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Anti-viral activity of Biostymina® (*Aloe arborescens folii recentis extractum fluidum*) against viruses causing upper respiratory tract infections tested *in vitro*

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PRZECIWWIRUSOWA AKTYWNOŚĆ BIOSTYMINY® (WODNEGO WYCIĄGU ZE ŚWIEŻYCH LIŚCI ALOESU DRZEWIASTEGO) PRZECIWKO WIRUSOM POWODUJĄCYM INFEKCJE GÓRNYCH DRÓG ODDECHOWYCH W BADANIACH IN VITRO

STRESZCZENIE

Wstęp. *Infekcje górnych dróg oddechowych, przeważnie o etiologii wirusowej, stanowią częsty problem zdrowotny we wszystkich grupach wiekowych. Stosowanie leków przeciwwirusowych w tych schorzeniach jest ograniczone zarówno ze względu na niewielką ilość substancji aktywnych dostępnych na rynku, jak i przez ich działania niepożądane i wysoki koszt terapii. Istotnym pozostaje więc badanie kolejnych substancji, szczególnie o większej dostępności cenowej i bardziej korzystnym profilu bezpieczeństwa.*

Cel pracy. *Celem badań było określenie aktywności przeciwwirusowej preparatu Biostymina® (będącego wodnym wyciągiem z A. arborescens), przeciwko wirusowi grypy typu A (hFluA), wirusowi RSV (Respiratory syncytial virus), wirusowi Coxsackie (CA9) i adenowirusowi (Adeno 5), w warunkach in vitro.*

Materiał i metody. *Dla oceny żywotności komórek przeprowadzono na wstępie badanie aktywności cytotoksycznej preparatu Biostymina® in vitro na linii komórkowej HEp-2 używając testu MTT i badania mikroskopowego. Natomiast aktywność przeciwwirusową Biostyminy® oceniano w teście redukcji łysinek wirusowych (ang. plaque-reduction assay) lub poprzez analizę efektu cytopatycznego. Ponadto, za pomocą specyficznego testu immunoenzymosorbcyjnego (ELISA) określano ilość nowosyntetyzowanych białek wirusa.*

Wyniki. *Najwyższe nietoksyczne stężenie preparatu Biostymina® (3,3%) prowadziło do 17% inhibicji hFluA. Aktywność antywirusowa została oceniona za pomocą redukcji łysinek wirusowych. Ponadto, roztwory o stężeniu 3,3% i 1,65% powodowały redukcję łysinek wirusowych CA9 (odpowiednio o 42,6% i 24%). Nie stwierdzono natomiast aktywności przeciwwirusowej badanego ekstraktu przeciwko RSV i Adeno 5.*

Wnioski. *W niniejszym badaniu stwierdzono aktywność przeciwwirusową preparatu Biostymina® w stosunku do wirusa grypy typu A (hFluA) oraz wirusa Coxsackie. W naszym poprzednim badaniu wykazaliśmy także istotną aktywność przeciw HRV14. Można więc przypuszczać, że hamujący wpływ Biostyminy® na podstawowe wirusy odpowiadające za rozwój infekcji górnych dróg oddechowych wynika głównie z immunomodulujących i przeciwzapalnych właściwości wyciągu z A. arborescens. Stwierdzenie tego działania jest bardzo istotne w kontekście braku bezpiecznych leków przeciwwirusowych. Dalsze badania są potrzebne w celu precyzyjnej oceny mechanizmu działania Biostyminy® w zapobieganiu i leczeniu infekcji wirusowych górnych dróg oddechowych.*

KEY WORDS: BIOSTYMINA® – ALOE ARBORESCENS – AKTYWNOŚĆ ANTYWIRUSOWA – CYTOTOKSYCZNOŚĆ – WIRUS GRYPY TYPU A – WIRUS COXACKIE – WIRUS RSV – ADENOWIRUS

