GENERAL INFORMATION

1. NAME OF THE CENTER AND LOCATION

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences - **Leading organization**

National Centre for Infectious and Parasitic Diseases (NCIPD), Sofia, Bulgaria - **Partner organization**

Individual participants from AgroBioInstitute-Sofia and Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences

2. TYPE OF THE RESEARCH INFRASTRUCTURE AND/OR SCIENTIFIC EXPERTISE

Identify the type of the RI, equipment/facilities/ specific research, and in particular linked to COVID-19:

Scientific equipment for: 1) analyzes of bio active substances by LC-MS/MS and GC-MS, validation of analytical procedures according to ICH requirements, plant metabolic profiling (ABI-Sofia); 2) plant biotechnology (primary and secondary metabolites, DNA markers: SSR's, AFLP's, RAPD's etc., systems for microclonal propagation directed to small fruits), genotyping, variety identification, genetic diversity assessment by dominant and co-dominant DNA marker systems (ABI-Sofia); 3) screening for antifungal and antibacterial effects of natural products of plant, microbial and algal origin or newly synthesized compounds, their toxicological profiling and cytotoxic potential (IMicB -BAS); 4) proving of viral, rickettsial, chlamydial diseases, Q-fever, evaluation of antiviral effect and cytotoxicity of newly synthesized compounds and natural products in cell cultures (NCIPD-Sofia); 5) laboratory diagnostics (serological and molecular biology screening of clinical samples from hospitalized and ambulatory patients for herpes viruses, influenza, measles, mumps, rubella, parvovirus B19, HIV and others (NCIPD-Sofia); 6) molecular docking methods and quantum-chemical calculations on the structure elucidation, spectral properties and structure–activity relationship of compounds isolated from natural products (IOCCP-BAS).

KEY WORDS:

Expertise in Metabolomics, Antibacterial assay, Antiviral assay, Quantum-chemical and molecular docking methods.

3. TYPE OF THE RESEARCH

Provide information on the research carried on or planned in regard with COVID-19 and other viruses

The proposed research with title *Chemical characterization and evaluation of antiviral and antibacterial activity of extracts from Graptopetalum paraguayense E. Walther (Crassulaceae)* was supported by the Bulgarian National Science Fund under Grant DN19/16/2017. The project is pioneering and is focused on the development of unified theoretical and experimental approaches for active components isolation and antiviral and antimicrobial activity evaluation of *Graptopetalum paraguayense* E. Walther (GP). The plant is native to Mexico but is popular in Chinese herbal medicine. It is a widely consumed plant food in Taiwan and it is used in folk medicine. It was found that GP extracts show anti-inflammatory, anti-conjunctivitis, antioxidant and antineoplastic activities. However, there is no information in the literature on the antiviral and antibacterial activity of GP and despite the intense study of the plant, little data exist regarding its chemical composition.

To evaluate the main organic groups, which had the leaf extract of the tested ethno plant metabolic profiling and analysis by GC-MS was performed. Next three main fractions were obtained - A (lipids), B (amino and organic acids, carbohydrates) and C (phenolic acids) and the composition of each were determined by GC-MS analysis. The cytotoxicity of GP extract was determined on Vero (Green monkey kidney), RD (human rhabdomyosarcoma) and MDCK (Madin-Darby canine kidney) cell lines. The total extract of *Graptopetalum paraguayense* E. Walther as well as the fractions has not cytotoxic effect on these cells.

To determine the capacity of the whole extract, as well as the three main fractions to inhibit the lytic activity we used the following strains:

1. **Herpes Simplex virus (HSV) strains** (collection): two wild-type (*wt*), sensitive to ACV Victoria (HSV-1) and Bja (HSV-2) and two resistant to ACV (ACVR) clinical isolates DD (HSV-1) and PU (HSV-2).

Antiviral activity of leaf extracts of GP against wt HSV strains sensitive to ACV (Victoria, HSV-1) and (Bja, HSV-2).

