IN SILICO AND *IN VIVO* STUDIES OF *ASTRAGALUS GLYCYPHYLLOIDES* SAPONIN(S) WITH RELEVANCE TO METABOLIC SYNDROME MODULATION

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Introduction & Aim

- Naturally-derived triterpenoids are well known modulators of metabolic syndrome. One of the suggested modes of action (MoAs) involves peroxisome proliferator-activated receptor gamma (PPARγ) binding [1, 2].
- In this study we aimed to: (i) evaluate *in silico* potential metabolites of the sapogenin of the main saponin present in a purified saponins' mixture (PSM) from *Astragalus glycyphylloides* and their PPARγ binding modes and (ii) investigate *in vivo* antihyperglycaemic, hypolipidaemic, antioxidant and hepato-protective effects of PSM on spontaneously hypertensive rats (SHRs) with streptozotocin-induced type 2 diabetes (T2D).

Data & Methods

- **Purified saponins' mixture:** A novel saponin has been detected in the PSM [3] (Fig. 1) and its sapogenin (oleanolic acid) was at the focus of the *in silico* studies. PSM was prepared as described in [2] and administrated orally to the model subjects.
- ***In silico* studies:** Knowledge-based expert system (Meteor Nexus, Lhasa Limited) was used for prediction of metabolites. Possible PPARγ binding modes of the metabolites were investigated by pharmacophore-based flexible docking (MOE, CCG Inc.) in the sub-pocket of PPARγ typically occupied by 18 weak partial agonists taken from PDB (with relative efficacy below 35%) [4] (Fig. 2).
- ***In vivo* experiments:** Spontaneously hypertensive rats (SHRs) serve as an experimental model of essential hypertension in human. PSM was investigated in SHRs with streptozotocin/nicotinamide-induced T2D (Fig. 3). For the biochemical analysis blood was collected from the tail vein after a local anestesia and the levels of glucose, triglycerides and total cholesterol were measured using a Multiparameter diagnostic device "MultiCare-in", Italy.
- **Determination of markers of oxidative stress:** malondialdehyde (MDA) quantity, reduced glutathione (GSH) level, catalase (CAT) and superoxide dismutase (SOD) activities were measured in liver homogenate.
- **Pathohistological assessment:** Liver tissues were fixed in 10% buffered formalin and thin sections (5 μm) were subsequently stained with hematoxylin/eosin for general histological features determination. Sections were studied under light microscope Euromex BioBlue equipped with photo camera.

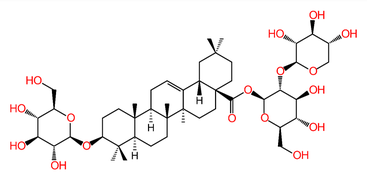
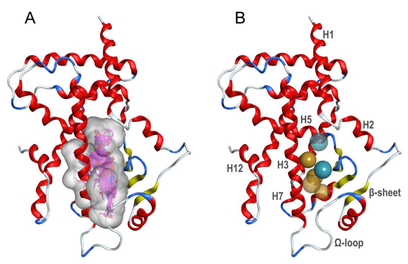


Figure 1. Major saponin in *A. glycyphylloides*



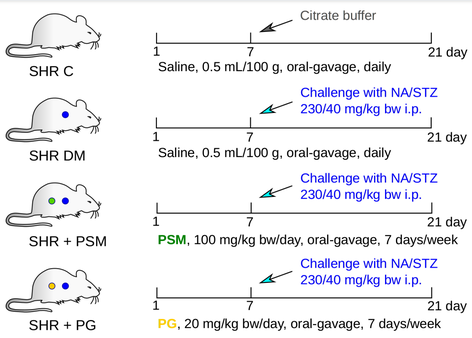


Figure 3. Design of the *in vivo* experiments

Figure 2. PPARγ ligand-binding domain: A. Docking site as defined by 18 weak partial agonists; B. Pharmacophore model of PPARγ weak partial agonists consisting of 3 essential (solid spheres) and 3 non-essential features; in cyan: hydrogen-bond acceptor features; in yellow: hydrophobic or aromatic features.

