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АБСТРАКТИ НА СТАТИИТЕ, ПРЕДСТАВЕНИ ЗА УЧАСТИЕ В КОНКУРСА ЗА ЗАЕМАНЕ НА АКАДЕМИЧНАТА ДЛЪЖНОСТ „ДОЦЕНТ“

1. Benedetti F., F.Berti, S. Budal, P. Campaner, F. Dinon,A. Tossi, R. Argirova, P. Genova, V. Atanassov, **A. Hinkov,** (2012): Synthesis and Biological Activity of Potent HIV-1 Protease Inhibitors Based on Phe-Pro Dihydroxyethylene Isosteres. *J. Med. Chem*. 2012, *55*, p. 3900−3910 DOI: 10.1021/jm3001136 **IF$_{2012}$=5.614**, **SJR$_{2012}$=2.343; Q1**

Abstract: Peptidomimetic inhibitors of HIV-1 PR are still a key resource in the fight against AIDS. Here we describe the synthesis and biological activity of HIV-1 PR inhibitors based on four novel dihydroxyethylene isosteres of the Phe-Pro and Pro-Pro dipeptides. The isosteres, containing four stereogenic centers, were synthesized in high yield and excellent stereoselectivity via the cyclization of epoxy amines derived from α-amino acids. The inhibitors were assembled by coupling the isosteres with suitable flanking groups and were screened against recombinant HIV PR showing activities in the subnanomolar to micromolar range. Two Phe-Pro-based inhibitors active at the nanomolar level were further investigated: both inhibitors combine the ability to suppress HIV-1 replication in infected MT-2 cells with low cytotoxicity against the same cells, thereby displaying a high therapeutic index. These results demonstrate the potential of the new Phe-Pro dihydroxyethylene isostere as a core unit of powerful HIV-1 PR inhibitors.

2. Stankova, I., K. Stanoeva, **A. Hinkov**, I. Alexiev, P. Genova-Kalou, R. Chayrov, R. Argirova. 2012. Amino acid and peptide esters of abacavir: synthesis and activity against human immunodeficiency virus type 1 in cell culture. *Medicinal Chemistry Research*, 21, 4053-4059. DOI: 10.1007/s00044-011-9956-y **IF$_{2012}$= 1.612, SJR$_{2012}$= 0.375; Q3**

Abstract: Abacavir (ABC) is clinically associated with hypersensitivity reactions, risk for cardiovascular disease, etc. A possible way to minimize side effects is by modifying chemical structure. Three esters of ABC containing amino acid (glycine) and dipeptide esters (glycyl-glycine) were synthesized and their activity on HIV-1 III B replication in MT-4 cells was evaluated. One of the newly synthesized esters—Gly-ABC—demonstrates low-cytotoxicity and high-anti-HIV-1 activity in MT-4 cells, as well as lowmitochondrial toxicity and high-genetic barrier to resistance.

3. Moyankova D., **A. Hinkov,** D. Georgieva, S. Shishkov, D. Djilianov . 2014. Inhibitory Effect of Extracts from Haberlea Rhodopensis Friv. against Herpes Simplex Virus. *Доклади на Българската академия на науките (Comptes rendus de l'Acad'emie bulgare des Sciences).* 2014, *63*(10), p. 1369-1376 **IF$_{2014}$=0.284; SJR $_{2014}$=0.205; Q3**

Abstract: Haberlea rhodopensis Friv. (Gesneriaceae) is a rare species, endemic for the Balkans. It belongs to the group of resurrection plants, able to withstand long periods of full desiccation. We tested the activity of total methanol, ethanol and water extracts from fresh and air-dried leaves of H. rhodopensis as well as apolar and polar fractions of methanol extracts against Herpes simplex virus 1 and 2. Cytotoxicity (maximum nontoxic concentration and cytotoxic concentration 50) against MDBK (Madin-Darby Bovine Kidney) cell line was determined by colourimetric MTT assays, modified for screening of anti-HSV compounds also in MDBK cells. Total methanol extract from dry leaves (MeDry) was the most active – 61% for HSV-1(F) and 60% for HSV-2(BA). Direct virus inactivating effect of the plant extracts showed no change in titre of treated virus against virus control. Polar and apolar fractions of the methanol extract from dry leaves showed lower cell protection compared to the total extract. It is tempting to speculate that the complex of all compounds in total methanol extract of H. rhodopensis possesses a unique synergetic inhibitory effect against herpes virus. The plant species could be considered as an interesting source of natural bioactivity substances with antiviral effect.