Introduction

Upper respiratory tract infections (URTIs) are among the most common reasons to visit a primary care provider. URTIs (known also as a "cold") are estimated to be the direct cause of seeking medical consultation in 60-90% of children (1). The easy transmission of viral respiratory pathogens, which can be carried in secretions as aerosols and droplets or via mucosal contact, allows for the rapid spread of the disease, especially among the family members of

the infected person (1-3). Uncomplicated URTIs last usually 7 to 10 days, and include a variety of symptoms, such as cough, sneezing, sore throat, rhinitis, and sometimes fever. In over 90% of cases URTIs are caused by viral infections and the systemic inflammatory response to them (2, 4). Rhinoviruses are considered to be responsible for up to 80% of URTIs. Other infectious agents include respiratory syncytial virus (RSV), influenza virus (hFluA), adenovirus (Adeno), and Coxsackie virus (CA) (1, 2, 5-10).

Unfortunately, despite the large scale of URTIs, which also has a serious negative economic impact (11), there are only a few antiviral drugs used for its treatment (including ribavirin – a nucleoside analog, adamantanes, and neuraminidase inhibitors (for hFluA), palivizumab – a monoclonal antibody (for RSV), pleconaril – an inhibitor of enterovirus replication (for CA), and cidofovir, a nucleoside phosphonate analog (for Adeno) (3, 7, 9, 10, 12)). However, the use of these therapeutics is limited by the high cost and limited effectiveness of therapy, as well as the potential health risk due to side-effects (11, 12). On the other hand, widely overused antibiotics have not been shown to treat URTI or even to prevent secondary bacterial infections. Moreover, the excessive use of antibiotics results in an increased risk of antibiotic resistance (2, 4, 11). Taken together, this illustrates that new drugs are needed in this area. One of their potential sources is herbal medicines with significant antiviral, immunomodulatory, or anti-inflammatory activity. They could be a good alternative in primary or adjunctive therapy and prophylaxis of URTI (13).

Aloe arborescens Mill. (*Asphodelaceae*: *Alooideae*) is a large, evergreen succulent, endemic to the mountainous regions of Southern Africa (14). It is known also as candelabra aloe or krantz aloe. This much-branched shrub grows up to 5 m tall. Its usually greyish green leaves with yellowish teeth form apical dense rosettes, whereas inflorescences consist of a characteristic elongated inverted-conical dense raceme with cylindrical flowers. However, *A. arborescens* hybridizes easily with other aloe species, which leads to many morphological variations (15). The aqueous extract from the leaves of this plant has been used to treat and prevent URTI in Central and Eastern Europe since the 1950s, when the first product Biostymina® was officially registered in Poland as an immunomodulatory agent, also for children. *A. arborescens* used for the production of Biostymina® is not collected from its natural habitat, but from the drug manufacturer's greenhouses where the cultivation is under controlled conditions. This procedure prevents from the formation of hybrids and provides a genetically uniform species through the years. *A. arborescens*, unlike other *Aloe* species, is characterized by a very low anthranoid content, which prevents the unwanted laxative effect (13). On the other hand, it contains many other substances responsible for its therapeutic value, such as glycoproteins, lectins P-2 and S-1, heteropolysaccharides, and phenolic compounds:, aloenin, and phenolic acids (15-18). The results of several studies have indicated immunomodulatory activity of *A. arborescens*, associated with stimulating B and T lymphocytes (13). The recent results confirm that Biostymina®, obtained in the current manufacturing process, influences the cellular response of the immune system, and accelerates the maturation of thymocytes and the acquisition by it of immune competence. These studies also demonstrated that stimulation of a cellular response by Biostymina® is comparable to that of synthetic immunostimulants: Levamisole and Isoprinosine (19). Candelabra aloe was also shown to inhibit cancer-cell-induced-angiogenesis, which suggests its possible role in immunocompromised neoplastic patients (20-22). *A. arborescens* is regarded also as an excellent appetite stimulant, demulcent, and allergy reducer (13, 23). Furthermore, the anti-inflammatory, antibacterial, antifungal, antioxidant, antidiabetic, radioprotective, and wound-healing properties of *A. arborescens* have been demonstrated (23-33). Recently, the antiviral activity of Biostymina® was shown *in vitro* against human rhinovirus (HRV14) (5). Its influence on other respiratory viruses has not been established yet, however. The aim of this study was an *in vitro* evaluation of Biostymina® with regard to its possible dose-dependent anti-viral activity against four human pathogenic RNA and DNA viruses: Influenza A (hFluA), Respiratory Syncytial Virus (RSV), Coxsackie (CA9), and Adenovirus (Adeno 5).

