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Correlation of Structure and Antiviral Activity of Alkaloids of *Polygonum* L. Plants Growing in Kazakhstan

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***Abstract*—**The article represents the results of isolation and component chromatographic analysis of essential oils of *Polygonym* L. plants growing in Kazakhstan in commercial reserves at the territory of Kazakhstan. The results of research of antiviral activity of isolated substances to flu virus have been represented in this article. The main pharmacophore groups in the structure of alkaloids have been identified.

***Keywords*—**Alkaloids, antiviral, bioactive substances, isolation, pharmacophore groups, *Polygonum* L.

I. Introduction

CURRENTLY to treat infectious diseases bioactive substances of plant origin having fewer side effects than synthetic medicines and medicines similar to natural components of a human body by the structure and action, become very important. Among bioactive substances, the most promising are such classes as alkaloids, isoprenoids, phenolic compounds and their derivatives. Most substances included by the mentioned classes of chemical compounds have anti antitumor, sedative, antibacterial, antimicrobial and antiviral effect [1]-[3].

Among substances of plant origin conditioning their curative effect, alkaloids take important place. Happy blend of low toxicity and high pharmacological activity make them extremely promising for prevention and treatment of a number of severe diseases. Therefore, a search for plants containing alkaloids, research of their chemical structure and development of simple and cost-effective ways of obtaining them for the purpose of creation of new effective medicines is a topical issue [4], [5].

Analyzing the scope of research in the field of chemistry, pharmacology and technology of alkaloids, we can make a conclusion about that there is no system approach during the research of relation structure-activity on different groups of these substances. It is connected not only with a complex structure of their molecules, but also with insufficient information on the nature of their effect on organs, tissues and other targets in organism. Moreover, during the research of such plant-based medicines researches face the issue of their bioavailability and passing biological membranes, experience difficulties in determination of vector of biological effect.

Considering the above, the research of influence of the structure of alkaloids on their antiviral properties is of significant and practical interest.

The purpose of this research was to identify pharmacophore groups in the structure of alkaloids of endemic *Polygonum* L. plants growing in Kazakhstan responsible for their antiviral action.

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II. Materials and Methods

A. Plant Materials

Plant raw materials (*Polygonum amphibium* L., *Polygonum minus* Huds. Fl. Angl.) were collected in the foothill of Zailiyskiy Alatau (the Republic of Kazakhstan) in blossoming period in July 2014.

B. Extraction and Chromatography

To isolate alkaloids modified methods were used [6], [7]: finely powdered dried herb was put in extraction unit. Alkaloids during their vigorous mixing were trice isolated by means of chloroform were mixed at ration 1:5 within 3 hours, after that the extract was drained and concentrated to a small volume at the temperature not higher than 50^0C.

After that the mixture was re-extracted by means of 3% HCl water solution. Salts of alkaloids formed during extraction are highly soluble in water and pass from chloroform phase to water solution. The obtained water solution of alcaloid salts was secondarily re-extracted by means of chloroform after preliminary alkalization of solution to pH 8-9. The extract of the sum of alkaloid base was divided from the water layer using water-soluble admixtures and was thoroughly flushed with water to neutral reaction of flush waters, concentrated in soft conditions.

Further, the obtained concentrate was divided on the column with silica gel, eluting alkaloids using ethylacetate-hexane mixtures of a composition from 1:9 to 3:7 with further re-chromatography of obtained fractions on silica gel, eluting by means of acetone-hexane mixture, with increase of the content of the latter in mixture from 1:4 to 1:1.

For HPLC of division, isolation and analysis of the obtained alkaloids reverse-phase HPLC on Zorbax ODS (5μm) column was used during gradient eluation with mixture 0.005M Na_2HPO_4 (pH 6) and acetonitrile from 80:20 to 20:80 for 20 min, using UV detector (306 nm), as well as the mixture of

acetonitrile with water if the content of acetonitrile is increased from 26 to 52% within 40 minutes using UV-detector with 280 nm in detection wavelength [6], [8].