4. Todorov, D., **A. Hinkov**, K. Shishkova, S. Shishkov. 2014. Antiviral potential of Bulgarian medicinal plants. *Phytochem. Rev*., 13 (2): 525-538. DOI: 10.1007/s11101-014-9357-1, **IF_{2014}=2.894, SJR_{2014}=0.923; Q1**

Abstract: Medicinal plants have been widely used to treat a variety of infectious and non-infectious diseases. Bulgarian flora includes 4,300 plant species, over 500 of which are rare or endemic to the country or the Balkan region. The aim of the present work is to summarize comprehensively the investigations on the antiviral activity of Bulgarian medicinal plants from the past three decades. The effect of different extracts derived from in vitro propagated plants has been examined as well. The phytochemical composition and its influence on specific steps of the viral life cycle have been discussed in this paper. The review includes the following families: *Amaryllidaceae, Fabaceae, Geraniaceae, Lamiaceae, Onagraceae, Ranunculaceae, Rosaceae, Crophulariaceae* and *Rhodophyta*. Special attention has been paid to viruses as important human pathogens.

5. Zahmanov, G., K. Alipieva, P. Denev, D. Todorov, **A. Hinkov,** S. Shishkov, M. I. Georgiev, 2015. Flavonoid glycosides profiling in dwarf elder fruits (Sambucus ebulus L.) and evaluation of their antioxidant and anti-herpes simplex activities. *Industrial Crops and Products*. 2015, *63*, p. 58-64 DOI:10.1016/j.indcrop.2014.10.053 **IF_{2015}=3.449, SJR_{2015}=1.049; Q1**

Abstract: Dwarf elder (Sambucus ebulus L.) is a popular medicinal plant, used for centuries in the folk medicine ofthe Balkan Peninsula. S. ebulus preparations have shown anti-inflammatory, anti-neoplastic and antimicrobial properties, besides abundant wound healing, antioxidant and anti-ulcerogenic activities. We developed a scheme for isolation of individual compounds utilizing different chromatographic techniques, while the structure elucidation was performed by means of 1D and 2D NMR. Five flavonoidglycosides, e.g. quercetin-3-O-laminaribioside [1], isorhamnetin-3-O-laminaribioside [2], quercetin-3-O-rutinoside [3], isorhamnetin-3-O-rutinoside [4], isorhamnetin-3-O-glucoside [5], were identified accordingly. Compounds 1 and 2 are reported for the first time in the genus Sambucus. Several triterpenes – ursolic, oleanolic and maslinic acid – were tentatively identified by GC–MS. The evaluation of anti-herpes simplex virus type 1 and antioxidant (in $ORAC_{FL}$and $HORAC_{FL}$)

properties suggests that the dwarf elder fruits might serve as a powerful source of valuable molecules for various purposes.

6. Todorov, D., K. Shishkova, D. Dragolova, **A. Hinkov**, V. Kapchina-Toteva, S. Shishkov. 2015. Antiviral activity of medicinal plant *Nepeta nuda*. *Biotechnol. Biotechnol. Equipment*, 29 (Suppl. 1): 39-S43. DOI:10.1080/13102818.2015.1047215. **IF$_{2015}$=0.379**, **SJR$_{2015}$=0.173; Q4.**

Abstract: *Nepeta nudasubsp.nuda* L. is a valuable medicinal plant well-known for its various therapeutic properties. This study assessed the antiviral activity of four extracts derived by methanol and chloroform extractions from in vivo grown and in vitro propagated plants. The cytotoxicity was tested on MadinDarby bovine kidney (MDBK) cell line. Maximal tolerated concentration (MTC) and cytotoxic concentration (CC_{50}) of both extracts were determined. Antiviral activity on viral replication was evaluated against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) in MDBK cell line. The 50%inhibitory concentrations (IC_{50}) and selective index (SI) of the extracts against both viruses were calculated. The methanol extract, derived from the native plant exhibited the greatest antiviral activity. The IC_{50} for both viruses was similar 320 mg/ml against HSV-1 and 510 mg/ml against HSV-2. The SI were 4.94 and 3.1, respectively. Applied in MTC, the extract inhibited viral replication by more than 95% in both HSV-1 and HSV-2. The virucidal effect was determined by thereduction of the infectious virus titer. All four extracts of *N. nuda* inactivated the extracellular form of HSV-2. The major virucidal activity was demonstrated by the chloroform extract from the native plant more than 99% viral inactivation.The extracts weakly affected the viral entry into the host cell. The highest inactivation was shown by the chloroform extract form the native plantmore than 90%.