Materials and methods

Test substance

Biostymina®, *Aloe arborescens folii recentis extractum fluidum* (0.25:1) from fresh leaves, in vials for oral intake, was provided by the manufacturer Phytopharm Klęka S.A. For the *in vitro* assays this test substance was diluted as described with the respective cell culture media.

Controls

Non-treated virus-infected cells cultivated in cell culture medium alone (MEM – Minimum Essential Medium Eagle), without active components, served as controls (virus-controls). Negative controls were

non-infected cells, and Ribavirin Viracole® (ICN Pharmaceuticals) (4-6 μg/ml) in RSV-infected HEp-2 or in CA9-infected BGM cell cultures and Amandatin hydrochloride (Glaxo Welcome) (4 μg/ml) used in hFluA-infected MDCK cells, and in Adeno 5 an internal standard-infected HEp-2 cells (7.5μg/ml) served as positive controls. The efficacy of all reference substances was confirmed in the anti-viral tests.

The cytotoxicity and cell viability of the test substance diluted in cell culture medium was investigated in untreated HEp-2 cells via MTT tests and morphological examinations.

Ribavirin (Virazole®), (3-6μg/ml), ICN Pharmaceuticals, effective against the RNA-viruses e.g. RSV and CA9, Amandatin hydrochloride (4 μg/ml), Glaxo Welcome, active in influenza virus (hFluA), and a laboratory standard (7.5μg/ml) against Adeno5 were used as positive controls. The efficacy of all reference substances was confirmed in the test systems.

Virus Strains and Cells

Four viruses responsible for URTI were used in the study:

- Human Influenza A/Chile/01/83 (H1N1, hFluA),
- Respiratory Syncytial Virus (RSV) – strain long,
- Coxsackie virus (CA9),
- Adenovirus C subtype 5 (Adeno 5).

The following cell lines were used in the anti-viral study:

- Madin Darbey Canine Kidney (MDCK) cells – selective for hFluA,
- Human-Epidermoid-Carcinoma (HEp-2) cells – selective for RSV and Adeno 5,
- Borgio Green Monkey (BGM) cells selective for CA9.

Cells used in the cytotoxicity study were Human-Epidermoid-Carcinoma (HEp-2) cell line.

All virus strains and cells were obtained from the Department of Medical Virology and Epidemiology of Viral Diseases of the Hygiene Institute of the University of Tübingen, Germany and Friedrich-Schiller-University, Jena, Germany.

Cytotoxicity tests in vitro

In order to determine the optimal concentration of Biostymina® for the anti-viral activity assay, a cytotoxicity study was performed to exclude its cytotoxic effects. Therefore, Biostymina® was tested for its *in vitro* cytotoxicity and metabolic effects on HEp-2 cell cultures (MTT test and morphological examination).

For determination of the highest non-toxic concentrations, the test substance (100%) was diluted 10:1 with ten-fold concentrated cell culture medium, and further diluted in log 2 and log 10 dilution steps. For analysis of the cytotoxicity of test substance, all examined dilutions were added in four replicates to growing HEp-2 cells. Thereafter, cells were incubated for 6 days at 37°C and 5% CO_2.

Analysis of the *in vitro* cytotoxicity was performed at days 1, 3 or 4, and 6 with the MTT test (34). For this purpose, cells were incubated for 1-2 hours with an MTT solution and after dissolution of the formazan crystals in DMSO, the optical density (OD) of the cell culture supernatants was analyzed with a photometer at 570 nm. OD values of the non-treated medium controls defined 100% viability (0% cytotoxicity). Cytotoxicity of the test substance determined in the MTT assay (% cytotoxicity) was the basis of the dose-response (toxicity) curves for the determination of the inhibitory concentrations showing 50% cytotoxicity (IC_{50}).

