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IN VITRO ANTIVIRAL ACTIVITY OF LEAF EXTRACTS OF *PLANTAGO MAJOR*, *PLANTAGO LANCEOLATA*, AND *RUBUS IDAEUS*

Advances in organic chemistry, biochemistry, biotechnology, and molecular virology have made it possible to synthesize a large number of antiviral drugs belonging to different pharmacological groups. However, one but the significant disadvantage of these drugs is their high toxicity. Therefore, along with the screening of new drugs among synthetic compounds, scientists are actively conducting research on antiviral agents of natural origin. Natural products with antiviral properties have advantages over synthetic compounds due to their low toxicity, minimal side effects, and mild action by various mechanisms. The ***aim*** *of the study was to investigate the properties of aqueous-alcoholic extracts of plantain leaves (Plantago major L. and Plantago lanceolata L.), wild and garden raspberry leaves (Rubus idaeus L.) and their fermented variants on the model of human adenoviruses (HAdV3, HAdV5, and HAdV7).* ***Methods.*** *Determination of cytotoxicity and antiviral action of extracts was performed by standard methods using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The titer of the virus synthesized in the presence of drugs was determined by the end of the virus dilution, which caused a 50% development of the cytopathic effect of the virus on cells (CPE). Neoflazid developed by Ecopharm (Ukraine) was used as a reference drug. It contained carboxylic acids and flavonoid glycosides isolated from wild cereals Deschampsia caespitosa L. (pike, turf) and Calamagrostis epigeios L. (dugout). All studies were performed in three replicates; the number of parallel determinations was 3—4. Calculated mean values, standard*

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deviation, and mean errors. Differences in the averages were considered significant at p<0.05. The research results were processed using Microsoft Office Excel 2010. ***Results.*** *Low cytotoxicity of extracts of Plantago major L. and Rubus idaeus L. (wild) leaves and their fermented variants was shown, CC_{50} was >3 mg/mL. The toxicity of extracts from the leaves of Plantago lanceolata L. and Rubus idaeus L. (garden) and their fermented variants, was slightly higher (CC_{50}=1.5 mg/mL). The extracts showed either a slight antiviral effect or its complete absence when used in a prophylactic regimen. We observed effective inhibition of reproduction of adenoviruses when used extracts after adsorption of viruses. Extract of plantain leaves in concentrations of 0.06—3 mg/mL inhibited the reproduction of HAdV5 by 68—83% and inhibited the reproduction of 3 mg/mL HAdV3 and HAdV7 by 55% and 11%, respectively. Extract of Rubus idaeus L. (wild) leaves in the concentration range of 0.06—3 mg/mL inhibited the reproduction of HAdV5 by 65—89%, HAdV3 by 41—84%, and HAdV7 by 22—59%. The maximum inhibition of reproduction of HAdV3 (by 34%) was shown for the extract from the leaves of Rubus idaeus L. (garden) at a concentration of 0.38 mg/m. The reproduction of the other viruses was suppressed by only 4—22%. It has been shown that the extracts of plantain and wild raspberry significantly affected the infectivity of viral offspring. Extract of plantain at a concentration of 3 mg/mL inhibited the reproduction of HAdV5 by 1.5 lg, and fermented extract of plantain — by 1 lg. The latter at a concentration of 0.06 mg/mL inhibited the formation of new viral offspring, the index of reproductive inhibition (IRI) being 1.6 lg. Both fermented and unfermented Rubus idaeus L. (wild) extracts had almost the same antiviral activity with IRI in the range 1.45 lg — 1.6 lg. Extracts of plantain and raspberry, regardless of the concentrations used, effectively inhibited the formation of infectious offspring of HAdV3. The maximum IRI was 1.44 lg for plantain extract and 1.5 lg for fermented plantain extract. Both the raspberry extracts (fermented and non-fermented) inhibited the synthesis of adenovirus serotype 3 by 1.46 — 1.54 lg. The drug Neoflazid completely inhibited the formation of infectious adenovirus at a concentration of 7.1 μg/mL. No virulicidal activity of all extracts against human adenoviruses 3, 5, and 7 serotypes was detected. We found different antiviral activities of extracts of wild and garden raspberry leaves, and we can assume that the flavonoid composition of the extracts plays an important role in their activity.* ***Conclusions.*** *Our new data on a wide range of anti-adenoviral activity of plantain and raspberry extracts are a prerequisite for further studies of the properties of individual components of extracts, in order to create an anti-adenoviral drug and give recommendations for its pharmacological use.*

Keywords: secondary metabolites, flavonoids, plantain and raspberry extracts, human adenoviruses, antivirus action.

Mankind is constantly exposed to viral infections that can cause pandemics (influenza, smallpox, HIV, polio, coronavirus), epidemics (dengue fever, yellow fever, chikungunya, West Nile), outbreaks, and sporadic diseases. Viruses cause about 70—90% of human infectious pathology [1]. Common infections are caused by viruses that can persist for life in the body (hepatitis, herpes, adenovirus). To date, human adenoviruses have been represented by more than 100 serotypes, and their number is steadily increasing [2]. They are the causative agents of a wide range of infectious diseases in humans with damage to the eyes, respiratory, enteric, and urogenital tract. They can be dormant in the human body and are risk factors for the development of severe generalized diseases in persons receiving immunosuppressive therapy after transplantation, and in patients with HIV [3—5]. They can retain infectious properties for a long time, being in the environment, in air, in liquid, or on the surfaces of subjects. Viruses are released from the water of rivers and seas throughout the year [6]. Adenoviruses enter the human body mainly by airborne droplets through the respiratory tract, in gastroenteritis — with food, in eye diseases — in the case of virus on the conjunctiva. In immunocompetent individuals, the disease manifests itself in the form of acute respiratory disease, often caused by 1, 2, 3, 5, 6, and 7 serotypes [7]. Typical outbreaks occur in closed groups at kindergartens and schools, among conscripts, and the so-called "hospital infections". At the same time, there is no licensed drug for etiotropic therapy of adenoviral diseases in the world [8]. Due to advances in organic chemistry, biochemistry, biotechnology, and molecular virology in recent years, a huge number of antiviral drugs belonging to different pharmacological groups have been synthesized. However, new viruses

appear, previously known ones mutate and become resistant, and therefore, the search for new antiviral drugs remains extremely important. A significant disadvantage of drugs based on chemical compounds is their significant toxicity. Along with the screening of new drugs among synthetic compounds, studies of antiviral agents of natural origin are being conducted. Natural products with antiviral properties have the benefits of minimal side effects, low toxicity, and gentle action by various mechanisms [9]. The European market for antiviral substances based on herbal raw materials is currently considered to be the only largest commercial market in the world for medicinal plants and herbal medicines. In France, Germany, Italy, Sweden, Switzerland, and the United Kingdom, such drugs are used as an adjunct to treatment with synthetic drugs. In Central and Eastern Europe, they are valued as an alternative to expensive drugs [10].