Tested agent	Antiviral activity					
	HSV-1 (strain Victoria)			HSV-2 (strain Bja)		
	% of protection of the cells in MNC	IC_{50} (mg/mL) ± SEM	SI	% of protection of the cells in MNC	IC_{50} (mg/mL) ± SEM	SI
GP extract	97.5	0.0001 ± 0.08	25 000	25.5	0.01 ± 0.03	250
Fraction C	94.5	0.01 ± 0.144	120	38.0	0.1 ± 0.005	12
ACV	100	0.0005 ± 0.02	800	95.7	0.005 ± 0.12	80

Antiviral activity of leaf extracts of GP against resistant to ACV HSV-1/HSV-2 ACV^R mutants (DD, HSV-1) and (PU, HSV-2).

Tested agent	Antiviral activity					
	HSV-1 (strain DD)			HSV-2 (strain PU)		
	% of protection of the cells in MNC	IC_{50} (mg/mL) ± SEM	SI	% of protection of the cells in MNC	IC_{50} (mg/mL) ± SEM	SI
GP extract	65.5	0.001 ± 0.01	2 500	13	0.1 ± 0.02	25
Fraction C	25.5	0.01 ± 0.004	21	10	0.1 ± 0.03	12
ACV	10.8	0.02 ± 0.05	20	0	0.02 ± 0.03	20

2. **Human influenza virus strains**: A/Puerto Rico/8/34 (H1N1) and B/Yamagata/16/88.

Antiviral activity of leaf extracts of GP against human influenza virus strain A/Puerto Rico/8/34 (H1N1).

Tested agent (MNC, mg/mL)	% of protection of the cells in MNC	IC_{50} (mg/mL)	CD_{50} (mg/mL)
Fraction C (0.01)	87.30%	0.01	20
Fraction B (0.1)	37.20%	1	20
GP extract (0.01)	76.80%	0.1	н.о*.
Tamiflu® (0.1)	94.90%	0.01	5.5

This is the first investigation on the anti-influenza and anti-HSV activity of the total extract and fractions isolated from *Graptopetalum paraguayense* E. Walther. The results of this study shed light that fraction C and total GP extract could be promising inhibitors of influenza A and HSV-1 viruses. The mechanism of the action of fraction C is not yet completely identified. Further studies are needed in order to verify which compounds could be responsible for this activity.

Based on the anti-viral results for GP extracts and especially phenol fraction C we performed theoretical investigations on the binding expedient of phenolic acids from this fraction to viral DNA polymerase amino acids. According to molecular docking methods used to model the interaction between a small molecule and a protein at the atomic level, *trans*-ferulic, gentisic and gallic acid have optimal interactions with the receptor. From the results based on the docking analyses, we have modeled some hydrogen-bonded complexes between phenolic and amino acids. The received data from our quantum-chemical calculations suggest that all phenolic acids could form stable complexes with amino acids from the DNA polymerase active site. The calculations were performed at B3LYP/6-31+G(d,p) level of theory using GAUSSIAN 09 software package.

Our planned tasks during the second part of the project are:

1. Evaluation of the antiviral activity of GP extracts, active fraction(s) and isolated compounds from active fraction(s) against panel of viruses which are relevant to human pathology: human immunodeficiency viruses (HIV), Epstein–Barr virus (EBV), measles virus (MeV), mumps virus, as well as some animal viruses such as bovine coronavirus (BCoV) and others.
2. The content of the products obtained after extraction will be clarified by spectral analysis. By means of different theoretical approaches IR, Raman, NMR, UV-vis and CD spectra of the isolated compounds of the active fractions will be simulated. Molecular docking and quantum-chemical methods will be applied to elucidate the structures of the active fractions and molecules as well as the probable mechanism of their antiviral effect.