Additionally, via phase contrast microscopy (magnification of 10x for general and 40x for intracellular view), the analysis of the cell cultures (HEp-2) was performed at day 6 after addition of the test substance, in order to verify the cytotoxic effects. The criteria for the microscopic analysis were as follows: altered cell morphology, generation of intracellular vacuoles, and destruction of cell monolayers.

Determination of anti-viral activity in vitro

The anti-viral activity of Biostymina® was assessed using plaque-reduction assays (FluA, RSV, CA9) and analyses of cytopathogenic effects (CPE) (Adeno 5). In addition, the amount of newly synthesized Adenovirus viral proteins was measured in a virus-specific enzyme-linked immunosorbent assay (ELISA), in a so-called "therapeutic approach", i.e. after adding the test substance to the cell cultures in semi-solid medium containing agarose 1 hour after virus infection. This part was run in four replicates and two (RSV, CA9, Adeno 5) or three (FluA) independent experiments. Experiments differed in the amount of virus used for the infection (multiplicity of infection, M.O.I.).

Plaque-reduction and CPE-based assays were performed on MDCK, HEp-2, BGM, and HeLa-cell cultures using standard procedures for the detection of infectious particles. The antiviral activity of the test substance was quantified in plaque-reduction assays by counting virus-derived plaques (plaque-forming units/ml, PFU/ml) for hFluA, RSV, and CA9. Further-

more, the cytopathogenic effect (CPE) for Adeno 5 was analyzed with a computer-based image processing system (AIDsystems). Virus plaques and CPE were quantified by employing an optical evaluation system (ELISpot reader and AID Diagnostika, respectively). The number of plaques or percent CPE of the test substance-treated cell cultures were compared to the number of plaques or percent CPE lesions of the virus controls, which determined 100% infection. For quantification of Adeno 5 antigens in the infected cell-culture supernatants, enzyme immunoassays (Merlin Diagnostika) were used.

Results

Cytotoxic properties in vitro

In order to exclude the cytotoxic effects of Biostymina®, the analysis of its *in vitro* cytotoxicity and metabolic effects on the virus-permissive cells HEp-2 was conducted. As previous research showed, Biostymina® did not cause cytotoxic effects on HeLa cells even in the 90% solution (4). Therefore, in this study a similarly high concentration of the extract was used.

Initially, the cytotoxicity study on HEp-2 cells did not show any cytolytic effect of Biostymina® in 90% to 5.6% solutions at day 1 or day 3. Therefore, the anti-viral studies with the test substance against the examined viruses, performed on MDCK, BGM, and HEp-2 cells, were started with solutions of 50% (1:2, FluA and CA9) and 90% (9+1, RSV and Adeno 5), followed by further log2 and log10 dilutions (tab. 1).

However, a highly sensitive reaction to Biostymina®, observed after using solutions of 50% (1:2) to 6.25% (1:16) on FluA-infected MDCK cells, as well as on CA9-infected BGM cells, was associated with the cytotoxic effects. The cytotoxic side effects of the test substance in solutions of 90% (9+1) to 5.6% (1:16) were revealed also in RSV- and Adeno 5-infected HEp-2 cells, which needed longer incubation times (6-7 days) until virus-caused lesions in the cell monolayer were visible. Therefore, an additional cytotoxicity study with Biostymina® on non-infected HEp-2 cells and longer incubation times (4-6 days) was performed to separate clearly cytolytic and anti-viral effects in the assays for the anti-viral activity.

The remarkable reduction of the HEp-2 cells metabolic activity from 33.9% to 12.1% was noticed at day 4 and day 6 while using dilutions of 90% (9+1) to 5.6% (1:16). Although no typical cytotoxic side effects were observed, the microscopic examination showed a slight but clear cellular modification of the cell monolayer. Only the solution of 3.3% (dilution of 1:30) when used for virus-infected cells did not cause visible cytotoxic side effects. Therefore, the final anti-viral studies with Biostymina® against hFluA, CA9, RSV, and Adeno 5, conducted on the MDCK, BGM, and HEp-2 cells, respectively, were performed with the 3.3% starting solution followed by further log 2 and log10 dilutions.