Phase I meta-bolites

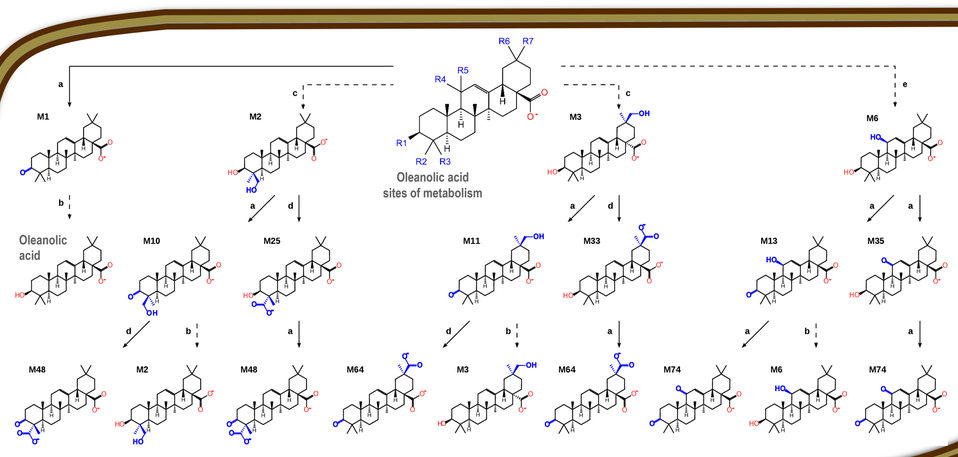


Figure 4. Diagram of the predicted metabolic transformations of oleanolic acid: a. oxidation of secondary (alicyclic) alcohols (alcohol dehydrogenase, ADH), b. reduction of alicyclic ketones (ADH), c. hydroxylation of methyl carbon adjacent to an aliphatic ring (CYP450), d. oxidation of primary alcohols (ADH), and e. allylic hydroxylation (CYP450).
The sites of metabolism (R1-R7) and the newly generated substituents are marked in blue. Biotransformations with probable and plausible likelihood levels are designated by solid and dashed arrows, respectively. For clarity, only the S forms of the 10 metabolites with stereocentres at the sites of metabolism are presented.

Figure 5. Predicted binding modes (BMs) by molecular docking simulations in the PPARγ binding pocket. A. Receptor occupation by representative poses of the oleanolic acid (OA) and its predicted metabolites (M) overlaid on caulophyllogenin, shown in magenta (PDB ID 5F9B, Ligand ID 5VN). B. Closer view of the representative poses – overlaid and separated. Numbers above the structures designate the 12 BM clusters to which the poses belong. ➢

- In total, 24 metabolites, 17 with probable likelihood of occurrence and 7 with plausible likelihood, were predicted. The central ring of the oleanolic acid (R4/R5) was metabolised in only 3 out of 20 predicted reactions generating M6, M13, M35, and M74, while the most frequently predicted sites of metabolism were at the peripheral rings of the structure (Fig. 4).
- 12 different binding modes (BMs) of the metabolites with characteristic receptor occupancy and distinct protein-ligand interaction fingerprints (PLIFs) were outlined (Fig.5, Table 1).
- BMs 1 – 5 have higher frequencies of occurrence (8 to 24 metabolites) and three of these reproduced excellently (BM 3) or resembled (BMs 1 and 2) the X-ray pose of caulophyllogenin (PDB ID 599B, Lig. ID 5VN); BMs 8 –12, which are characteristic for a single metabolite, are termed "singular".
- Metabolites M13.R and M64.S have the broadest spectrum of binding modes – 7 and 6, correspondingly. M64.S results from targeting the peripheral rings of the query molecule and displays the most frequent BMs. M13.R shares the rarely occurring BMs 6 and/or 7 with M6.R, M35 and M74. These metabolites are related to biotransformations at the central ring of the oleanolic acid (Fig. 4, Table1).
- A high number of non-shared BMs is predicted for the stereoisomeric couples of M6, M13, M64, and M33 (shaded, Table 1). These metabolites possess a carboxyl substituent at positions R6/R7 (M64, M33) or hydroxyl substituent at positions R4/R5 (M6, M13), which are rarely occurring substructures within the predicted series of metabolites and might be the reason for their diverse behaviour in the receptor pocket.

Figure 6. Protein-ligand interactions of dicarboxylic metabolites: A. M64.S, displaying BM 5, performs simultaneous interactions with H3 and the β-sheet by performing a contact with Ser289 (H3), in addition to the commonly predicted water-mediated contact with Glu343 (β-sheet), allowing the bulky part of the structure to fit in the Ω-loop at the bottom. B. M33.R with a "singular" BM 9, forms contacts with Leu228 (H1-H2 loop), Ser342 (β-sheet), and Glu343 (β-sheet), leaving the hydrophobic part of the structure in the sub-pocket outlined by H3, H5 and H7. C. Stabilising intra-protein interaction of Arg288 (H3) and Glu343 (β-sheet), triggered by the two metabolites.

