ENDEMIC PLANT SPECIES IN BULGARIA: OVERVIEW ON CONSERVATION STATUS AND *IN SILICO* PREDICTION OF POTENTIAL ANTI-CORONAVIRUS ACTIVITY

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INTRODUCTION & AIMS

- The Bulgarian flora includes 270 Balkan and 174 Bulgarian endemic species [1]. Assessing the conservation status and the phytochemical composition of these plants are important aspects of the sustainable preservation, popularization and utilization of such rich natural source of compounds with unexplored biological activities.
- The aims of this study were to: (i) summarize conservation-relevant data on endemic plants found in Bulgaria, (ii) extract and organize data about their phytochemical profiles and (iii) compare them to naturally-occurring compounds, investigated for potential relevance to different coronaviruses.

DATA & METHODS

- The NIH PubMed and PubChem systems (https://www.ncbi.nlm.nih.gov/) were used to prepare a virtual library of phytochemicals from endemic plant species found in Bulgaria. An *in house* virtual library of 32 naturally-occurring compounds with known activities against human and animal coronaviruses was used (see Poster MB&BT-11).
- 3D structure preparation and *in silico* screening were performed using OpenEye Scientific Software (https://www.eyesopen.com/). Multi-conformer structure databases (50 conformers / structure) were created using OMEGA v.3.1.1.2 [2]. Shape- and chemical features-based overlays of the conformers were performed in ROCS v.3.3.1.2 [3]. Analysis of van der Waals interaction surfaces was performed by MOE software v. 2019.0102.

References: *[1]. Petrova A., Vladimirov V., Balkan endemics in the Bulgarian flora, Phytologia Balcanica 16, 2010, 293 – 311.*
[2]. Hawkins P.C., A.G. Skillman, A. Nicholls. J Med Chem., 50, 2007, 74-82.
[3]. Galluzzo P., P. Ascenzi, P. Bulzomi, M. Marino. Endocrinology., 149, 2008, 2567-75.

RESULT 1. FOUR ENDEMICS IN BULAGRIA: BRIEF CONSERVATION DATA OVERVIEW

Achillea thracica Velen.

***Asteraceae*; CR, BULGARIAN ENDEMIC**

- in dry grassy places along roads, in scrub and forest glades in the xerothermic oak forest belts
- Thracian Lowland, Toundzha Hilly Country; 150–300 m alt.
- National Biodiversity Act.; 1997 IUCN Red List of Threatened Plants; Annex 1 of the Bern Convention (1979); Natura 2000

Centaurea parilica Stoj. & Stef.

***Asteraceae*; EN, BALKAN ENDEMIC**

- in stony meadows and slopes, including in rock crevices close to the timberline and in the subalpine belt, calcicole
- Slavyanka Mt, Pirin Mts; (1000)1500–2100 m alt.
- National Biodiversity Act.; partially in Alibotush and Orelyak Strict Nature Reserves; Natura 2000

Micromeria frivaldszkyana (Degen) Velen.

***Lamiaceae*; EN, BULGARIAN ENDEMIC**

- in rocky and grassy places, scrub, usually on limestone; in the beech forest belts and in the subalpine grasslands
- Balkan Range (central, eastern); 900–1800 m alt.
- National Biodiversity Act.; Central Balkan National Park, Kozya Stena Strict Nature Reserve, Sinite Kamani Nature Park; Natura 2000

Sideritis scardica Griseb.

***Lamiaceae*; EN, BALKAN ENDEMIC**

- in open, dry, stony places, on limestone, shallow and eroded soil; subalpine/alpine vegetation belts
- Slavyanka Mt, Pirin Mts, Rhodopi Mts; 1000–2200 m alt.
- List of medicinal plants under special regime for conservation and use; partially in Alibotush Strict Nature Reserve; in Slavyanka Mt., Pirin National Park, Trigradsko Zdrelo Protected Site in Rhodopi Mts.; Natura 2000