The results of the assays of cytotoxicity and the cell viability of the test substance for HEp-2 cells, obtained from MTT tests and morphological examinations, are summarized in table 2 and figure 1.

Anti-viral properties in vitro

The initial incubation of the infected virus-sensitive cells with the high concentrations (90% and 50%) of Biostymina® led to a strong growth inhibition, but on the other hand, it resulted in parallel cytotoxic side effects, as described above. Therefore, the experimental concentration of Biostymina® was decreased to allow the unequivocal separation between cytolytic and anti-viral effects (tab. 3).

The anti-viral activity of the test substance against all four tested viruses hFluA, RSV, CA9, and Adeno 5 was measured. In the 3.3% solution inhibition of circa 17% against hFluA was detected in all three independent experiments. However, this result was under the EC50 value (the effective concentration of the test substance leading to a 50% reduction of virus infections). Two further essays of the anti-viral activity against CA9 (3.3% - 1:30) and 1.65% (1:60) showed a reduction of 42.6% and 24% of the CA9- specific viral plaques, respectively. With regard to RSV as well as Adeno 5, no anti-viral activity was determined in all studies (PFU, CPE, ELISA) with all tested substance concentrations (3.3% to 0.02% solutions).

The details of the anti-viral activity of Biostymina® are presented in table 4 and 5, and in figure 2.

Table 1. Summary of the concentrations of the test substance (Biostymina® – 100% solution in water) used in the experiments for the quantification of the cytotoxicity on HEp-2 cells *in vitro*.

	Dilution (1/x) / concentrations (% solution, v/v) in cell culture media (MEM)									
1/x dilution	9+1	2	4	8	16	32	64	128	256	2560
Percent of solution	90	45	22.5	11.3	5.6	2.8	1.4	0.8	0.4	0.04

Table 2. HEp-2 cells: Examination of cytotoxicity and viability using MTT-assay and microscopic examination of Biostymina®.

Percent of cytotoxicity												control	IC_{50}% solution
Dilution (1/x) / concentration (% solution, v/v) in cell culture media (MEM)													
Biostymina®	1/x dilution	9+1	2	4	8	16	32	64	128	256	2560	MEM	
	Percent of solution	90	45	22.5	11.3	5.6	2.8	1.4	0.8	0.4	0.04		
Hep-2	Day 1	9.8	4.1	2.7	3.2	2.8	2.8	0.7	0.5	1.9	1.3	0.0	≥ 90
	Day 4	**21.5**	**18.0**	12.5	8.0	9.8	9.8	2.6	3.1	1.1	0.3	0.0	
	Day 6	**33.9**	**29.2**	**24.9**	**15.1**	12.1	9.3	9.3	8.4	7.0	3.9	0.0	
	Morphology	+/-	+/-	+/-	-	-	-	-	-	-	-	-	

Grey – highest substance concentrations without any substance-induced cytotoxicity (based on MEM values); Bold – microscopic examination: affected cell monolayer and some vacuoles but no typical toxic effects visible; (-) unaffected cells; (+) cells with moderate vacuoles.