As a result, 15 individual alkaloids were isolated from *Polygonum* L. plants: hydroxypolginone, dihydropolgridine, polgridine, lelobandine, polgacrine, methoxypolgacrine, hydroxypolgamine, polginine, lobeline, polginone, polgamine, lobelandine, hydroxylelobandine, 6-methoxylelobandine, 8-propyl-10-phenyllobeliolone.

C. Antiviral Examinations

Antiviral activity of alkaloids of *Polygonum* L. plants was researched in the Institute of Microbiology and Virology of the Ministry of Education and Science of the Republic of Kazakhstan.

The following viruses were used in the work:

1) Orthomyxoviruses: Bird flu virus, strain A/FPV/Rostock/ 34, human flu viruses - strains A/MRC/11, A/Leningrad/ 54/1, A/X-7, A/X-73, swine flu viruses A/swine/Iowa/30.
2) Paramyxoviruses: Newcastle disease virus, strains A/ PMV-1/chicken/Beaudette, A/PMV-1/chicken/LaSota/46, PVM-1/chicken/Almaty/47/98, Sendai parainfluenza virus (strain 960).

Virus titer in allantois fluid was 10^7-10^9 ID_{50}/ml.

Hemagglutinating activity of viruses was determined as per standard methods using chick-embryo suspended material.

Neuraminidase activity was determined using standard thiobarbituric method as per Amoniff using fetuin as a substrate.

Virus-inhibiting properties of compounds were studies in experiments with ortho- and paramyxoviruses on the model of chick-embryos. Anti-viral properties were determined using 'screening test' method designed to neutralization of a virus at the amount of $100EID_{50}$ with set concentrations of medicines. The difference of virus titer compared to control group was deemed as the criterion of antiviral action. At that, as a rule, only full inhibition of titer of virus was taken into consideration.

Virucidal activity of substances researched was determined by treatment of virus-containing material with chemical compounds at 37^0C within 30 minutes with further titration of infectivity of treated material. The difference between the titer in a virus in sample without exposition and its titer after it was taken for real virucidal action. If the difference in titers was 1.0-2.0 lg, a substance was deemed having moderate or expressed activity [9].

Inhibition of neuraminidase activity: 0.1ml of phosphate-salt buffer containing different concentrations of substances researched was added to 0.1 ml of solution of virus or glycoproteins. The mixture was incubated within 30 min at 37^0C, after that 0.1 ml of phosphate-salt buffer (pH-5.9) and 0.1ml of fetuin solution. The mixture was incubated again with periodic shaking within 18 hours at 37^0C. The reaction was being stopped by adding 0.1 ml of periodate. It was left for 20 minutes at room temperature and added 1.0 ml of sodium arsenite. After that, 2.5 ml of thiobarbituric acid was added and was being boiled within 7-15 minutes, then it was cooled and added 4.3 ml butanol, shook intensively within 3 minutes to discolor butanol layer to pink. The obtained solution was centrifuged at 2000 rpm within 5 minutes. The activity of enzyme was judged based on optical density measured on spectrophotometer at wavelength equal to 549 nm [10].

Infectious titer of viruses was determined based on Reed and Muench method [11].

D. Statistical Analysis

For mathematic processing of the results methods of setting average values and their average errors were used. The results were statistically processed using 'Statistica 6.0' software package. The reliable values were those at achieved significance point $p<0.05$.

III. Results and Discussion

Currently different medicines are used in complex treatment of viral infections: virus-specific substances acting directly on viruses; interferons and interferon inducers able to inhibit synthesis of viral particles; immune response modifiers correcting dysimmunities arisen against the background of viral infections; symptomatic medicines influencing on general symptoms (temperature, pain syndrome, cough); pathogenetic medicines used in case of intoxication, dehydration, allergic reactions etc.

The best results in treatment of viral diseases are achieved in affecting of all range of antiviral medicines on viral infection. However, pathogenetic and symptomatic medicines are most frequently used whereas virus-specific and biologic response modifier medicine has not taken yet the leading place in therapy of viral infections.