4. WEBSITE

Provide the internet address:	*URL: http://www.orgchm.bas.bg/pocc_en.html*

5. BACKGROUND, PUBLICATIONS AND OPEN DATA REPOSITORY

leading research team AND Scientific publications of the research group on the topics of related to coronaviruses research results**;** **link to open data repository**	Assist. Prof. Dr Nadezhda Markova, IOCCP - BAS Prof. DSc Venelin Enchev, IGIC - BAS Assoc. Prof. Dr Snezhanka Bakalova, IOCCP - BAS Assist. Prof. Dr Miroslav Rangelov, IOCCP - BAS As. Nina Stoyanova – Nankova, IGIC - BAS Assist. Prof. Dr Ivayla Dincheva, ABI - Sofia Assoc. Prof. Dr Ilian Badjakov, ABI - Sofia Assist. Prof. Dr Maya Zaharieva, IMicB-BAS Corr. Member Prof. DSc Hristo Najdenski, IMicB-BAS Assoc. Prof. Dr. Petia Genova - Kalou, NCIPD - Sofia Assist. Prof. Dr Stefka Ivanova, NCIPD – Sofia PhD student Radoslav Marinov, NCIPD – Sofia **Published articles on the subject of the project** M. M. Zaharieva, P. Genova-Kalou, I. Dincheva, I. Badjakov, S. Krumova , V. Enchev, H. Najdenski, N. Markova, "Anti-Herpes Simplex Virus and antibacterial activities of *Graptopetalum paraguayense* E. Walther leaf extract: a pilot study" *Biotechnology & Biotechnological Equipment* 33 (2019) 1251-1259. https://www.tandfonline.com/doi/full/10.1080/13102818.2019.1656108 Stoyanova, N., Rangelov, M., Genova-Kalou, P., Enchev, V., Markova, N. "Binding Expedient of Phenolic Acids From the Plant *Graptopetalum Paraguayense* E. Walther to Viral DNA Polymerase Amino Acids: A Theoretical Insight", *Reports Awarded with "Best Paper" Crystal Prize'19, 58th Annual scientific conference of University of Ruse and Union of Scientists with international participation* (2019) 134-139. http://conf.uni-ruse.bg/bg/docs/cp19/bp/bp-19.pdf N. Markova, D. Batovska, E. Kozuharova, V. Enchev, "Anti-conjunctivitis effect of fresh juice of xGraptoveria (*Crassulaceae*) – a phytochemical and ethnobotanical study", *Journal of Intercultural Ethnopharmacology* 4(1) (2015) 24-28. https://www.ncbi.nlm.nih.gov/pubmed/26401380

The results received have presented on 11 national and international scientific forums as: *30th International Symposium on the Chemistry of Natural Products*, 2018, Athens, Greece; *International Agricultural, Biological and Life Sciences Conference*, 2018, Edrine, Turkey; *7th European Congress of Virology*, 2019, Rotterdam, Netherlands; *International Biological, Agricultural and Life Science Congress*, *BIALIC*, 2019, Lviv, Ukraine; *Chemistry As Innovating Science*, CHAINS 2019, Veldhoven, Netherlands and others.

Other papers related to virology

Genova-Kalou P., Vladimirova N., Stoitsova S., Krumova S., Kurchatova A., Kantardjiev T. (2019). "Q fever in Bulgaria: laboratory and epidemiological findings on human cases and outbreaks, 2011 to 2017". *Euro Surveill*., 24(37): pii = 1900119. *https://doi.org/10.2807/1560-7917*

Krumova St., Pavlova A., Yotovska K., Genova-Kalou P. (2019): "Combined laboratory approach to detection of Parvovirus B19 and *Coxiella burnetii* in patients with fever of unknown origin". *Clinical Laboratory*, 65: 69-76.

Argirova, R., Nenova, R., Ivanov, D., Genova-Kalou, P., Raleva, S. (2016). "Experimental model to study co-infection of human immunodeficiency virus – type 1 (HIV-1_{IIIB}) and influenza virus in cell culture". *Biotechnology & Biotechnological Equipment*, 30, 1, 100-105. *doi: org/10.1080/13102818.2015.1091273*

Stankova I., Stanoeva K., Hinkov A., Alexiev I., Genova-Kalou P., Chayrov R., Argirova R. (2012). "Amino acid and peptide esters of abacavir: synthesis and activity against human immunodeficiency virus type 1 in cell culture". *Medicinal Chemistry Research*, 21, 12, 4053-4059.,*doi:10.1007/s00044-011-9956-y*