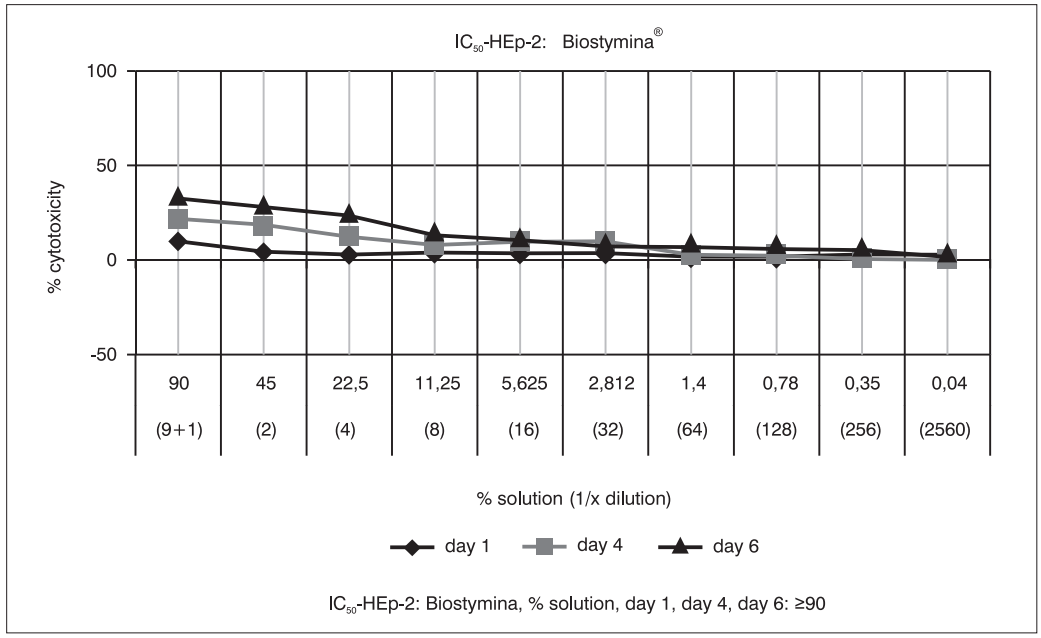

Fig. 1. Determination of the anti-cytotoxic activity of Biostymina®.

Table 3. Summary of the final concentrations of Biostymina® used in the respective tests for quantification of its antiviral activity *in vitro* against RSV or Adeno 5 infected HEp-2, hFluA infected MDCK, and CA9 infected BGM cells.

Cells	IC50			No cytotoxicity range			Final concentrations of the test substance					
	percent solution (1/x dilution of the 100% stock solutions)											
	Day 1	Day 3	Day 6	Day 1	Day 4	Day 6	log2 steps					log10 step
Hep-2	≥90 (9+1)			22.5 (4)	11.3 (8)	2.8 (32)	3.3 (30)	1.65 (60)	0.82 (120)	0.41 (240)	0.2 (480)	0.02 (4800)
	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5	log2 steps					log10 step
MDCK	32.3 (3.1)	>90 (9+1)	n.d.	6.5 (16)	50 (2)	n.d.	3.3 (30)	1.65 (60)	0.82 (120)	0.41 (240)	0.2 (480)	0.02 (4800)
BGM	>90 (9+1)			6,5 (16)	>90 (9+1)							

IC50: inhibitory concentrations showing 50% cytotoxicity (day 1, day 3/4, day 5, day 6); n.d. – not done.

Table 4. Summary table: Anti-viral activity "therapeutic" approach.

		Highest concentrations used in the antiviral tests "therapeutic approach"			
		antiviral studies	**study 1 M.O.I. 0.0006**	**study 2 M.O.I. 0.0004**	**study 3 M.O.I. 0.0004**
		% solution	3.3	3.3	3.3
hFluA	percent of inhibition		12.0	18.6	20.5
	percent of solution	EC_{50}	>3.3	>3.3	>3.3
		IC_{50}_day 3 MDCK	32.3	32.3	32.3
RSV	percent of inhibition		-1.8	3.5	n.d.
	percent of solution	EC_{50}	negative	negative	n.d.
		IC_{50}_day 3 MDCK	≥90	≥90	n.d.
CA9	percent of inhibition		44.2	41.0	n.d.
	percent of solution	EC_{50}	≥3.3	≥3.3	n.d.
		IC_{50}_day 3 MDCK	≥90	≥90	n.d.
Adeno 5	percent of inhibition		1.3	0.0	n.d.
	percent of solution	EC_{50}	negative	negative	n.d.
		IC_{50}_day 3 MDCK	≥90	≥90	n.d.

n.d. – not done; M.O.I. – multiplicity of infection (virus infection); EC_{50} – effective concentration (anti-viral activity); IC_{50} – inhibitory concentration (cytotoxicity); negative – no anti-viral effect.

Table 5. Detailed results of the anti-viral tests "therapeutic" approach of Biostymina® against hFluA, RSV, CA9 and Adeno 5.