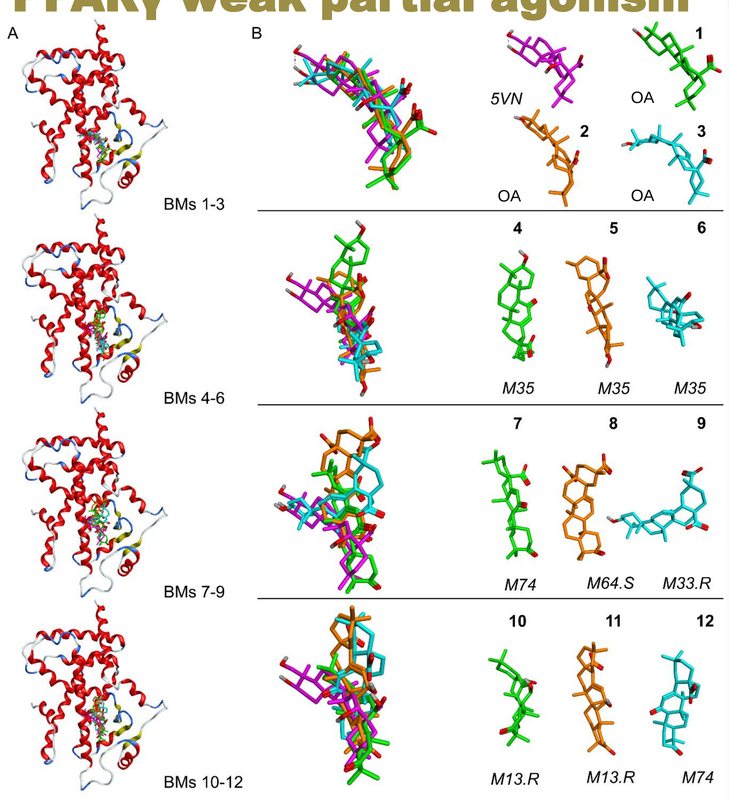
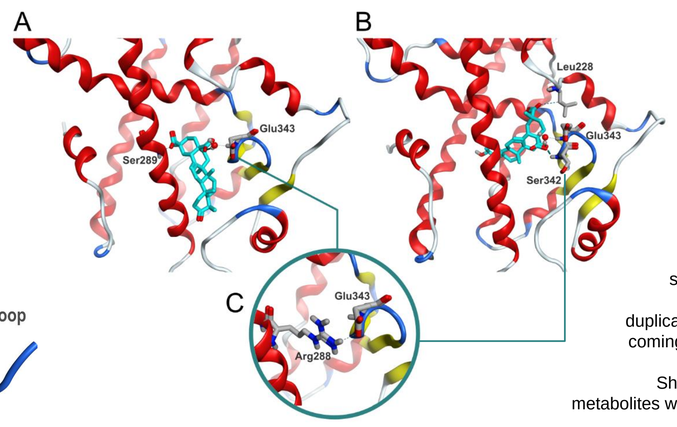


Figure 7. Representative protein-ligand interactions of the three most frequent binding modes among the predicted metabolites (Table 1). A. BM 3, M10.S, B. BM 3, M11.R. C. BM 1, M48.S; D. BM2, M33.S; Rendering: ligands (cyan carbon atoms) and amino acids (element type colouring) are displayed in a stick mode, water molecules (element type colouring) – in a ball and stick mode.



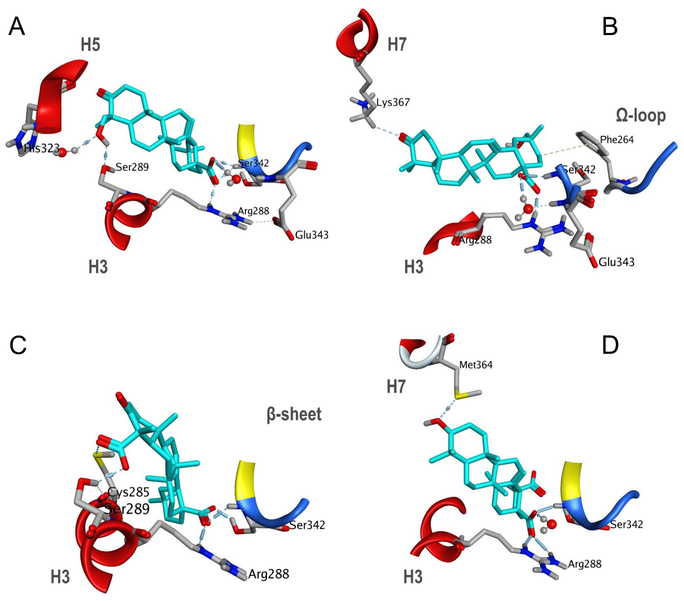


Table 1. Full list of the 12 PPARγ binding modes (BMs) displayed by the 24 predicted metabolites (oleanolic acid, Ms). BM clusters marked in red hold the highest numbers of structures displaying non-shared BMs, within a stereoisomeric couple of metabolites, compared to the total structure count for the respective BM; BM clusters marked in blue include duplication in the total count of metabolites, coming from shared BMs by stereoisomers in the corresponding couples. Shaded stereoisomeric couples include metabolites with high number of non-shared BMs.