The aim of our work was to study the cytotoxic, virucidal, and antiviral effects of aqueous-alcoholic extracts of plantain leaves (*Plantago major* L.), plantain lanceolata (*Plantago lanceolata* L.), wild and garden raspberry leaves (*Rubus idaeus* L.) and their fermented variants against human adenoviruses (HAdV3, HAdV5, and HAdV7).

Materials and methods. *Preparation of plant extracts.* Dry leaves of plantain and raspberry were ground to a powdery state and passed through a sieve with a pore diameter of 1 mm. The material (5 g) was extracted for 2 h with distilled water (150 mL) in a water bath at 80 °C (reflux) and filtered. Ethanol was added to the filtrates to a final concentration of 20%. Polysaccharides and alcohol-insoluble macromolar compounds were removed by centrifugation (5 min at 5000 rpm). The supernatant was transferred into tubes and stored at 4 °C. To improve the functional organs and reduce toxic side effects, the method of fermentation of extracts was used. The fermentation was performed for 2 hr at a temperature of 37 °C using the exoenzyme culture fluid *Saccharomyces cerevisiae*, assigning filtration by short-term heating of the substrate to 90 °C. The fermented liquid was centrifuged for 10 min at 8000 rpm. Supernatants were collected and stored in a refrigerator at 4 °C. The following leaf extracts were studied*:* plantain (*Plantago major* L. and *Plantago lanceolata* L.), wild and garden raspberries (*Rubus idaeus* L.), and their fermented variants. The concentration of all extracts was 30 mg/mL. The drug Neoflazid developed by Ecopharm (Ukraine) was used as a reference drug. It contained carboxylic acids and flavonoid glycosides isolated from wild cereals *Deschampsia caespitosa* L. (pike, turf) and *Calamagrostis epigeios* L. (dugout). The content of flavonoids in the drug Neoflazid was not less than 4.0 mg/g in terms of rutin. *Chromatography (HPLC).* Separation of the secondary metabolites of the leaves was performed using the 2-eluent scheme, which we present in the relevant section: (eluent I = 5 g/L aqueous solution of orthophosphoric acid; eluent II = acetonitrile) on a column Agilent Zorbax SB-C18, 5μm, 4.6 × 250 mm. Sample volume 5 μL, column temperature 20 °C, flow rate 1.0 mL/min. Basic detection was performed at wavelengths 205 and 254 nm [11]. *Cell culture and viruses.* Reference strains of human adenoviruses 3, 5, and 7 serotypes (HAdV3, HADV5, and HADV7), supported by the D.K. Zabolotny Institute of Microbiology and Virology of NAS of Ukraine, were cultured in human laryngeal carcinoma cells Hep-2 (ECACC N86030501). The cytotoxicity of the extracts was tested *in vitro* using the MTT method [12]. Cells in 96-well plates were incubated with the extracts (two-fold dilutions in the concentration range from 0.188 to 3 mg/mL) for 48 hr, made 20 μL/well of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma) at a concentration of 5 mg/mL, was cultured for 2—4 hr at 37 ºC in atmosphere of 5% CO_2. The culture medium was removed, made at 100 μL/well 96º ethyl alcohol. The study was performed in 3 replicates. The optical density of the samples

was determined spectrophotometrically on a Multiskan FC reader (Thermo Fisher Scientific, USA) at a wavelength of 538 nm. The percentage of living cells was calculated for each concentration of substance compared to control cells (without the addition of extracts). The concentration at which 50% inhibition of cell population growth (CC_{50}) occurs was determined based on the dose-response curves using Microsoft Excel 2010 software for Pentium Pro. *Studies of the virucidal action of extracts* were performed according to the classical scheme [13]. Extracts at the maximum non-toxic concentrations previously determined were mixed with equal volumes of an undiluted virus and maintained at 37 ºC for 2 hr. Next, Hep-2 cells in 96-well plates were infected with serial 10-fold dilutions of the virus-extract suspension (50 µL/well) in 3 replicates. A mixture of the virus with an equal volume of support medium was maintained as a control one under the same conditions. After 4—5 days of cultivation, with the appearance of pronounced cytopathogenic action (CPD) of the virus in the control, further processing was performed according to the standard method of MTT. Virus titers were determined at the end point of the dilution, which causes 50% CPD. Using the prediction function of Microsoft Excel, virus titers and the index of inhibition of virus reproduction (IRI) were calculated as follows:

Virus titer control (lg) —
Virus titer experiment (lg)

The antiviral activity of the extracts was investigated by the MTT method, using the following cell treatment regimens: 1) 1 hr before infection with the virus; 2) during the adsorption of the virus; 3) after infection of cells with a virus [14]. To do this, after 24 hr of cell growth in 96-well plates and the formation of 90% of the monolayer, the growth medium was removed. According to scheme 1, different concentrations of extracts (200 µL/well) were added to cells, according to Scheme 2 were added to cells 50 µL/well of the viral mixture with different concentrations of extracts, and according to scheme 3 were added to cells virus in the amount of 50 µL/well. The multiplicity of infection was determined in advance. After 1 hr of contact ~~to~~ with the wells (according to Scheme 1, after removal of the medium with extracts), made 50 µL/well of virus. After 2 hr of adsorption, the virus was removed according to all schemes, the cells were washed with Hanks' solution (Sigma, USA), and 200 µL of support medium (Schemes 1 and 2) or medium containing extracts in different dilutions (Scheme 3) were added. Then the plates were incubated at 37 °C in an atmosphere of 5% CO_2 for 4 days. The contact time of the studied extracts with infected Hep-2 cells (Scheme 3) was 96 hr. Next, 20 µL/well of MTT solution was added to the wells. The percentage of protection against the virus was calculated by the formula [15]:

(ODexp.) — (ODcv) / (ODcc) —
(ODcv) × 100%,

Where ODexp. is the optical density in the wells with hoods, Odcv is the optical density of virus control, and ODcc is the optical density of cell control.