Virus	**Anti-viral test**	**Percent of reduction**								
		percent of solution (1/x dilution)							**positive control**	**virus control**
	(1/x)	15	30	60	120	240	480	4800	Amandatine 4 μg/ml	MEM
	percent of solution	6.6	3.3	1.65	0.82	0.41	0.2	0.02		
hFluA	Study 1 MOI 0.0006	tox	12.0	1.0	0.0	0.0	-1.8	0.0	59.3	0.0
	Study 2 MOI 0.0004	n.d.	18.6	7.6	-4.2	1.9	-0.4	-0.2	66.5	0.0
	Study 3 MOI 0.0003	n.d.	20.5	6.6	-1.3	4.8	-0.5	0.3	63.3	0.0
RSV									Ribavirin 3 μg/ml	MEM
	Study 1 MOI 0.00078	0.5	-1.8	0.0	0.0	0.0	1.5	0.0	73.6	0.0
	Study 2 MOI 0.0004	n.d.	3.5	-0.4	3.3	1.9	0.8	1.2	71.2	0.0
CA9									Ribavirin 6 μg/ml	MEM
	Study 1 MOI 0.0008	n.d.	44.2	27.2	19.2	6.1	3.7	1.4	41.6	0.0
	Study 2 MOI 0.0004	n.d.	41.0	20.7	2.1	0.0	-1.6	1.4	59.7	0.0
Adeno 5									Lab-Std. 7.5 μg/ml	MEM
	Study 1 MOI 0.004	CPE	n.d.	1.3	-1.3	0	0	-1.3	1.3	40.3	0.0
		ELISA	n.d.	0.2	4.4	-1.7	-2.2	2.4	-2.4	48.4	0.0
	Study 2 MOI 0.002	CPE	n.d.	0	-1.4	2.9	5.8	-1.4	0	49.3	0.0
		ELISA	n.d.	1.1	-3.1	-0.3	1.8	3.3	-1.1	64.1	0.0

n.d. – not done.