Metabolite	BM 1	BM 2	BM 3	BM 4	BM 5	BM 6	BM 7	BM 8	BM 9	BM 10	BM 11	BM 12	BMs count
Oleanolic acid	1	1	1		1								4
M1	1	1	1		1								4
M2.S	1	1		1									3
M2.R	1	1	1										3
M3.S	1	1	1	1	1								5
M3.R	1	1	1										3
M6.S	1	1	1	1	1								5
M6.R	1	1	1			1	1						5
M10.S	1	1	1	1									4
M10.R	1	1	1										3
M11.S	1	1	1		1								4
M11.R	1	1	1										3
M13.S	1	1	1	1									4
M13.R	1	1	1			1	1			1	1		7
M25.S	1	1	1										3
M25.R	1	1	1	1									4
M33.S	1	1	1	1	1								5
M33.R	1	1							1				3
M35	1	1		1	1	1							5
M48.S	1	1	1										3
M48.R	1	1	1	1									4
M64.S	1	1	1	1	1			1					6
M64.R	1	1											2
M74	1	1				1	1					1	5
Ms count	**24**	**24**	**19**	**10**	**8**	**4**	**3**	**1**	**1**	**1**	**1**	**1**	

- All described poses are suggested to induce a moderate activation of PPARγ.
- The presence of two carboxylic substituents at the small distance of four C-atoms is characteristic only for the stereoisomeric couples of M64 and M33. Such a topological feature points to the engagement of both carboxylic groups in specific PLIFs (Fig. 6).
- Although the BM clusters possess characteristic PLIFs, contacts involving Arg288 (H3) and Ser342 (β-sheet), which are typical for the weak partial agonists (Al Sharif et al., 2018) and water-mediated interaction with Glu343 (β-sheet) are the most frequently predicted ones (Fig. 7A-D). The BM 3, holding the highly scored poses, is associated with all aforementioned contacts plus two additional stabilising HB acceptor interactions with His323 (H5, water-mediated, Fig. 7A) or even more often, Lys367 (H7, Fig. 7B). The latter is an amino acid residue which, due to its flexible and thus highly adaptive side chain, participates in a stabilising intra-protein network that involves Met364 and Phe363 (turn in H7) and His449 (H11).
- In the PLIFs of the "singular" BMs 9-12, Arg288 and Ser342 can: (i) be the only amino acid residues performing specific interactions that stabilise the ligand in the pocket, (ii) interact with the ligand in combination with other amino acids like Cys285 (H3) or Leu228 (H1-H2 loop), or (iii) be substituted by alternative pose anchoring points, related to hydrogen bonding with Leu330 or Ile326 from H5.

In vivo effects and pathohistology

Table 2. Blood level of glucose, triglycerides and total cholesterol.

Group	Blood glucose level (mmol/L)	Triglycerides (mmol/L)	Total cholesterol (mmol/L)
SHR C	4.90 ± 0.48	0.48 ± 0.03	1.25 ± 0.09
SHR DM	9.92 ± 0.81*	0.77 ± 0.07*	2.34 ± 0.20*
SHR DM + PSM	7.34 ± 0.51*+	0.61 ± 0.05*+	1.97 ± 0.10*+
SHR DM + PG	6.24 ± 0.75*+	0.52 ± 0.09+	1.69 ± 0.19*+

Data are expressed as mean ± SEM of six rats (n = 6). *p < 0.05 vs SHR control group; +p < 0.05 vs diabetic SHR group

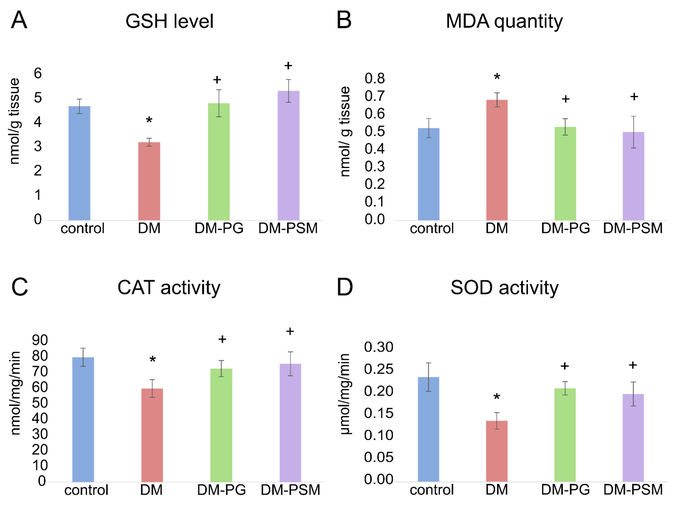
Table 3. Changes in body weight and systolic blood pressure.