The infectious titer of the virus synthesized in the presence of extracts (introduced according to Scheme 3) was determined by the end point of dilution of the virus, which causes 50% CPD [13]. Dilution of the virus, which reduces the optical density of the sample compared to the optical density of cell control by 50% and is the titer of the virus and is expressed in lg TCD_{50}/mL (decimal logarithms of 50% tissue cytopathic doses per milliliter). After 5 days of culturing the cells with virus-containing material, samples were taken, frozen three times, thawed, and centrifuged for 20 min at 3.000 rpm. The cell pellets were removed, serial 10-fold dilutions were prepared, and the monolayer of daily Hep-2 cells was infected. After 5 days of cultivation, MTT solution was added to the plate, and the optical densities of the samples were determined. Then

the infectious titer of the virus and the index of inhibition of virus reproduction (IRI) were calculated. *Statistical processing of the results*. All studies were performed in 3 replicates; the number of parallel determinations was 3—4. Mean values, standard deviation, and mean errors were calculated. Differences in averages were considered significant at $p<0.05$. The research results were processed using Microsoft Office Excel 2010.

Using both schemes, we showed a slight anti-adenoviral activity of the extracts, which did not exceed 7%. According to the prophylactic scheme of the study (Fig. 1, *a*), the activities of *Plantago major* L. plantain leaf extract and the fermented extract were more pronounced. In the case of the presence of extracts during the adsorption of the virus (Fig. 1, *b*), we also showed a slight antiviral activity of fermented extract from the leaves of wild raspberry (*Rubus idaeus* L.), which had a slight concentration dependence. Extracts from the leaves of *Plantago lanceolata* L. and garden raspberries and their fermented variants did not show antiviral properties when applied according to the prophylactic scheme and in the case of the presence during the virus adsorption. Thus, the lack of a pronounced antiviral effect of the extracts in the prophylactic mode and during the virus adsorption indicates that the extracts do not block or modify cellular receptors and do not affect the ability of cells to adsorb the virus. The activity of unfermented extracts of *Plantago major* L. and *Plantago lanceolata* L. as well as wild and garden *Rubus idaeus* L. in the reproduction of human adenoviruses of 3, 5, and 7 serotypes when introduced into Hep-2 cells immediately after adsorption of viruses was studied. This scheme allows one to detect the effect on the viral genome replication, protein expression, and virus formation in the cell [14]. *Plantago major* L. extract at a concentration of 0.06—3 mg/mL inhibited the reproduction of HAdV5 by 68—83% (Fig. 2). It was slightly less effective against HAdV3 reproduction because only at the maximum concentration 3 mg/mL did the extract inhibit the CPD of the virus by 55%. Regarding HAdV7, the extract did not show significant antiviral activity: it inhibited the reproduction of the virus by 11% only at the maximum concentration (Fig. 2).

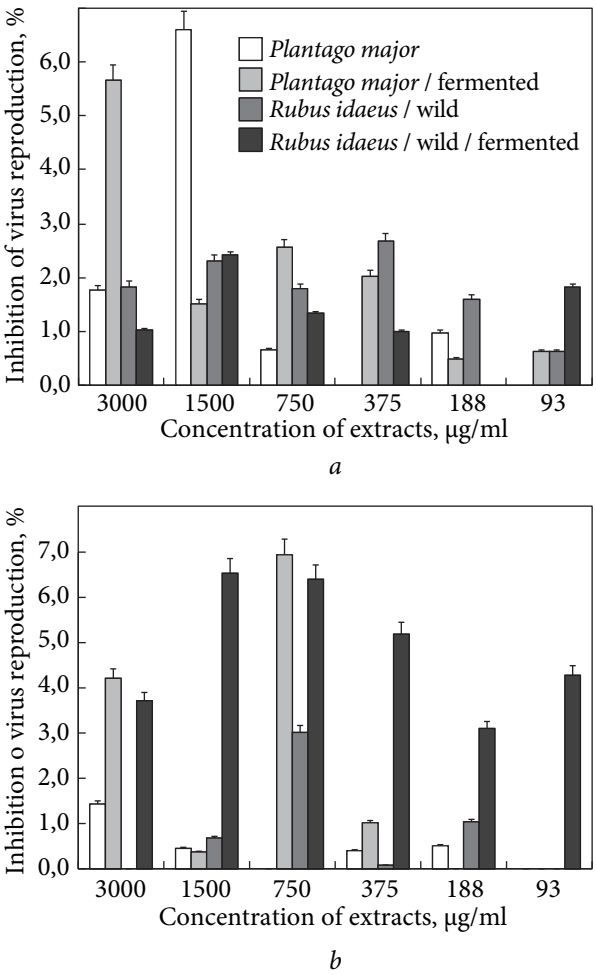


Fig. 1. Inhibition of HAdV5 reproduction by plantain leaf extracts, wild raspberries, and their fermented variants (*a*) according to the prophylactic application scheme 1 hr before infection and (*b*) in the case of the extract presence during virus adsorption

The extract of wild raspberry leaves inhibited the reproduction of HAdV5 by 65—89% in the concentration range of 0.06—3 mg/mL (Fig. 3). It had a pronounced antiviral effect on HAdV3 (inhibition of the CPD of the virus by 41—84%).

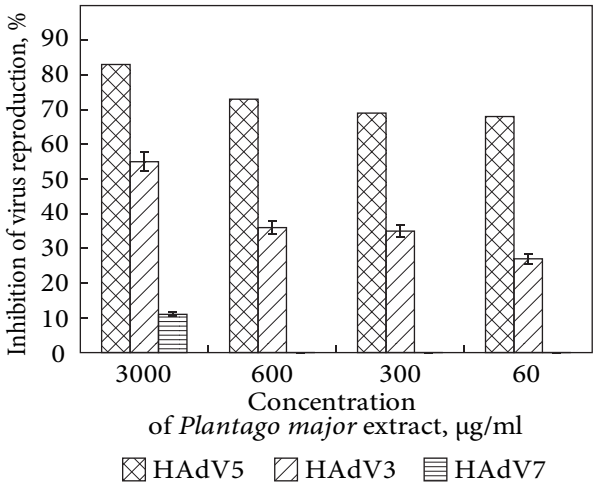


Fig. 2. Inhibition of reproduction of human adenoviruses (HAdV3, HAdV5, and HAdV7) in cell culture Hep-2 extract from the leaves of *Plantago major* L.