7.Todorov, D., D. Pavlova, **A. Hinkov**, K. Shishkova, D. Dragolova, V. Kapchina-Toteva, S. Shishkov. 2015. Effect of *Teucrium chamaedrys* L. on Herpes simplex virus type 2. *Compt. rend. Acad. bulg. Sci.*, 68 (12): 1519-1526. **IF$_{2015}$= 0.233**, **SJR$_{2015}$=0.205; Q3.**

Abstract: *Teucrium chamaedrys* L. (Wall germander) is a widely distributed species of *Teucrium* (*Lamiaceae*) found in the spontaneous flora of Bulgaria, and some Central and South European countries as well. This is a medicinal plant with a history of traditional use in Bulgaria and in other Balkan countries for herbal tea and basic medical healing treatments. In our *in vitro* study, chloroform and methanol extracts received by soxhlet extraction as well as methanol extract obtained by thermostat extraction were tested for antiviral activity. Two of studied extracts inhibited the replication of Herpes simplex virus type 2 (HSV-2) in MDBK cells significantly without apparent cytotoxicity. The 50% effective concentration (EC_{50}) of the chloroform extract was 350 μg/ml. The replication of the virus was suppressed over 82% from the extract applied in maximum tolerable concentration (MTC). The methanol thermostat extract showed weak antiviral effect (EC50 = 680 μg/ml). The extracts applied in MTC inactivated extracellular virus, viral adsorption and entry of HSV-2. Antiherpes activity by crude extracts from *Teucrium chamaedrys* is observed for the first time.

8. Angelova, P., **A. Hinkov**, V. Tsvetkov, D. Todorov, K. Shishkova, D. Dragolova, S. Shishkov, V. Kapchina-Toteva. 2018. Antiherpes virus activity of extracts from Artemisia chamaemelifolia *Vill. Compt. rend. Acad. bulg. Sci.*, Vol 72, No11, pp.1475-1483. **IF$_{2018}$=0.251; SJR$_{2018}$=0.21; Q2.**

Abstract: Human alphaherpesvirus (HHV) type 2 is the causative agent of genital herpes. The inhibitory effect of water and chloroform soxhlet extracts from the aerial parts of Bulgarian plant *Artemisia chamaemelifolia* Vill. (*Asteraceae*) against the replication of HHV type 2 strain BA (sensitive to acyclovir) and strain DD (resistant to acyclovir) was investigated in vitro. Results from the antiviral experiments with water extract showed that it achieved protection of the cell monolayer ~70% (for both used strains) according to modified MTT test and inhibited production of infectious virus yield ~80% for strain BA and ~70% for strain DD according to yield reduction assay. The inhibition was dose-related. The water extract also exhibited weak virucidal activity in maximal nontoxic concentration. The chloroform soxhlet extract had no effect on the replication of the two strains used, nor did it affect the infectivity of extracellular virions.

9. **Hinkov A.**, Angelova P., Marchev A., Hodzhev Y., Tsvetkov V.,Dragolova D, Todorov D, Shishkova K., Kapchina-Toteva V., Blundell R., Shishkov S., Georgiev. M., 2020. *Nepeta nuda ssp. nuda* L. water extract: Inhibition of replication of some strains of Human Alpha herpes virus (genus Simplex virus) in vitro, mode of action and NMR-based metabolomics, *Journal of Herbal Medicine* (2020), doi: https://doi.org/10.1016/j.hermed.2020.100334. **IF$_{2020}$=1.554, SJR$_{2020}$=0.483; Q2.**