Animal group	Systolic blood pressure (mm Hg)	Change %		Mean body weight (g)		
		vs SHR C	vs SHR DM	Initial	Final	Change
SHR C	226±11.4			336±8.2	360±4.8	+ 24
SHR DM	194±8.3	-14*		340±6.3	312±6.2	- 28
SHR DM+PSM	201±13	-11	+4	338±9.7	342±4.6	+ 4
SHR DM+PG	169±6.6	-25*	-13+	326±9.2	332±6.8	+ 6

Data are expressed as mean ± SEM of six rats (n = 6). *p < 0.05 vs SHR control group; +p < 0.05 vs diabetic SHR group

- The blood glucose levels were significantly ($p < 0.05$) elevated in the STZ-induced diabetic SHRs (by 102.4%), compared to their matched controls. PSM decreased in statistically significant manner blood glucose level by 26%. The hypoglycaemic effect of PSM was comparable to the effect of the positive control pioglitazone (Table 2).
- The decreased body weight of STZ treated SHRs, compared to the controls is probably the reason for the reduction of the blood pressure by 14% ($p < 0.05$) in SHR DM group compared to non-treated SHRs. PSM treatment did not change significantly the blood pressure in diabetic SHRs compared to SHR DM group. Pioglitazone treatment additionally reduced blood pressure by 13%, compared to SHR DM (Table 3).
- The serum levels of triglycerides and total cholesterol in the SHR DM group were increased by 60% ($p < 0.05$), and by 84.2% ($p < 0.05$) respectively, compared to control SHRs. PSM treatment decreased the levels of triglycerides by 21% and total cholesterol by 16% compared to diabetic SHRs in a manner comparable to the effects of pioglitazone (Table 2).
- The beneficial effect of PSM treatment in diabetic animals was evidenced by significant increase ($p < 0.05$) in GSH levels by 66%, while MDA production was decreased ($p < 0.05$) by 27%. The effects of NA/STZ on the antioxidant enzymes activity of CAT and SOD were prevented by PSM treatment. CAT activity was 26% higher ($p < 0.05$) and SOD – 44% higher ($p < 0.05$) in PSM-treated group compared to SHR DM group. The antioxidant effect of PSM was comparable to that of pioglitazone (Fig. 8).
- The control group showed normal histological and cellular architecture with distinct hepatocytes, spaces of Disse, central veins and tracts (Fig. 9A). In the livers of rats treated with NA/STZ the hepatic cells were disarranged (swollen appearance, cloudy cytoplasm and microgranulations). The swollen cells affected space of Disse and the lumen of the sinusoidal capillaries was narrowed (Fig. 9B). In the livers of the rats from the group with induced type 2 diabetes mellitus and treated with PSM, gross morphology was spared and abnormal hepatocytes are observed only around the terminal hepatic venulae (Fig. 9C). Livers from animals of the positive control group reviled similar histological parameters as well. In the livers of diabetic SHRs, treated with pioglitazone, in the centrilobular areas only single swollen cells were observed (Fig. 9D). These findings suggest that PSM had a protective effect on the liver at histological level commensurable to pioglitazone.

Figure 8. Antioxidant effects of PSM and pioglitazone in control and diabetic SHRs: A. Levels of GSH, B. Quantity of MDA, C. CAT activity, D. SOD activity. Data are expressed as mean ± SEM of six rats (n = 6); *p < 0.05 vs SHR control group; +p < 0.05 vs diabetic SHR group.



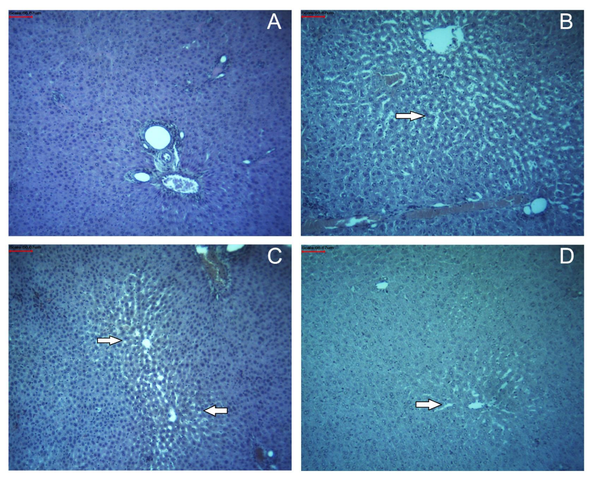


Figure 9. Pathohistological analysis of livers (haematoxylin and eosin, line bar length – 66.67 μm) of SHRs: A. Liver of a rat from control group. Normal histological structure; B. Liver of a rat treated with NA/STZ. Hepatocytes with unclear borders and microgranular cytoplasm; C. Liver of a diabetic rat, treated with PSM; D Liver of a diabetic rat, treated with pioglitazone.