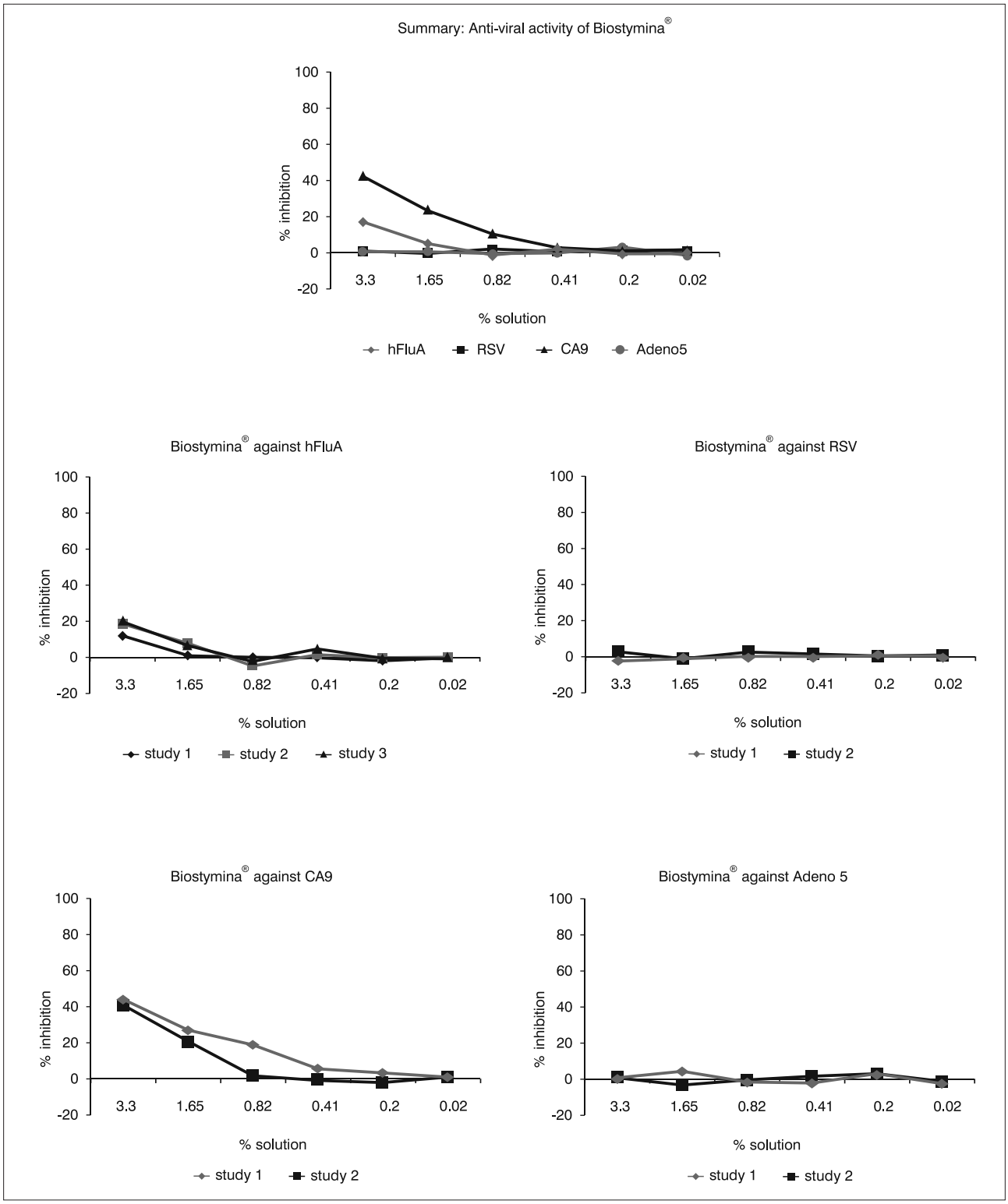

Fig. 2. Determination of the anti-viral activity of Biostymina® against hFluA, RSV, CA9 and Adeno 5.

Discussion

This is the first study carried out to evaluate the anti-viral activity of an aqueous extract of *A. arborescens* (Biostymina®) against URTI-causing viruses (FluA, CA9, RSV and Adeno 5), while no anti-viral activity was observed against RSV and Adeno 5. The stimulating effect of Biostymina® on the immune system was documented before (13, 19), and

this extract has also been proved to be effective in prophylaxis, as well as in shortening duration and reducing severity of URTIs (35). URTIs are currently considered to be caused by viral-caused-upregulation of certain cytokines, rather than by the virus itself (2). Thus, the decrease of the proinflammatory cytokine level can result in the inhibition of infection development (4). Proof of the anti-viral activity of Biostymina® against FluA (even though below EC50 value), HRV14, and CA9 further supports the use of Biostymina® as a valuable immunomodulator and anti-viral compound in URTIs.

Although previous studies indicated the anti-viral activity of *Aloe* species (the hot glycerin extract of *Aloe vera* gel) against herpes simplex virus type 2 (36), *Aloe vera* gel in HIV-infection (37), and an ethanolic extract of *Aloe hijazensis* against haemagglutinating viruses (38), the results were often ambiguous.

At this stage it remains open which constituents are responsible for the observed antiviral activity. In this research an anti-viral activity of an aqueous *A. arborescens* extract – Biostymina® against CA9, and less pronounced inhibitory activity against hFluA, was demonstrated, and the 90% solution of Biostymina® was shown recently to cause a 60% reduction in virus-derived plaques (HRV14) (4). Taken together, these results encourage further research on the role and mechanisms of anti-viral activities of *Aloe arborescens* extracts.

Conclusions

In this study, Biostymina® in a 3.3% and 1.65% solution, (1:30) and (1:60) dilutions, respectively, was shown to exert an anti-viral effect against CA9, causing a reduction by 42.6% and 24% of the CA9-specific viral plaques, respectively. It also demonstrated a less pronounced anti-viral activity in the 3.3% concentration, resulting in a 17% inhibition of hFluA. Further studies are needed to investigate the potential effect of *A. arborescens* aqueous extract on other pathogenic viruses.

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