel model", in which the peptide spirals form a bundle with a cavity, while the hydrophobic regions of the AMP indicate the core region of the phospholipid bilayer, and the hydrophilic regions form the inner region of the pore. The second is a "carpet model", where the peptide aggregates and envelops the surface of the phospholipid bilayer, creating a carpet of destruction. Finally, the "toroidal pore model", in which a polypeptide helix inserts in a plastid bilayer and causes the phospholipid molecule of the monolayer to twist and to bend, forming a hydrophilic phosphate head. Moreover, several AMPAS can also exhibit antimicrobial activity because of the membrane's thinning/thickening, targeting models for oxidized lipids and etc, while phospholipid composition, membrane protein content, pH, peptide size and amphipathy will be affecting their antimicrobial activity (Yasir et al. 2018). The data, obtained on the antimicrobial

activity of the peptide, match the data, presented in the database for APD3 antimicrobial peptides, where the studied peptide is identified as AP01406 peptide with bactericidal properties to *E. coli*, *S. aureus* and *P. aeruginosa*. Peptides, derived from milk proteins, particularly, CAMP211-225, have an antibacterial effect to *E. coli* and *Yersinia enterocolitica* (*Y. enterocolitica*) (Wang et al. 2020), which is confirmed by our research. A feature of an AMP is the presence of specific peptide sequences, which are crucial for a certain activity. One of these important features is the presence of a glycine residue in the peptide chain (Tossi et al. 2000). Our peptide contains glycine. It should be pointed that glycine at the end of the peptide chain prevents the peptide cleavage by peptidases (Andreu et al. 1998).

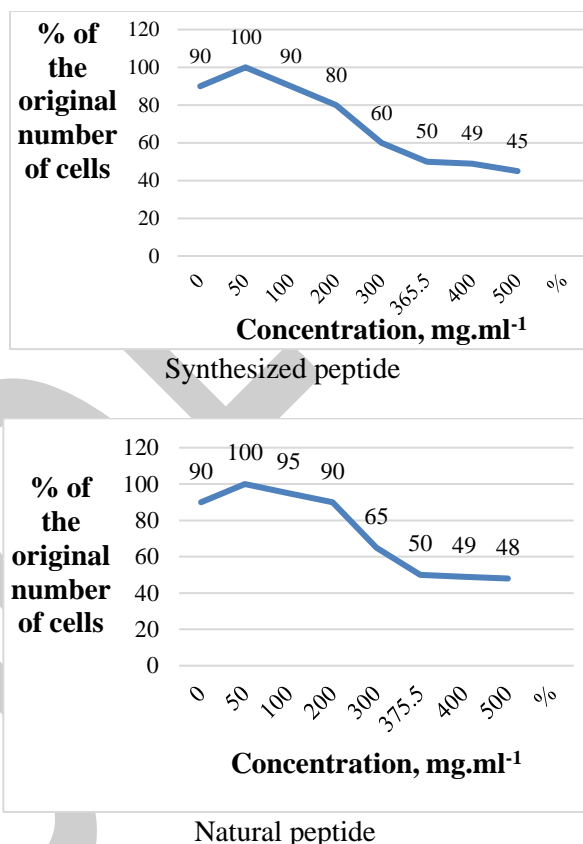
As a result of our research, it was proved that natural and synthesized peptides have an antitumor effect on C6 cells, and the synthesized peptide has a higher activity.

After 48 h, tumor cells decrease by 50% at the concentration of the natural peptide of  $375.5 \pm 3.8 \mu\text{g} \cdot \text{ml}^{-1}$ , and the synthesized peptide –  $356.7 \pm 3.5 \mu\text{g} \cdot \text{ml}^{-1}$  ( $R^2 = 0.7217$ ) (Fig. 3).

The antitumor activity of peptides is consistent with the data (Teixeira et al. 2019; Naydenova 2022) showing that biopeptides obtained by hydrolysis of milk proteins cause apoptosis in malignant cells. Milk proteins is becoming increasingly attractive as it possesses antimicrobial, antiviral, anti-inflammatory, anti-proliferative. Since biopeptides can pass through the blood-brain barrier, they can be used for treatment of brain tumors (Cutone et al. 2020).

The obtained data are consistent with studies (Cheever 2011) showing that antimicrobial peptides are effective for treatment of glioblastoma melanoma and gastric cancer. It should be pointed out that some peptides based on anticancer vaccines have a function of stimulating or increasing the number of tumor-specific T-cells. Although peptide vaccination cannot be sufficient for cancer regression, promising results from a phase II in individuals with incurable cancers related to human papilloma virus 16 (HPV-16) confirm the effectiveness of long-term peptide immunization (Rini et al. 2016). Peptide vaccines such as IMA901 and IMA950 used for the treatment of glioblastoma

contribute to the development of cancer therapy peptides (Massarelli et al. 2019). Hilf et al. (2019) showed the peptide antitumor vaccines for glioblastoma to be highly effective.



**Figure 3.** Nonlinear regression curves, with it helping the value of the ID 50% for each of the peptides was determined

The obtained data are consistent with the results of studies showing that the low molecular mass of peptides, the relatively small content of amino acids and their repeatability ensure the penetration of the peptide into cancer cells, and it will lead to their death (Liu et al. 2017).

### Conclusions

The complex biological activity of the natural peptide and its synthesized analogue were analysed and compared. The natural peptide was isolated from bovine colostrum. The artificial analogue was obtained by the three-phase synthesis and provided by Pepmic Co., Ltd (Suzhou, China). Both peptides have ACSAG acid sequence and a molecular weight. The chromatogram and mass spectrum of the synthesized peptide have shown that they are



identical to the natural one. The isoelectric point of the synthesized peptide is 5.22 and theoretically it could be antimicrobial. It has been proven that natural and synthesized peptides have antitumor activity against C6 cells, antimicrobial activity against *E. coli* ATCC 25922 *B. subtilis*, and antifungal activity as well. The synthesized peptide showed the highest antitumor activity (ID 50%) when its concentration in the culture medium with tumor cells is 17%. The antimicrobial activity of the synthesized peptide against *E. coli* ATCC 25922 and *B. subtilis* is 11.7 and 14.6%, which is higher, than that of the natural one. We have shown that synthesized peptides are no less effective than natural ones in their antimicrobial and antitumor properties. Natural and synthesized ACSAG peptides are recommended for use as antimicrobial and antitumor drugs and functional ingredients in food products for disease prevention.

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