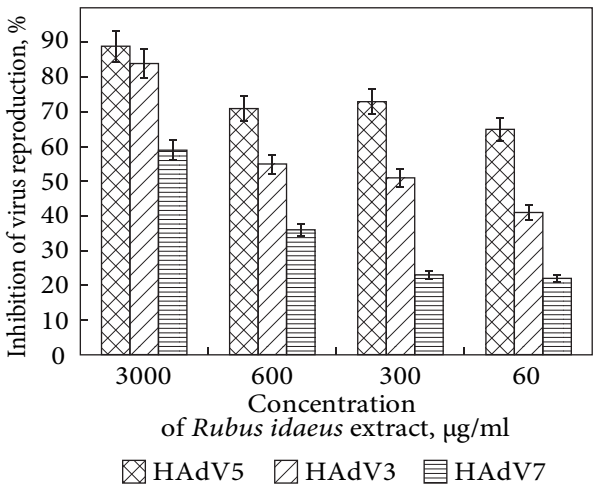


Fig. 3. Inhibition of reproduction of human adenoviruses in Hep-2 cells by the extract of wild raspberry (*Rubus idaeus* L.)

Regarding HAdV7, the action was more effective than by extracts from the leaves of plantain: in the concentration range of 0.06—3 mg/mL, inhibition of virus reproduction was by 22—59%. Thus, wild raspberry leaf extract had a wider range of activity and more effectively inhibited the reproduction of adenoviruses. As for extracts of *Plantago lanceolata* L. and garden raspberry *Rubus idaeus* L., their antiviral activity was insignificant: the maximum inhibition of reproduction of HAdV3 by 34% is shown for the extract of raspberry leaves at a concentration of 0.38 mg/mL, and the reproduction of other viruses was suppressed only by 4—22% (not illustrated).

Using the prediction function of the computer program Microsoft Excel 2010, concentrations of extracts were determined, which suppressed the reproduction of viruses by 50% (EC_{50}). The results of the analysis of antiviral activity show that the most effective inhibition of the reproduction of all adenoviruses was demonstrated by wild raspberry leaf extract (Table 1).

The influence of extracts on the synthesis of adenoviruses *de novo* was studied. It has been shown that *Plantago major* L. and *Rubus idaeus* L. (wild) not only inhibited the reproduction of viruses but also significantly influenced the formation of viral offspring. Extract of *Plantago major* L. at a concentration of 3 mg/mL inhibited the reproduction of adenovirus by 1.5 lg, fermented extract — by 1.0 lg (Fig. 4). With decreasing concentration of unfermented extract, such intense titer suppression was no longer observed. Fermented plantain extract at a concen-

Table 1. **Concentrations of extracts that inhibited the reproduction of adenoviruses 3, 5, and 7 serotypes in Hep-2 cells**

Extract from leaves	Inhibition of reproduction (EC_{50}), mg/mL		
	HAdV3	HAdV5	HAdV7
Plantago major L.	2.3	<0.06	<3.0
Plantago lanceolata L.	<3.0	<3.0	<3.0
Rubus idaeus L. (wild)	0.4	<0.06	2.1
Rubus idaeus L. (garden)	<3.0	<3.0	<3.0

tration of 0.06 mg/mL inhibited the formation of new viral offspring more intensely than at higher concentrations; its IRI was 1.6 lg. When studying the effect of extracts from raspberry leaves, there was observed a slightly different picture. It was found that the fermentation of raspberry leaf extract did not affect its activity in any way. That is, both fermented and unfermented raspberry extracts had almost the same antiviral activity, and IRI was in the range of 1.45 lg — 1.6 lg (Fig. 4). Due to the low antiviral activity of extracts from the leaves of plantain lanceolate and garden raspberries, their effects on infectious titers of adenovirus were not detected.

The results of the study of the effect on the titers of adenovirus 3 serotype synthesized de novo in the presence of plantain and raspberry extracts are presented in Figure 5. *Plantago major* L. and *Rubus idaeus* L. extracts, regardless of the concentrations used, effectively inhibited the formation of the infectious progeny of the virus. The maximum IRI was 1.44 lg for *Plantago major* L. and 1.5 lg for fermented *Plantago major* L. extract. Both (fermented and non-fermented) *Rubus idaeus* L. extracts inhibited the synthesis of adenovirus serotype 3 by 1.46—1.54 lg. The drug Neoflazid completely inhibited the formation of infectious adenovirus at a concentration of 7.1 μg/mL.

The virulicidal activity of all extracts and Neoflazid against human adenoviruses 3, 5, and 7 serotypes was not detected.

In the course of our research, we found a difference in the activity of extracts from plants of different species of the same genus, i.e. from the leaves of garden varietal *Rubus idaeus* L. and wild raspberry *Rubus idaeus* L. or plantains *Plantago major* L. and *Plantago lanceolata* L. Extracts of raspberry *Rubus idaeus* L. (wild) and plantain *Plantago major* L. were active against adenoviruses 3,5, and 7 serotypes, while extracts from leaves of raspberry *Rubus idaeus* L. (garden) and plantain *Plantago lanceolata* L. did not have such activity. Studies of chromatographic profiles of

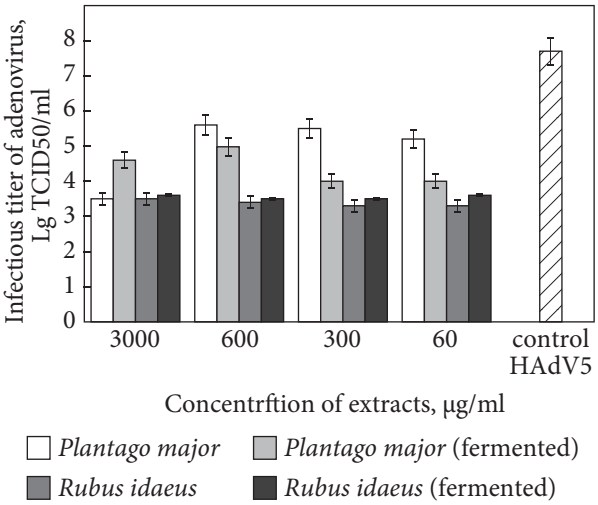


Fig. 4. The titer of adenovirus 5 serotype synthesized in Hep-2 cells in the presence of extracts from *Plantago major* L. and *Rubus idaeus* L. (wild)