Abstract: *Nepeta nuda* L. has been used in traditional folk medicine for its diuretic, anti-asthmatic, antioxidant, spasmolytic, sedative and analgesic properties (attributed to the nepetalactones). In the present study, water extract from *Nepeta nuda ssp. nuda* L. was tested in order to study its' effect on the replication of Human Alpha herpes virus (HHV) type 1, strain F (ACV-sensitive) and type 2, strain DD (ACV-resistant) in vitro. Toxicity was measured at 48 h (CC_{50} = 7.35 mg/ml±0.06) and 72 h (CC_{50} = 4.488 mg/ml±0.308) after infection. The extract showed potent anti-herpesvirus activity in both antiviral tests performed (MTT-based colorimetric assay and yield reduction assay). By the time the extract was added, two experimental arrangements were applied. The antiviral activity increased when water extract was added simultaneously with the inoculation of the cell monolayer (EC_{50} of 0.66 mg/ml±0.04 and 0.788 mg/ml±0.009 for the F and the DD strains, respectively, measured via colorimetric assay). The IC_{50} value was 0.181 mg/ml±0.073 and 0.0888 mg/ml±0.014 for the F and the DD strains, respectively, measured via yield reduction assay. Unfortunately, selectivity for viral versus cellular molecular targets (SI) was low except for the SI values (40.60 and 82.77 for the F and the DD strains, respectively) obtained via the yield reduction assay when water extract was added simultaneously with the inoculation of the cell monolayer. In both types of antiviral assays water extract retained activity against the ACV-resistant DD strain. The virucidal assay showed that water extract did not reduce the infectivity of either of the strains used at a concentration equal to the maximum non-toxic concentration. Therefore, the above-mentioned rise in the antiviral activity detected in experimental settings when the extract was added immediately after inoculation is not due to direct inactivation of the extracellular virions. Rather, it is due to interference with the adsorption but not the penetration (according to the results of the conducted experiment). The time of addition test demonstrated that the water extract continued to exhibit antiviral activity even when added 10 h after infection. All these observations suggest that water extract exibits its anti-herpesvirus activity by influencing both early (adsorption) and late events of HHV replication. Metabolomic studies of the extract showed that the major phenolic acids present in the extract include rosmarinic, chlorogenic, gallic, vanillic, caffeic, protocatechuic, ferulic and cinnamic acids; while the presence of flavonoids was marked by cirsimaritin, chrysoeriol, vanillin, rutin and quercetin.

10. Chuchkov K., R. Chayrov , **A. Hinkov**, D. Todorov, K. Shishkova, I. G. Stankova. 2020. Modifications on the heterocyclic base of ganciclovir, penciclovir, acyclovir - syntheses and antiviral properties, *Nucleosides, Nucleotides & Nucleic Acids*, 39:7, 979-990. DOI: 10.1080/15257770.2020.1725043 **IF_{2020}=1.381, SJR_{2020}=0.27; Q3.**

Abstract: Esters of the antiherpetic drugs ganciclovir, penciclovir with the bile acids (cholic, chenodeoxycholic and deoxycholic) and amino acid esters of acyclovir were generated and evaluated for their in vitro antiviral activity against herpes simplex viruses type 1 and type 2 (HSV-1, HSV-2). The antiviral assays demonstrated that modified analogs of ACV and PCV are less active compared to the initial substances against HSV-1and HSV-2. CC_{50} for ganciclovir-deoxycholate corresponded to the CC_{50} of the other analogs and its activity is lower than ganciclovir. Obtained results show that tested modification do not impro0ve bioavailability of nucleoside analogs in cells.

11. Todorov, S. D., V. Q. Cavicchioli, M. Ananieva, V. P. Bivolarski, T. A. Vasileva, **A. V. Hinkov,** D.G. Todorov, S. Shishkov, T.f Haertlé, I.N. Iliev, L.A. Nero, I. V. Ivanova, (2020). Expression of coagulin A with low cytotoxic activity by Pediococcus pentosaceus ST65ACC isolated from raw milk cheese. *Journal of applied microbiology*, *128*(2), 458-472. DOI 10.1111/jam.14492 **IF_{2020}=3.066, SJR_{2020}=0.889; Q2**