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Stanchev S., Jensen F., Hinkov A., Atanasov V., Genova-Kalou P., Argirova R., Manolov I. (2011). “Synthesis and inhibiting activity of some 4-hydroxycoumarin derivatives on HIV-1 protease”. *ISRN Pharmaceutics*, Article ID 137637, 9 pages. *doi:org/10.5402/2011/137637*

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Genova-Kalou, P., Dundarova, D., Idakieva, K., Mohmmed, A., Dundarov, S., Argirova, R. (2008). “Anti-herpes effect of the hemocyanin derived from mollusc *Rapana thomasiana”*. *Zeitschrift fur Naturforschung*, 63c, 5 – 6, 429 – 434

Kovala-Demertzi, D.,Varadinova, T.,Genova, P., Souza, P., Demertzis, M. (2007). “Platinum(II) and Palladium(II) Complexes of Pyridine-2-carbaldehyde Thiosemicarbazone as Alternative anti-Herpes Simplex Virus Agents”. *Bioinorganic Chemistry and Applications*, 2007, Article ID 56165, 6 pages. *doi:org/10.1155/2007/56165*

Manolov, I., Raleva, S.,Genova, P., Savov, A., Froloshka, L., Dundarova, D., Argirova, R. (2006). “Antihuman Immunodeficiency Virus Type 1 (HIV-1) Activity of Rare Earth Metal Complexes of 4-Hydroxycoumarins in Cell Culture”. *Bioinorganic Chemistry and Application*, 2006, Article ID 71938, 1 - 7. *(ISSN: 15653633),doi: 10.1155/BCA/2006/71938*

Kostova, I., Raleva, S., Genova, P., Argirova, R. (2005). “Recent advances in the discovery and development of plant-derived natural coumarins and their analogues as anti-human immunodeficiency virus – type 1 (HIV-1) agents”. *Biotechnology and Biotechnology Equipment*, 19, 1, 16 – 22

Varadinova,T., Vilhelmova, N., Badenas, F., Terron, A., Fiol, J., Garcia-Raso, A., Genova,P. (2005). “Effect of metal complexes of acyclovir and its acetylated

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Quideau, S., Varadinova, T., Karagiozova, D., Jourdes, M., Pardon, P., Baudry, C., Genova, P., Diakov, T., Petrova, R. (2004). Main structural and stereochemical aspects of the antiherpetic activity of nonahydroxyterphenoyl-containing C-glycosidic ellagitannins. *Chemistry and Biodiversity*, 1, 2, 247 – 258. *doi: 10.1002/cbdv.200490021*

6. COORDINATOR

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7. POSIBLE PARTNERS

Indicate the partner organizations

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8. IMPLEMENTED AND RUNNING PROJECTS

Projects related to virology, vaccines, infection diseases …	"Chemical characterization and evaluation of antiviral and antibacterial activity of extracts from *Graptopetalum paraguayense* E. Walther (Crassulaceae)" supported by the Bulgarian National Science Fund under Grant DN19/16/2017, IOCCP - BAS, Leading organization and NCIPD – Sofia, Partner organization. The project started the second part of implementation. "Molecular-genetic identification and creation of an archive genomic bank of the circulating human and animal *C. burnetii* genotypes and determination of their role as particularly dangerous infectious agents causing epidemiological outbreaks on the territory of Bulgaria" supported by the Bulgarian National Science Fund under Grant КП-06-Н33/3/13.12.2019; Project leader: Assoc. Prof. Dr Petia Dinkova Genova – Kalou (NCIPD). "DRIED BLOOD SPOTS (DBS) AS ALTERNATIVE, ARCHIVAL MATERIAL FOR DETECTION OF VIRAL AGENTS (MEASLES, MUMPS, RUBELLA, HEPATITIS B VIRUS) IN BULGARIAN POPULATION" supported by the Bulgarian National Science Fund under Grant DM03/1,12.12.16 – completed; Project leader Assist. Prof. Dr Stefka Krumova Ivanova (NCIPD).