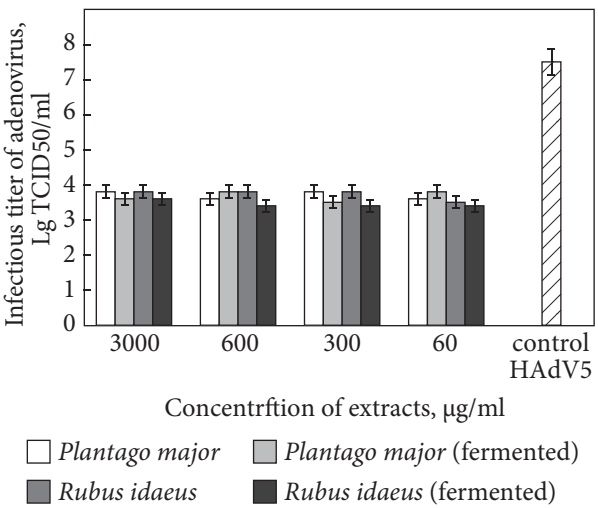


Fig. 5. The titer of adenovirus 3 serotype synthesized in Hep-2 cells in the presence of extracts from *Plantago major* L. and *Rubus idaeus* L. (wild)

hoods revealed some differences in their composition (Fig. 6). The chromatogram (b) of *Plantago major* L. extract indicates the presence of three main components with a delay of 5.918, 6.097, and 7.048 min. Similar three components were found in the chromatogram (a) of *Plantago lanceolata* L. extract, but with a slightly shorter retention time. The major component, determined

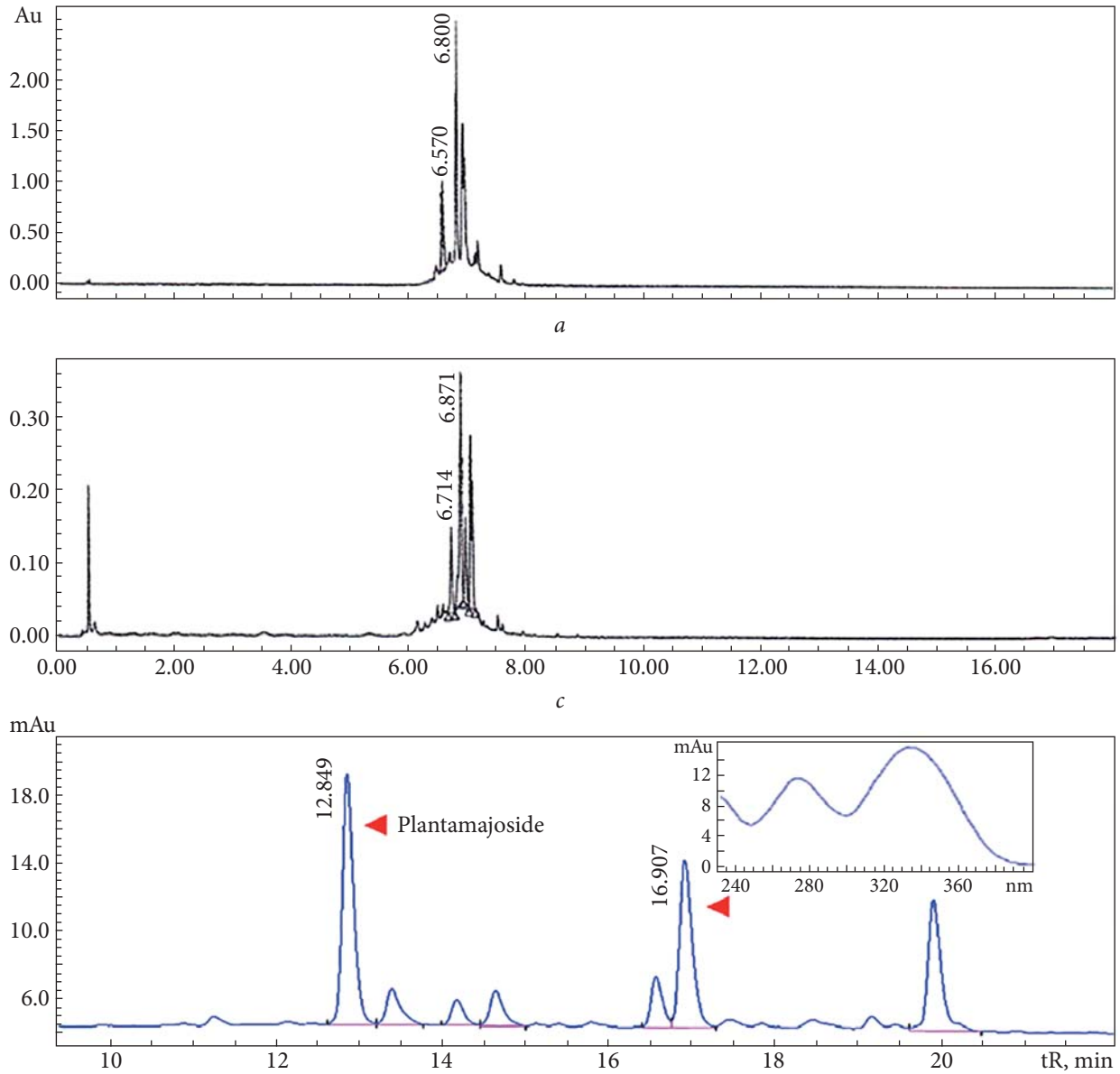
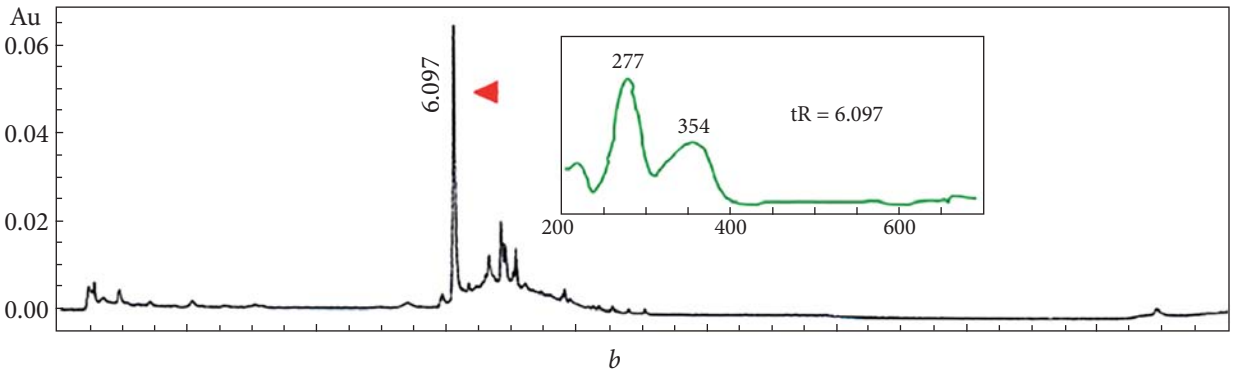