Conclusions

- On the basis of molecular docking simulations, a PPARγ weak partial agonism was suggested for the sapogenin of the main saponin in PSM from *Astragalus glycyphylloides* and for its *in silico* predicted metabolites. Clustering of docking poses according to the binding mode and the protein-ligand interaction fingerprints underlined the role of the predicted sites of metabolism for the diversity of the PPARγ binding.
- The developed novel protocol for *in silico* prediction of possibly coexisting metabolites and their therapeutic MoAs could further be broadened to aid the prioritisation of naturally-derived molecular scaffolds bearing optimal potential to serve as drug-discovery leads, nutraceuticals, or as components of new functional foods.
- Based on the *in vivo* investigation of antidiabetic, antioxidant and antihypertensive effects of PSM in model of NA/STZ-induced type 2 diabetes in spontaneously hypertensive rats, it could be concluded that administration of PSM to diabetic rats improved their glycaemic and liver biochemical and antioxidant status. The histological examination proved that the PSM had hepatoprotective effect which was less pronounced compared to pioglitazone, in rats with artificially induced type 2 diabetes mellitus.

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References: 1. Kwon, DY. et al., 2012, Eur. J. Nutr. 51:529-540; 2. Al Sharif M. et al., 2017, Food Chem Toxicol. 112:47-59; 3. Shkondrov A., et al., 2018, Phytochem. Letters 26:44-49.44; 4. https://www.rcsb.org/

PREDICTING PPARγ PARTIAL AGONISM OF NATURAL TRITERPENOIDS: DEVELOPMENT OF A PHARMACOPHORE-BASED VIRTUAL SCREENING PROTOCOL

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Introduction & Aims

- The metabolic syndrome is a complex condition associated with hypertension, type 2 diabetes and obesity [1].
- Saponins and sapogenins are shown to ameliorate the metabolic syndrome and modulation of the nuclear receptor PPARγ activity has been suggested as one of the possible mechanisms of the amelioration [2-3].
- **In this study we aim to (i) develop and validate a virtual screening protocol for PPARγ weak partial agonists and (ii) screen naturally-derived triterpenoids with relevance to metabolic syndrome and PPARγ signalling.**

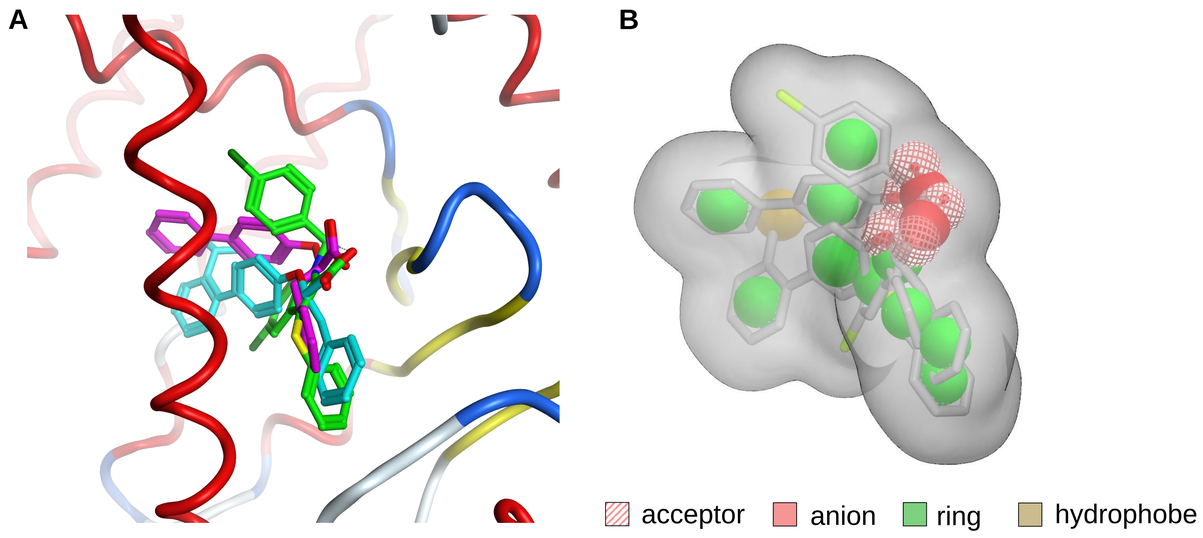
Data & Methods

- PPARγ weak partial agonists used for the training set were extracted from their corresponding X-ray complexes with the receptor from the Protein Data Bank (PDB; http://www.rcsb.org). Structures of the screened compounds were retrieved from the NIH PubChem/NIH PubMed systems and from the DUD-E database (www.dude.docking.org/) and geometrically optimized in the MOE software (CCG Inc.).
- The OpenEye software packages (https://www.eyesopen.com/) were used for generation of up to 10 conformers per structure using the default settings (OMEGA), for development of the virtual screening protocol as well as for screening of the triterpenoid structures (ROCS). ROCS TanimotoCombo scoring was used for estimation of shape and atom "color" (electrostatics, H-bonding, lipophilicity) similarity.