One of the groups of secondary metabolites of the plants - alkaloids can be related the number of the most promising sources of medicines of plant origin. Currently, the structure of more than 7500 compounds has been identified [12], [13].

Antioxidant activity and related ability of many metabolites of this class to act as agent preventing or inhibiting formation of tumors, strengthening blood vessels, protecting gastrointestinal tract and liver, stimulating brain and heart function has been established for the researched compounds of this group [14]-[16].

During experiments it has been demonstrated that during research of compounds in maximum concentration that nine substances (polgridine; polginine; polginone; polgacrine; hydroxypolginone; dihydropolgridine; lobeline; lelobandine; and 8-methyl-10-phenyllobelidiol) 100% inhibiting effect relating to bird flu virus. Hydroxypolgamine and methoxy-polgacrine, lobelandine, 8-propyl-10-phenyl-lobeliolone demonstrated antiviral activity to the lesser degree, decreasing virus reproduction in allantois cavity of chick-embryos by в 60, 40 and 20% respectively. Polgamine, 6-methoxy-lelobandine and hydroxylelobandine did not demonstrate virus-inhibiting action within the researched interval of concentrations. It is probable that effective dose of these substances is in the other concentration range than 0.0025-2.5%. In addition, it has been found that decrease in the

concentration of the compounds has resulted in decrease in their virus-inhibiting activity. Thus, in concentration equal to 0.25% only seven substances had virus-inhibiting action, at that 100% effect was demonstrated only by polgridine, dihydropolgridine. The three other compounds - polginine, hydroxypolginone and lelobandine decreased reproduction of virus by 40%, and polginone - by 20%.

Further decrease in concentration of the researched substances down to 0.025% has resulted to much bigger decrease in their virus-inhibiting activity. Out of twenty compounds, only four had virus-inhibiting effect, at that dihydropolgridine maximally inhibited reproduction of bird flu virus. Polgridine inhibited virus reproduction by 80%, and hydroxypolginone only by 20%. Research of compounds in the least concentration – 0.0025% demonstrated virus-inhibiting activity to bird flu virus only by dihydropolgridine – 40% [see Table I].

During research of virus-inhibiting effect of alkaloids using Newcastle disease virus model, the following results have been obtained. Research of the compounds in maximum concentration– 2.5%, has identified antiviral effect of sixteen substances out the them: dihydropolgridine, polgridine, hydroxypolginone - 100%-of reproduction of virus; lobeline, polginine (80%), polginone, polgacrine, lelobandine - 60% inhibition, polgamine, hydroxypolgamine, methoxypolgacrine (40%), 6-methoxylelobandine - 20%. No inhibiting effect of lobelandine, hydroxylelobandine and 8-propyl-10-phenyl-lobeliolone was found.

With decrease in concentration of the medicines down to 0.25% it has been proven that polgridine and hydroxypolginone inhibited reproduction of virus by 80%. Medicines polginine, polginone, lobeline and lelobandine, methoxypolgacrine decreased virus reproduction by 60 and 40% respectively. Other seven substances in concentration equal to 0.25% demonstrated 20% level of inhibition of viral particles in allantois cavity of chick-embryos.

At researched concentration equal to 0.025%, ten compounds non-sensitive to Newcastle disease virus were found: polgamine, polginine, polgacrine, 6-methoxy-lelobandine, 8-propyl-10-phenyllobeliolone, lobelandine and hydroxylelobandine. The rest ones continued to keep antiviral effect, although to relatively lesser extent. Compounds polginone, hydroxypolginone, lobeline and polgridine inhibited reproduction of virus by 60, 60, 40 and 40% respectively.

Thus, during the conducted researches is has been demonstrated that the group of alkaloid compounds in the researched range of concentrations had high virus-inhibiting effect both on bird flu virus and on Newcastle disease virus.

In the result, it has been demonstrated that dihydropolgridine showed the maximum antiviral activity. During experiments it was shown that at 2.5% concentration of dihydropolgridine, there is a full inhibition of a reproduction of all above strains of viruses [see in Fig. 1].