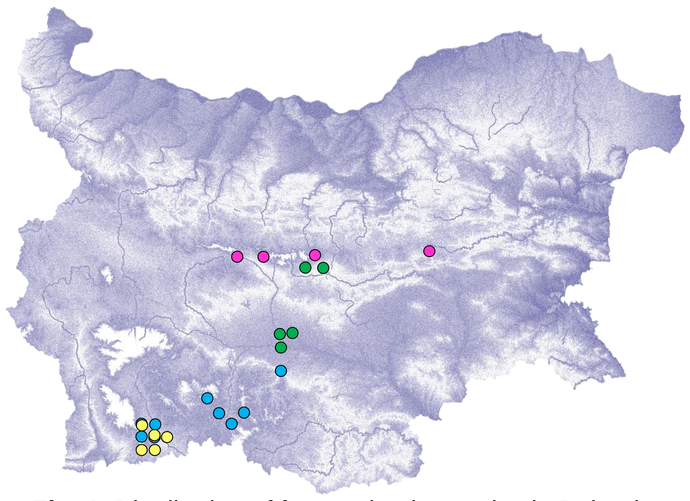


Fig. 1. Distribution of four endemic species in Bulgaria.
Source: http://e-ecodb.bas.bg/rdb/bg/vol1/

RESULT 2. PHYTOCHEMISTRY: VIRTUAL LIBRARY GENERATION

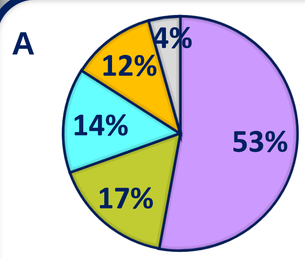
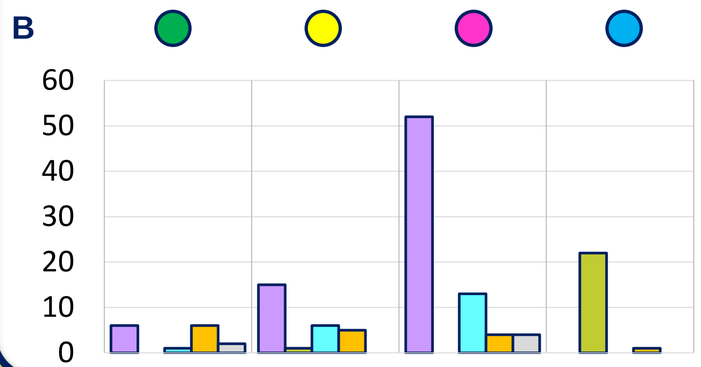


Fig. 2. Chemical composition of the virtual library of 132 compounds: A. Distribution of chemical classes within the library; B. Distribution of chemical classes by species (color coding: see "Result 1").

Terpenes:	monoterpenes, sesquiterpenes, diterpenes, carotenoids
Phenols:	phenolic acids, flavonoids, phenylethanoid glycosides
Hydrocarbons:	cyclohexanes, aromatic hydrocarbons
Lipids:	fatty acids, fatty esters, fatty alcohols
Others:	alcohols, aldehydes, ketones, furans