Result 1. Developed and validated virtual screening protocol for PPARγ weak partial agonists

- **Training set selection and generation of ROCS query**
  - The VS protocol was developed using 20 known PPARγ weak partial agonists (E_{max} ≤ 35%) from the PDB.
  - Multiple queries were generated with a maximal number of structures set to 3. The best query consisting of the ligands from PDB complexes 2Q5S, 3D6D and 4E4K (Fig. 1), was selected based on its performance in the validation step.
- **Construction of the test sets**
  - The following test sets including PPARγ decoys and PPARγ ligands (EC_{50} ≤ 5μM) were prepared:
    - 101 known PPARγ weak partial agonists (WPA, E_{max} ≤ 35%) collected based on literature search of homologues of the selected compounds in the training set as well as data extraction from a freely available PPARγ ligands' database developed previously (PPARγ-LigDB, http://biomed.bas.bg/qsarmm/);
    - 48 PPARγ strong partial agonists (SPA, E_{max} = 45% – 65%) from the PPARγ-LigDB;
    - 167 PPARγ full agonists (FA, E_{max} ≥ 75%) from the PPARγ-LigDB;
    - 21045 PPARγ decoys extracted from the DUD-E database.

Fig. 1. Query generation: A. X-ray poses of the three weak partial agonists composed the best query: 2Q5S, NZA (in green), 3D6D, LRG (magenta), and 4E4K, RRG (cyan) overlaid in the protein template of 3D6D, B. Best query (consisting of shape and "color" atoms) with overlaid X-ray poses of the three structures



- **Validation of the VS protocol**
  - The enrichment in ROCS represents the ratio of the true positive rates (sensitivity) to the false positive rates (Fig. 2, Table 1). As seen from the enrichment curve for the weak partial agonists the VS protocol performs with a good specificity, allowing for discrimination between PPARγ decoys and weak partial agonists. The curves for the strong partial agonists and for the full agonists are near or below the random (dashed) line, underlining the selectivity of the developed VS protocol.

Fig. 2. Performance of the VS protocol: A. Enrichment curves of the three activity classes resulting from validation runs in the presence of decoys, B. Discrimination of activity classes using ROCS TanimotoCombo scoring

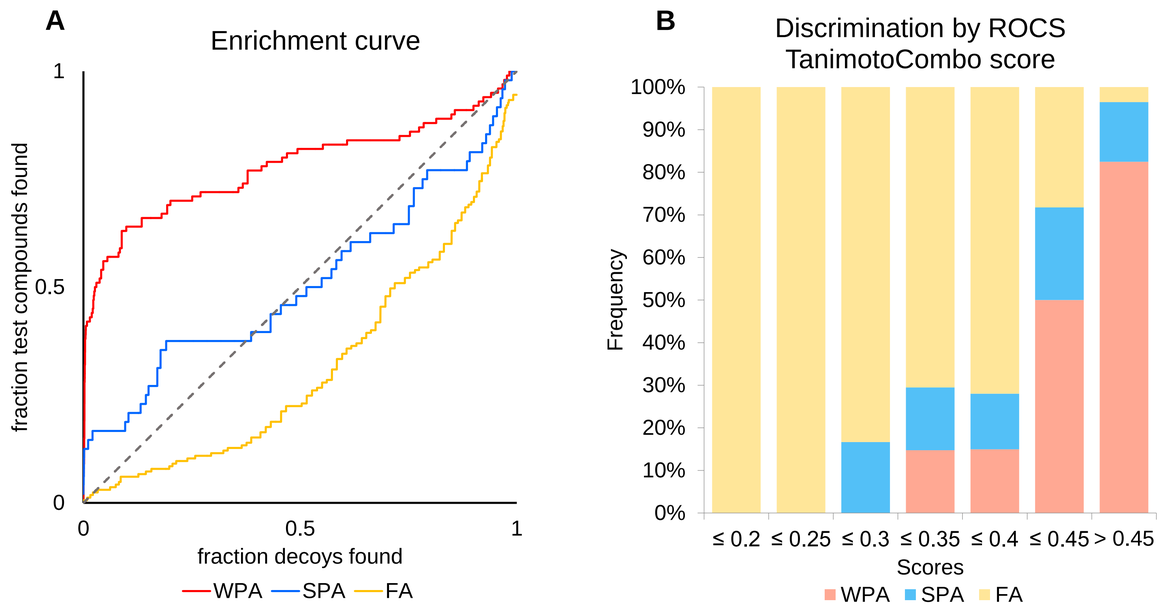


Table 1. Statistics of the validation runs

Set	No of cmpds	Enrichment in top 0.5%	No of cmpds in top 0.5%
WPA	101	77.5	39
SPA	48	24.6	6
FA	167	1.2	1

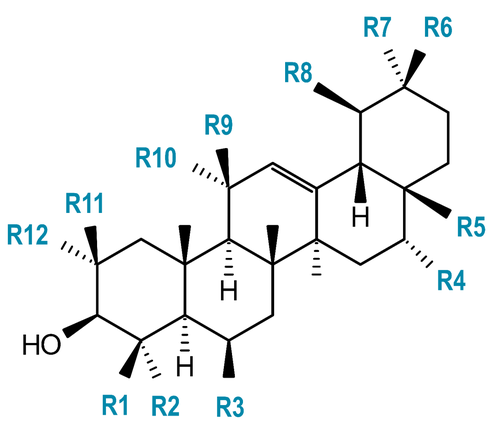
- **Selection of TanimotoCombo threshold for VS**
  - A cutoff value of 0.40 (Fig. 2B) for the ROCS TanimotoCombo score allows for discrimination between the different activity classes. Such low similarity score is considered reasonable since the query is composed of 3 structures and high scores in terms of shape or "color" atoms are quite improbable.