Abstract: Aims: We aimed to evaluate some specific conditions for growth of Pediococcus pentosaceus ST65ACC and its bacteriocin expression through ABC transporters; to purify the bacteriocin and determine its sequence; and to evaluate the cytotoxicity potential of the purified bacteriocin(s).
Methods and results: The results presented for growth behaviour of P. pentosaceus ST65ACC showed that the bacterial growth was slightly influenced when cultured in MRS broth with different amounts of inoculum: 1, 2, 5 and 10%. The bacteriocin activity increased when 5 and 10% inocula were used. The carbon source (glucose) used in different amounts (1, 2, 3 or 4%) had no significant effect on growth and bacteriocin production. The studied strain P. pentosaceus ST65ACC was able to metabolize xylooligosaccharide (XOS) as the sole carbon source, resulting in the production of an antimicrobial peptide. The genes involved in the ABC transport system and sugar metabolism of P. pentosaceus ST65ACC were expressed at different levels. The bacteriocin produced by P. pentosaceus ST65ACC was partially purified by precipitation with ammonium sulphate (40% saturation), followed by reversed-phase liquid chromatography, resulting in the identification of an active bacteriocin. Tandem mass spectrometry was used to identify the partial sequence KYYGNGVTCGKHSCSVDWGK sharing high similarity to coagulin A. The semi-purified bacteriocin had low cytotoxicity based on estimated values for maximal nontoxic concentration (MNC) and cytotoxicity concentration (CC_{50}).
Conclusions: The bacteriocin produced by P. pentosaceus ST65ACC is similar to coagulin, with low cytotoxicity, strong antimicrobial activity and possible additional metabolite routes in the producer cell. In addition to MRS broth, bacteriocin was produced also in medium containing XOS (as the single carbon source).
Significance and impact of the study: To the best of our knowledge, this is the first report of evaluation of the role of ABC transporters in the expression of bacteriocin by P. pentosaceus, cultured in MRS and XOS.

12. Tsvetkov, V., **A. Hinkov**, D. Todorov, E. Benova, I. Tsonev, T. Bogdanov, S. Shishkov & K. Shishkova. 2020. Effect of Plasma-Activated Medium and Water on Replication and Extracellular Virions of Herpes Simplex Virus-1. *Plasma medicine*, 10, 1, 15-26. DOI: 10.1615/PlasmaMed.2020033626**. SJR$_{2020}$=0.228; Q3.**

Abstract: We use a surface-wave sustained discharge (SWD) in argon at atmospheric pressure (using a plasma torch) in these experiments. The plasma torch is sustained using a 2.45-GHz electromagnetic wave with applied microwave powers of 13, 15, and 20 W. At these discharge conditions, the length of the plasma torch outside of the quartz tube is ~1–1.5 cm, and the gas plasma temperature does not exceed 40°C. This allows direct treatment of samples using the active zone of the discharge. In the cytotoxicity study, only two of the experimental settings achieve up to 50% survival of the cell monolayer after adding plasma-treated medium. Examining the effect of the plasma torch treatment media on herpes simplex virus-1 replication, we found that none of the applied experimental assays show significant protection on the cell monolayer. In a study of the virucidal action of a plasma-treated viral suspension diluted with sterile water at a ratio of 1:2 that was treated for 300 s at 13-W wave power, a decrease in the viral sample titer occurred unlike in the 1.67 log10 control. Using optical emission spectroscopy, we found that OH intensity increases at the contact point between plasma and the water surface. Intensity of NO-γ also increases to the contact point with applied power. We also monitored the amount of peroxide radicals in plasma-treated water and nutrient medium in the presence of lucigenin.

13. Tsvetkov, V., D. Todorov, **A. Hinkov**, K. Shishkova, L. Velkova, A. Dolashki, P. Dolashka, S. Shishkov. 2021. Effect of extracts of some species from Phylum Mollusca against the replication of Human Alphaherpesviruses. *Bulgarian Chemical Communications*, 53, Special Issue-A, 66–72. **IF$_{2021}$=1.554, SJR$_{2021}$=0.168; Q4.**