TABLE I
VIRUS-INHIBITING ACTIVITY OF POLYGONUM ALKALOIDS ON THE MODEL OF MYXOVIRUSES

Alkaloid	Concentration	A/FPV/ Rostock/34	APMV-1/chicken/ La Sota/46
Hydroxypolginone	2.5%	100	60
	0.25%	0	20
	0.025%	0	0
	0.0025%	0	0
Dihydropolgridine	2.5%	100	100
	0.25%	100	20
	0.025%	100	20
	0.0025%	40	0
Polgridine	2.5%	100	100
	0.25%	100	80
	0.025%	60	40
	0.0025%	0	0
Polgacrine	2.5%	100	100
	0.25%	40	80
	0.025%	20	60
	0.0025%	0	0
Polginine	2.5%	100	80
	0.25%	40	60
	0.025%	0	0
	0.0025%	0	0
Lelobandine	2.5%	100	60
	0.25%	40	40
	0.025%	0	40
	0.0025%	0	0
Lobeline	2.5%	100	80
	0.25%	0	60
	0.025%	0	60
	0.0025%	0	0
Polginone	2.5%	100	60
	0.25%	20	60
	0.025%	0	60
	0.0025%	0	0
Methoxypolgacrine	2.5%	60	40
	0.25%	0	40
	0.025%	0	20
	0.0025%	0	0
Hydroxypolgamine	2.5%	60	40
	0.25%	0	20
	0.025%	0	20
	0.0025%	0	0
Polgamine	2.5%	0	40
	0.25%	0	20
	0.025%	0	0
	0.0025%	0	0
Lobelandine	2.5%	40	0
	0.25%	0	0
	0.025%	0	0
	0.0025%	0	0
Hydroxylelobandine	2.5%	0	0
	0.25%	0	0
	0.025%	0	0
	0.0025%	0	0
6-methoxylelobandine	2.5%	0	20
	0.25%	0	20
	0.025%	0	0
	0.0025%	0	0
8-propyl-10-phenyl-lobeliolone	2.5%	20	0
	0.25%	0	0
	0.025%	0	0
	0.0025%	0	0

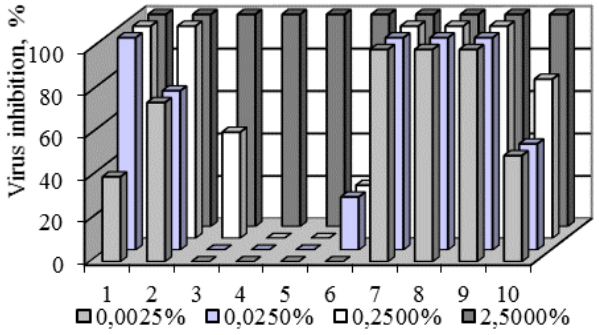

Fig. 1 Inhibition of viruses by various concentration of dihydropolgridine (1- A/FPV/Rostock/34, 2- A/Leningrad /54/1, 3- A/swine/Iowa/30, 4- A/MRC/11, 5- A/X-7, 6- NDV(L Sota), 7- A/X-73, 8- Sendai (960), 9- NDV(Beaudette), 10- NDV/47/98)

During the analysis of the obtained data, it has been established that antiviral activity of alkaloid compounds depends on their structure to a great extent. Thus, increase in molecular weight of a compound decreases the ability of a medicine to inhibit reproduction of viruses (up to 40%).

Glycosidation of an alkaloid, irrespective of its nature and point of addition of carbohydrate component to molecules of tested compounds, results in enhancement in amphiphilic properties, and as a result, enhances the ability of compounds to inhibit reproduction of viruses.