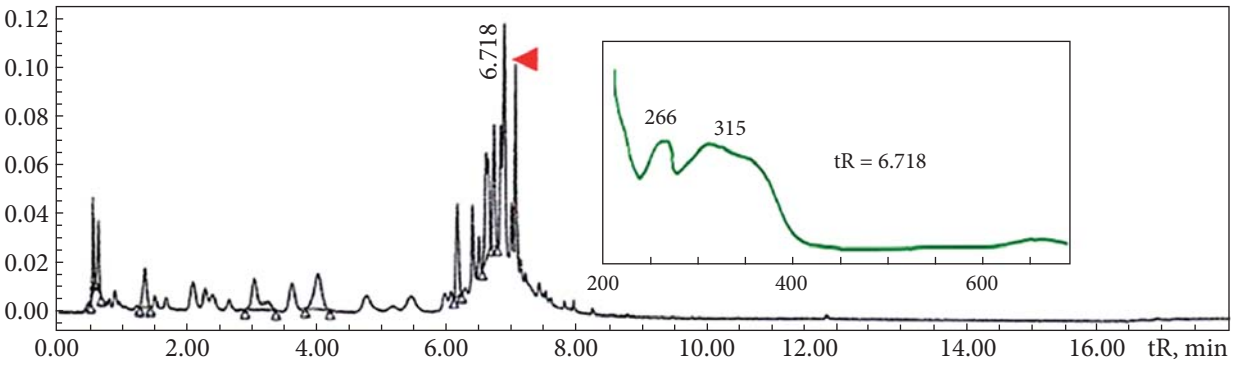
Fig. 6. Chromatographic profiles of plant extracts: *a* — *Plantago lanceolata* L.; *b* — *Plantago major* L.; *c* — *Rubus* equipped with a diode detector. Immediately before analysis, the samples were filtered through a 0.2 μm syringe umn, 150 × 2.1 mm, 5μm. Thermostating of the column 30 °C, flow rate 0.5 mL/min Program for gradient I (*a*—*d*) II (*e*) A in B: 0—20 min, 5—25% A; 20—25 min, 25—70% A; 25—30 min, 70% A. Sample volume 5 μL. Detection

with a delay of 6.097 min, exhibits two major absorption peaks at 277 and 354 nm, characteristic for phenols such as caffeic and chlorogenic acids.

These compounds are known as effective antiviral agents [16—18]. In addition, the extract of leaves of *Plantago major* L. contains plantamayoside, a derivative of caffeic acid, which has high biological activity [19]. The chromatograms (c, d) of extracts of varietal and wild raspberry leaves *Rubus idaeus* L. showed significant differences



b



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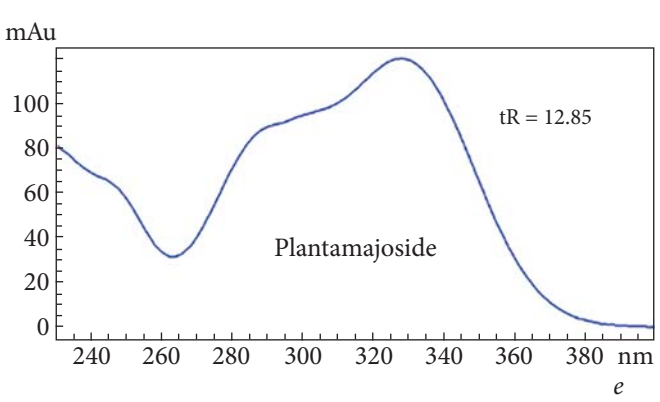

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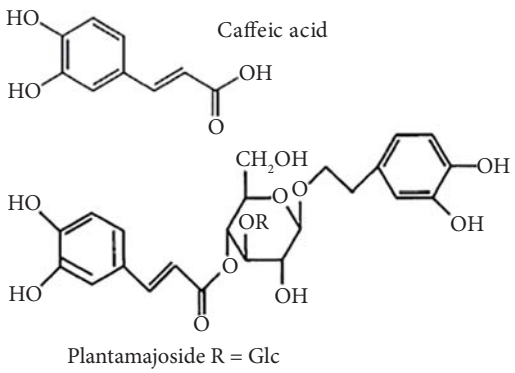

idaeus L. (natural form); *d* — *Rubus idaeus* L.(cultivar). Liquid chromatography was performed on an Agilent 1100 filter. A 2-eluent scheme (eluent A = acetonitrile, B = 2% acetic acid) was used on an Agilent Zorbax XDB-C18 col-A in B: 0—20 min, 5—70% A. Sample volume 1 μL. Basic detection at a wavelength of 254 nm. Program for gradient at wavelengths — 205, 254, 300 and 350 nm.

in the total content of flavonoids. The chromatogram of wild raspberry extract (d) has a peak with a retention time of 6.718 min, which shows two absorption peaks at 266 and 315 nm. Since we found different antiviral activities of extracts of wild and varietal raspberry leaves, we can assume that the flavonoid composition of the extracts plays an important role in their activity.