Abstract: In the present study, hemolymph from Rapana venosa (hRv), Helix lucorum (Hl) and Eriphia verrucosa (hEv), mucus from Helix aspersa (Ha) and structural subunit α-HaH from hemocyanin of H. aspersa (sHa) were tested against replication of antiviral drugs acyclovir (ACV) sensitive strain F and BA of human alphaherpesvirus type 1 and type 2 in vitro. All six extracts showed no anti-herpesvirus activity using an MTT-based colorimetric assay to detect inhibition of Human Alphaherpesviruses (HHV) replication. In a virus analysis, the six extracts tested reduced the infectivity of both viruses from the two strains used to varying degrees and applied at maximum non-toxic concentrations. Fractions from hemolymph from R. venosa (MW 30-100 kDa) and from E. verrucosa (MW 3-100 kDa) showed the highest activity (over 99% inhibition of extracellular virions infectivity by first and second type of viruses respectively), sufficient to be considered pharmacologically significant. Hemolymph from R. venosa and E. verrucosa and mucus from H. aspersa have little effect on the adsorption of BA strain of human alpha herpesvirus type 2, and strain F of the HHV 1. The effect on the first type being more pronounced.

14. Petrova, D., U. Gašić, L.Yocheva, **A. Hinkov**, Z. Yordanova, G. Chaneva , D. Mantovska, M. Paunov, L. Ivanova, M. Rogova, K. Shishkova, D.Todorov, A.Tosheva, V. Kapchina-Toteva, V.Vassileva, A. Atanassov, D. Mišić, G. Bonchev and M. Zhiponova. 2022. Catmint (Nepeta nuda L.) Phylogenetics and Metabolic Responses in Variable Growth Conditions. *Front. Plant Sci*. 13:866777. doi: 10.3389/fpls.2022.866777. https://doi.org/10.3389/fpls.2022.866777. **IF$_{2021}$=6.627, SJR$_{2021}$=1.359; Q1.**

Abstract: Nepeta nuda (catmint; Lamiaceae) is a perennial medicinal plant with a wide geographic distribution in Europe and Asia. This study first characterized the taxonomic position of N. nuda using DNA barcoding technology. Since medicinal plants are rich in secondary metabolites contributing to their adaptive immune response, we explored the N. nuda metabolic adjustment operating under variable environments. Through comparative analysis of wild-grown and in vitro cultivated plants, we assessed the change in phenolic and iridoid compounds, and the associated immune activities. The wild-grown plants from different Bulgarian locations contained variable amounts of phenolic compounds manifested by a general increase in flowers, as compared to leaves, while a strong reduction was observed in the in vitro plants. A similar trend was noted for the antioxidant and anti-herpesvirus activity of the extracts. The antimicrobial potential, however, was very similar, regardless the growth conditions. Analysis of the N. nuda extracts led to identification of 63 compounds including phenolic acids and derivatives, flavonoids, and iridoids. Quantification of the content of 21 target compounds indicated their general reduction in the extracts from in vitro plants, and only the ferulic acid (FA) was specifically increased. Cultivation of in vitro plants under different light quality and intensity indicated that these variable light conditions altered the content of bioactive compounds, such as aesculin, FA, rosmarinic acid, cirsimaritin, naringenin, rutin, isoquercetin, epideoxyloganic acid, chlorogenic acid. Thus, this study generated novel information on the regulation of N. nuda productivity using light and other cultivation conditions, which could be exploited for biotechnological purposes.

15. Todorov, D., V. Tsvetkov, **A. Hinkov**, K. Shishkova, S. Shishkov, D. Pavlova, & D. Mantovska. 2022. Effect of the soil type over the antiherpes activities of *Teucrium chamaedrys*. *Fresenius environmental bulletin*, 31(9), 9831-9838. **IF$_{2021}$=0,61, SJR$_{2021}$=0.182; Q4.**

Abstract: Soil composition is one of the most important factors determining the metabolism of the plant life and from that - the amount of the active molecules - influencing the antiviral activity of the extracts. Our study tries to quantify its influence over the antiviral effect of the plant *Teucrium chamaedrys* L. over the human pathogen human alphaherpes virus. To get wider scope, our specimens have been collected from eastern Rhodope Mountain, Rila mountain and Sofia region - getting in our specter of research both mountain and lowland climate and serpentine, silicate and calcareous soils. The most active extracts are - the chloroform soxhlet extract from the region of Dobromirtzi village - inactivating extracellular virions of HHV-2 up to 99,57% and methanol thermostat one from the same region with more than 99,99% activity. The influence over viral replication cycle is weak, with most active extract have selective index just 1,87. As a result of the conducted experiments we suggest may influence the antiviral activity of *T. chamaedrys* populations in comparison to non-serpentine populations. This underlines the need to control the origin of the medicinal plants when used to test for antiviral activities.