As a result of the conducted research we have also established that the value of antiviral activity is increased owing to carbonyl group in molecule, at that this increase in some cases reaches 12% of the activity of the same molecule which has no carbonyl group. Moreover, phenolic hydroxyls have positive influence on the value of antiviral activity, at that, antiviral action increased on 14 researched strains of viruses by 3.0-3.2% when every new phenolic hydroxyl group appeared in molecule. Ortho-position of phenolic hydroxyl and carbonyl group of ketonic type also had significant influence on the value of activity, increasing it up to 8%. The presence of different types of carbohydrate substitutes had no serious influence on the value of activity, although for medical purposes and application in veterinary medicine glycosidation is very important, as such substances are more soluble and, hence, less therapeutic concentration compared to aglycons of the same type. Moreover, the activity of methoxylated, ethoxylated and prenylated derivatives isolated us has been researched. It was established that methoxylation decreases antiviral activity quintessentially – down to 40% (for permethyl derivative) and in case of mono-methoxylation - down to 9.0-9.5%; ethoxylation of molecule did not have such serious influence on the value of activity, in this case decrease in the value of antiviral effect did not exceed 2.0-2.7%. Prenylated derivatives as well as glycosidated forms demonstrated comparable results with non-prenylated forms of compounds identical on the structure.

IV. Conclusion

Using combination of chemical and chromatographic methods, individually 15 alkaloids of plant origin of different structural groups were isolated from *Polygonum* L. plants growing in Kazakhstan.

It has been established that *Polygonum* L. alkaloids has high antiviral effect to influenza and parainfluenza viruses.

The analysis of correlation of the structure and antiviral activity of alkaloids allowed identifying the main pharmacophore groups, among which the most important are glycosidation, the presence of carbonyl and hydroxyl groups, molecular weight and molecular size.

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References

[1] P. M. Dewick, *Medicinal Natural Products,* N.-Y.: John Wiley & Sons Ltd, 2002.
[2] M. Heinrich, J. Barnes, S. Gibbons, *Fundamentals of Pharmacognosy and Phytotherapy*, N.-Y.: Elsevier, 2012.
[3] M. Wichtl, *Herbal drugs and phytopharmaceuticals*, Stuttgart: CRC Press, 2007.
[4] B. Ozcelik, M. Aslan, I. Orhan, T. Karaoglu, *Microbiol. Res.*, vol. 160, pp. 159-164, 2005.
[5] B. S. Min, Y. H. Kim, M. Tomiyama, N. Nakamura, *Phytother. Res.*, vol. 15, pp. 481-486, 2001.
[6] R. A. Muzychkina, D. Yu. Korulkin, *Bioactive constituents of plants. Extraction, separation and analysis*, Almaty: Atamura, 2006.
[7] R. A. Muzychkina, D. Yu. Korulkin, *Methodology of research of natural metabolites*, Almaty: MV-Print, 2012.
[8] L. Kursinszki, H. Hank, I. Laszlo, *J. Chromatogr. A*, vol. 1091, pp. 32–39, 2005.
[9] H. Balfour, M. E. M. Benwell, P. E. Holtom, *New Engl. J. of Med.,* vol. 340, no. 16, pp. 1255-1268, 1999.
[10] E. Clerco, M. Vandewalle, *J. Pharmacol. Exper. Therap*., vol. 297, no. 1, pp. 1-10, 2000.
[11] J. M. Hu, G. D. Hsiung, *Antiviral. Res*., vol. 11, no. 5-6, pp. 217-232, 1989.
[12] E. Fattorusso, O. Taglialatela-Scafati, *Modern Alkaloids: Structure, Isolation, Synthesis and Biology*, Weinheim: Wiley-VCH, 2008..
[13] L. J. Cseke, A. Kirakosyan, P. B. Kaufman, *Natural Products from Plants*, London: CRC Press, 2006.
[14] J. Dai, R. J. Mumper, P. E. Larsen, J. C. Zwick, *Molecules*, vol. 15, pp.7313-7352, 2010.
[15] K. M. Marshalla, S. S. Matsumotoa, J. A. Holdenb, *Biochem. Pharmacol*., vol. 66, pp. 447-462, 2003.
[16] A. Stepanchikova, A. Lagunin, D. Filimonov, V. Poroikov, *Current Med. Chem.*, vol.10, pp. 225-232, 2003.