Discussion. Biologically active substances (BAS) of natural origin as a source of vari-

ous chemical compounds have a wide range of pharmacological actions. BAS contained in rapidly dividing plant cells have unique regulatory properties [20—22]. today About 50% of known anti-cancer therapies are derived from plants [23]. For example, compounds such as taxol and periwinkle alkaloids Vinca minor destabilize tumor cell microtubules and prevent rapid tumor spread. The world is actively studying the effects of compounds of natural origin against viruses of different families [24—29]. The antiviral properties of Folia Theae green tea have been shown as inhibitors of various stages of reproduction of RNA and DNA-containing viruses. It was established that the main antiviral components of green tea are catechins, which contain residues of gallic acid [10]. *Epilóbium angustifolium* has a high anti-influenza activity, similar to that of the commercial drug Tamiflu. The extract contains a large number of tannins related to antioxidant polyphenols, which have the ability to ensure human health [24].

The objects of our research plantain (*Plantago major* L.), plantain lanceolate (*Plantago lanceolate* L.), and raspberry (*Rubus idaeus* L.) are medicinal plants that have been used in traditional medicine for thousands of years to treat various human diseases. They have antipyretic, antitussive, hemostatic, anti-infective, and other useful properties. Some of the secondary metabolites identified in these plants include lignin alkaloids, catechins, terpenoids, hydrolyzed tannins, flavonoids, quercetin, quercetin glycosides, polyphenols, phenolic acids such as gallic, elogic, caffeic, and chloro [10]. We present the results of the study of cytotoxic, virucidal, and antiadenoviral effects of extracts and their fermented products. The absence of toxic effects of the extracts on Hep-2 cells indicates the prospects of their use as possible antiviral substances The lack of virucidal and prophylactic properties indicates that the extracts do not act on the extracellular virus, do not modify the cell membrane, blocking cellular receptors of the virus, which could lead to loss of infectivity of viral particles or prevent adsorption of the virus on the cell membrane. Significant effects of extracts on adenovirus reproduction, especially on the infectious titer of the virus, indicate inhibition of late stages of reproduction, which may be associated with viral nucleic acid replication, protein expression, or viral particle formation. Previously, the authors [30] have studied the effect of aqueous extract of plantain on the reproduction of human adenoviruses 3, 8, and 11 serotypes and herpes simplex viruses 1 and 2 types. It was concluded that a wide range of antiviral activity of the extract and purified compounds from *Plantago major* L. extract, which have high antiviral activity, are mainly obtained from phenolic compounds, especially caffeic acid. Purified from the extract of Plantago major L. caffeic acid showed high activity against herpes simplex viruses type 1 and 2 and human adenovirus type 3, whereas purified chlorogenic acid effectively inhibited the reproduction of adenovirus serotype 11. The literature data are confirmed and supplemented by the results of our studies on the inhibition of reproduction of adenoviruses 3, 5, and 7 serotypes. The obtained results confirm the assumption that plants of different species of the same genus can differ significantly in the contents of biochemical components, which in turn affects the presence, severity, or the absence of their antiviral properties. Thus, we found a difference in the activity of extracts from leaves of the plantain *Plantago major* L., active against adenoviruses 3, 5, and 7 serotypes and the lanceolate *Plantago lanceolata* L., which was not active. Further studies have shown that *Plantago major* L. leaf extract contains plantamayoside, a caffeic acid derivative, which is absent in *Plantago lanceolata* L.

There have been many publications since the 70s of the last century [10] devoted to the leakage of plantain, but despite the widespread use of raspberries in folk medicine, the effect of raspberries on the inhibition of viruses is insignificant. Bulgarian scientists [31] studied the activity of metalo-

logical extracts of wild berries *Rubus idaeus* L. and found the selective antiviral activity of their associations against poliovirus type 1 (PV-1), coxsackie virus B1 (105-B1), respiratory syncytial virus HR (HRSV influenza A/H3N2 virus). A number of publications are devoted to the study of anti-influenza, anti-herpes, anti-rabies, and anti-respiratory syncytial activities of extracts of *Rubus coreanus*, *Rubus ulmifolius Schott,* and *Rubus imperialis* [32]. The authors argue about the prospects of developing antiviral drugs based on them.

Conclusions. Therefore, we have obtained priority data on the anti-adenoviral activity of water-ethanol extracts from the leaves of wild raspberries. Differences in the qualitative and quantitative composition of flavonoids were revealed in extracts of varietal and wild raspberry leaves using the HPLS method, which affected their antiviral activity and thus the authors' data on the relationship between the severity of antiviral action and total polyphenols in extracts [30]. Native complexes of medicinal plants and their components are substances, and their combinations belong to different classes of chemical compounds. These can be polyphenols, terpenoids, alkaloids, organic acids, and others. In this regard, phytochemical studies of plants are of great importance. Equally important is the question of the taxonomic position of plants, as the analysis of the distribution of natural compounds in taxa of different levels can serve as a basis for predicting the search for biologically active substances that have required properties [33]. Plant species with high antiviral activity can be recommended for deeper study as a base in the creation of phytopreparations of antiviral action, taking into account the taxonomic position of the species, type of extraction, phase of plant development, and its part taken for analysis.

Today in medical practice there is a possibility to choose an extremely wide range of drugs. At the same time, there is no safe synthetic drug for the etiotropic therapy of viral infections, and therefore the search for new effective and safe drugs remains relevant. Natural plant compounds can be used as inhibitors of various viral infections at different stages of their manifestation and development; they can be used for a long time without causing addiction, as they act gently and safely [33]. According to the WHO, the use of phytopreparations is appropriate for about 75% of patients [34]. Thus, our new data on a wide range of anti-adenoviral activity of plantain and raspberry leaf extracts is a prerequisite for further studies of the properties of their individual components in order to create an anti-adenoviral drug and to make recommendations for its pharmacological use. The wide prevalence of these plants in Ukraine, the low toxicity of extracts, and the shown antiviral activity can further contribute to their effective use in clinical pharmacology. Another important argument for the study of plant extracts is the need of special medical rules that will facilitate their use in clinical practice. Herbal preparations already sold as dietary supplements may be admitted to clinical trials without preclinical pharmacological/toxicological trials if they have already been shown to be safe (Guide to the Development of Botanical Preparations for Industry, December 2016, Pharmaceutical Quality/CMC) [33].