RESULT 3. SIMILARITY-BASED VIRTUAL SCREENING IN ROCS

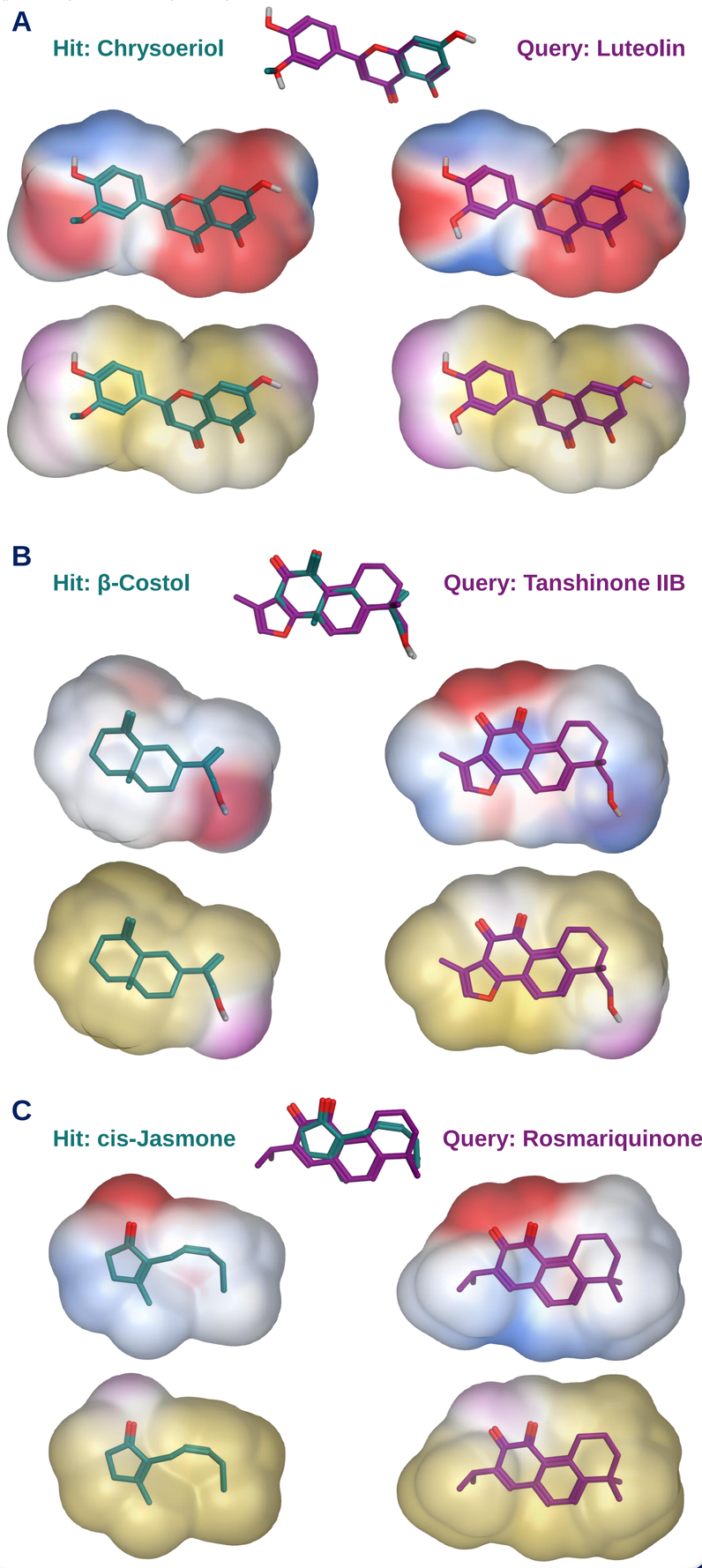
Table 1. Top-scored (S > 1) hit-query couples, representative for different chemical classes and endemic species. Hit – structure from an endemic plant; Query – structure tested for targeting SARS-CoV; S – Score (TanimotoCombo coefficient) for shape and chemical features' similarity estimation [0÷2]. Hits' color coding: chemical classification (* see "Result 2"); source endemic plant (** see "Result 1").

Hit compound	*	**	Query compound	S
Chrysoeriol			**Luteolin**	**1.89**
Chlorogenic acid			Luteolin	1.11
5-O-feruloylquinic acid			Luteolin	1.10
5-O-feruloylquinic acid			Hesperetin	1.05
Caffeic acid			Luteolin	1.05
β-Costol			**Tanshinone IIB**	**1.11**
β-Bourbonene			Rosmariquinone	1.01
1,5-Di-epi-β-bourbonene			Rosmariquinone	1.01
Guaiol			Rosmariquinone	1.01
cis-Jasmone			**Rosmariquinone**	**1.03**
cis-3-Hexenyl isovalerate			Rosmariquinone	1.01

- 132 endemic plants-derived compounds (Fig.2) were aligned on 32 naturally-occurring compounds with known activities against various coronaviruses.
- Luteolin is known for inhibiting viral replication, while the rest of the query compounds are known inhibitors of the SARS-CoV 3-chymotrypsin-like protease.

RESULT 4. ANALYSIS OF MOLECULAR SURFACES OF THE STRUCTURES

Fig. 3. Molecular overlays of selected top-scored hit-query couples (see Table 1): comparative analysis of the electrostatic and the lipophilic properties mapped on compounds' van der Waals interaction surfaces. Color coding for electrostatics: - (red), + (blue), 0 (white). Color coding for lipophilicity: hydrophilic (violet), lipophilic (yellow), neutral (white).



CONCLUSIONS

- **Phytochemical data regarding four endemic species found in Bulgaria** and belonging to two families, i.e. ***Asteraceae*** (*Achillea thracica* Velen. and *Centaurea parilica* Stoj. & Stef.) and ***Lamiaceae*** (*Micromeria frivaldszkyana* (Degen) Velen. and *Sideritis scardica* Griseb.), were collected and organized in a **virtual library of 132 compounds** including terpenes, phenols, hydrocarbons, and lipids. A brief overview on conservation status, distribution, habitats and conservation actions associated with these endemic plant species was made.
- Based on the estimated structural similarity with naturally-occurring compounds tested against SARS-CoV replication or SARS-CoV 3-chymotrypsin-like protease activity, we **outlined promising endemic plant-derived molecular scaffolds, i.e. phenols (*S. scardica*), terpenes (*C. parilica* and *M. frivaldszkyana*), and lipids (*M. frivaldszkyana*), which could be subjects of further *in silico* drug design studies targeting coronaviruses**.

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