16. Hoxha, R.; Todorov, D.; **Hinkov, A.**; Shishkova, K.; Evstatieva, Y.; Nikolova, D. In Vitro Screening of Antiviral Activity of Lactic Acid Bacteria Isolated from Traditional Fermented Foods. *Microbiol. Res.* 2023, 14, 333–342. https://doi.org/ 10.3390/microbiolres14010026 **IF$_{2022}$=1.5, SJR$_{2020}$=0.483, Q4.**

Abstract: Studies of newly isolated strains of lactic acid bacteria (LAB) are a good basis for expanding the potential for their applications in functional foods, probiotic food supplements, and other probiotic products. They exhibit various functional properties, including such with antiviral activity. Probiotic strains can manifest their antiviral effects by various mechanisms, including direct interaction with viruses, production of antiviral compounds, or immune system modulation. Ten newly isolated LAB strains from traditional fermented food products have been tested for the determination of their antiviral activity. This study was performed to evaluate the effect of cell-free supernatants (CFSs) from the studied strains for the effect on viral replication of Human alphaherpesvirus—HHV-1 and HHV-2 as well as for direct virucidal activity. The CFSs of the LAB strains were used in non-toxic concentrations of 25%, 6.25%, and 1.6%. No direct virucidal activity was observed in tested CFSs, but five of the strains observed a well-defined effect of viral replication inhibition with the selective index (SI) from 4.40 to >54. For two of these five strains, Lactobacillus delbrueckii subsp. Bulgaricus KZM 2-11-3 and Lactiplantibacillus plantarum KC 5-12 strong activity against HHV-2 with a selective index (SI) over 45 was detected, which is a good basis for further research.

17. Shkondrov, A., **A. Hinkov**, V. Cvetkov, K. Shishkova, D. Todorov, S. Shishkov, I. Stambolov, K. Yoncheva & I. Krasteva. 2023. *Astragalus glycyphyllos* L.: antiviral activity and tablet dosage formulation of a standardized dry extract. *Biotechnology & Biotechnological Equipment*, 37, 1, 2221752, https://doi.org/10.1080/13102818.2023.2221752. **IF_{2022}=1,762, SJR_{2022}=0.317; Q3.**

Abstract: *Astragalus glycyphyllos* L. is widely used in Bulgarian folk medicine. Extracts from this plant have antiproliferative, antitumor and immune stimulating effects *in vitro* and *in vivo*. The aim of this study was to examine the potential antiviral activity of a standardized extract from the aerial parts of the plant and to formulate it in a suitable dosage form. A dry extract was obtained, and its phytochemical composition was determined by ultra-high-performance liquid chromatography– high resolution electrospray ionization mass spectrometry (UHPLC-HRESIMS). The extract contained flavonoids and predominantly saponins. 17(R),20(R)-3β,6α,16β-trihydroxycycloartanyl-23-carboxylic acid 16-lactone 3-O-β-D-glucopyranoside was used as a reference for quantitation. The extract had 3% saponins. The antiviral activity of the extract was tested against Simplexvirus humanalpha type 1 (acyclovir sensitive) and 2 (acyclovir resistant) in vitro. Between 60% and 70% of protection was reached against both virus strains when the extract (at the maximal non-toxic concentration of 0.6 mg/mL) was added simultaneously with and 1 h after Madine and Darby bovine kidney cell inoculation. The extract was also tested in combination with acyclovir only against Simplexvirus humanalpha type 1 *in vitro*. Further, the lyophilized extract was mixed with appropriate excipients aiming to prepare tablets via direct compaction. The optimized tablets fulfilled the requirements of the pharmacopoeia for disintegration and friability. Thus, the study demonstrated the possibility to formulate a standardized extract of *A. glycyphyllos* in tablets and their potential for adjuvant antiviral application in further trials.