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IN VITRO АНТИВІРУСНА АКТИВНІСТЬ ВИТЯЖОК З ЛИСТЯ *PLANTAGO MAJOR, PLANTAGO LANCEOLATA* ТА *RUBUS IDAEUS*

Досягнення органічної хімії, біохімії, біотехнології та молекулярної вірусології дали змогу синтезувати велику кількість противірусних препаратів, що належать до різних фармакологічних груп. Поряд із скринінгом нових синтетичних сполук активно проводяться дослідження противірусних засобів природного походження. Натуральні продукти з противірусними властивостями мають переваги перед синтетичними сполуками завдяки низькій токсичності, мінімальним побічним ефектам та м'якій дії за різними механізмами. **Метою** дослідження було вивчити цитотоксичну, віруліцидну та противірусну дію водно-спиртових витяжок з листя подорожника великого (*Plantago major* L.), подорожника ланцетолистого *(Plantago lanceolata* L.*),* малини дикої і садової (*Rubus idaeus* L.) та їхніх ферментованих варіантів щодо аденовірусів людини — 3, 5, 7 серотипів (HAdV3, HAdV5, HAdV7). **Методи.** Отримували водно-спиртові витяжки з листя подорожника великого та ланцетолистого, малини дикої та садової. Ферментацію витяжок проводили з використанням екзоферментів культуральної рідини *Saccharomyces cerevisiae*. Концентрація витяжок становила 30 мг/мл, вміст етилового спирту — від 10 до 20 %. В якості референс-препарату використовували препарат Неофлазид, розроблений НВК «Екофарм» (Україна), що містить карбонові кислоти та флавоноїдні глікозиди, виділені з диких злаків

Deschampsia caespitosa L. (щучка дерниста) та C*alamagrostis epigeios L.* (війник наземний). Вміст флавоноїдів у препараті Неофлазид — не менше 4.0 мг/г у перерахунку на рутин. Структурний склад витяжок визначали з використанням високоефективної рідинної хроматографії з оберненою фазою в системі Agilent 1260. Вивчення цитотоксичності та антивірусних властивостей проводили стандартним колориметричним методом з використанням МТТ. При дослідженні анти-аденовірусної дії були використані три схеми додавання витяжок до клітин: за 1 год до інфікування; під час адсорбції вірусу (як профілактичні схеми) та введення після інфікування клітин вірусом (лікувальна схема). Досліджували віруліцидну активність витяжок. Титр вірусу, синтезований в присутності витяжок, визначали за кінцевою точкою розведення, що спричиняє 50% розвитку цитопатичної дії вірусу. Усі дослідження проводили з трьома повторами та трьома паралельними визначеннями. Відмінності середніх показників вважали прийнятними при $p<0,05$. Обробку результатів проводили з використанням програми Microsoft Excel 2010 для Pentium Pro. **Результати.** Витяжки з листя подорожника великого (*Plantago major* L.*)*, малини дикої (*Rubus idaeus* L.) та їхні ферментовані варіанти в концентрації 3 мг/мл були не токсичні для клітин Hep-2 (CC_{50} становила >3 мг/мл). Токсичність витяжок з листя подорожника ланцетолистого (*Plantago lanceolata* L.), малини садової (*Rubus idaeus* L.) *та їхніх* ферментованих варіантів була дещо більшою (CC_{50} = 1.5 мг/мл). CC_{50} для препарату Неофлазид становила 10 мкг/мл. При внесенні витяжок за 1 год до зараження клітин чи присутності їх під час адсорбції аденовірусів (профілактичні схеми) показана незначна анти-аденовірусна активність, що не перевищувала 7 %. Висока антивірусна активність витяжок показана при внесенні їх після адсорбції вірусів (лікувальна схема). Витяжка з листя подорожника великого у концентрації 3 мг/мл пригнічувала репродукцію HAdV5 на 83%, HAdV3 — на 55 %, а HAdV7 — на 11 %. Витяжка з листя малини дикої в діапазоні концентрацій 0.06—3 мг/мл пригнічувала репродукцію HAdV5, HAdV3 та HAdV7 на 65—89 %, 41—84 % та 22—59 %, відповідно. Активність витяжок з листя подорожника ланцетолистого та листя малини садової була низькою, максимальне пригнічення репродукції HAdV3 на 34 % показане для витяжки з листя малини садової в концентрації 0.38 мг/мл, репродукція інших аденовірусів пригнічувалась лише на 4—22 %. Досліджували вплив витяжок на інфекційність аденовірусів. Показано, що витяжки з листя подорожника великого та малини дикої суттєво впливали на утворення інфекційного вірусного потомства, а саме: витяжки з подорожника великого (ферментована та неферментована) пригнічували репродукцію аденовірусу в середньому на 1,5 lg. Ферментована і неферментована витяжка з листя малини пригнічували репродукцію аденовірусу на 1.45 lg — 1.6 lg. Препарат Неофлазид в концентрації 7.1 мкг/мл повністю інгібував репродукцію HAdV5. Віруліцидної дії всіх витяжок та препарату Неофлазид відносно аденовірусів людини виявлено не було. Встановлено різницю в активності витяжок рослин різних видів одного роду, тобто з листя садової сортової *Rubus idaeus* L. (garden) і дикої малини *Rubus idaeus* L. (wild); подорожників *Plantago major* L. та *Plantago lanceolata* L. Витяжки малини *дикої* та подорожника *великого* були активні відносно аденовірусів 3, 5, 7 серотипів, у той час як витяжки з листя малини *садової* та подорожника ланцетолистого такої дії не мали. Дослідження хроматографічних профілів витяжок подорожників показали відмінності у складі фенолів, таких як кофеїнова та хлорогенова кислоти. Витяжка листя *Plantago major* L. містить плантамайозид — похідне кофеїнової кислоти, що має високу біологічну активність. Хроматограми витяжок листя сортової та дикої малини мали значні відмінності у загальному вмісті флавоноїдів. **Висновки.** Отримані нами нові дані щодо широкого спектру антиаденовірусної активності витяжок з листя подорожника та малини є передумовою для устворення лікарського препарату та рекомендацій для його фармакологічного застосування. Широка розповсюдженість рослин подорожника та малини на території України, низька токсичність витяжок та виявлена антиаденовірусна активність можуть в подальшому сприяти ефективному використанню їх у клінічній фармакології.

Ключові слова: *вторинні метаболіти, флавоноїди, екстракти подорожника і малини, аденовіруси людини, антивірусна активність.*