Result 2. Virtual library of sapogenins

- A virtual library (VL) of 24 triterpenoids was constructed. The criteria for compound selection were:
  - i. relevance to the metabolic syndrome
  - ii. experimental evidence for positive modulation of PPARγ activity/expression
  - iii. evidence for mono-treatment with a pure compound
- Within the distribution of the compounds by activity type:
  - two of the compounds are known to increase PPARγ expression (esculentoside A and glycyrrhizic acid);
  - one displays both PPARγ agonism and upregulation (asiaticoside);
  - the rest are known as PPARγ agonists.
- The VL includes one known PPARγ weak partial agonist from PDB – caulophyllogenin, with E_{max} = 9.4% and EC_{50} = 12.6μM.
- The triterpenoids share 10 common aglycons (sapogenins), described in Table 2.

Result 3. Virtual screening of sapogenins with ROCS

Table 2. Aglycons of 24 triterpenoids, and TanimotoCombo scores



Name[i]	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	Tanimoto Combo score
Compounds **9**[ii]; 1, 2, **3-6**[iii]; Oleanoic acid	CH_3	CH_3	H	H	COOH	CH_3	CH_3	H	H	H	H	H	**0.465**
Glycyrrhizic acid	CH_3	CH_3	H	H	CH_3	COOH	CH_3	H	= O		H	H	**0.438**
Esculentoside A	CH_3	CH_2OH	H	H	COOH	CH_3	$COOCH_3$	H	H	H	OH	H	**0.421**
Compound 2[ii]	CH_3	CH_3	OH	OH	COOH	CH_3	CH_3	H	H	H	H	H	**0.418**
Compounds **3, 5-8, 10, 11**[ii]; Hederagenin	CH_3	CH_2OH	H	H	COOH	CH_3	CH_3	H	H	H	H	H	**0.414**
Compound 1[ii]	CH_3	CH_2OH	OH	OH	COOH	CH_3	CH_3	H	H	H	H	H	**0.406**
Caulophyllogenin	CH_3	CH_2OH	H	OH	COOH	CH_3	CH_3	H	H	H	H	H	0.400
Platycodin D	CH_2OH	CH_2OH	H	OH	COOH	CH_3	CH_3	H	H	H	OH	H	0.390
Platyconic acid	COOH	CH_2OH	H	OH	COOH	CH_3	CH_3	H	H	H	OH	H	0.374
Asiaticoside	CH_3	CH_2OH	H	H	COOH	H	CH_3	CH_3	H	H	H	OH	0.362

i. when multiple compounds are listed, those in bold are related to the reported effect on the receptor; ii. Quang TH et al., 2011, Carbohydr Res. 346:2567-75; iii. Nhiem NX et al., 2011. Bioorg Med Chem Lett., 21:6143-7

- Considering the selected TanimotoCombo scoring threshold, 6 out of 10 studied sapogenins passed successfully the VS with scores between 0.406 and 0.465.
- Since the VS protocol was trained over selected weak partial agonists with a high potency (EC_{50} ≤ 5μM) it is suggested that the reason for the marginal value of the known PPARγ weak partial agonist (caulophyllogenin) is its lower potency.

Conclusions

- The developed VS protocol proved its ability to discriminate PPARγ weak partial agonists from receptor's decoys as well as from other activity classes like strong partial agonists and full agonists.
- Our results from the application of the VS protocol to sapogenins suggest possible PPARγ weak partial agonistic activity for 6 out of 10 studied sapogenins.
- This research contributes to the mechanistic explanation of the effects of triterpenoid saponins/sapogenins by a potential PPARγ-weak partial agonism and can direct further studies of these compounds for their use as metabolic syndrome modulators. The approach can be applied for VS of other naturally-derived compounds to predict their PPARγ weak partial agonism.

Acknowledgements: Support from the National Science Fund of Bulgaria (grant DM 01/1/2016) is acknowledged. The authors wish to thank the OpenEye Free Academic Licensing Program for providing a free academic license for molecular modeling and chemoinformatics software.

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FOOD, MEDICINAL, AROMATIC AND SPICE PLANTS IN BULGARIAN HOME GARDENS – INTERDISCIPLINARY PERSPECTIVES

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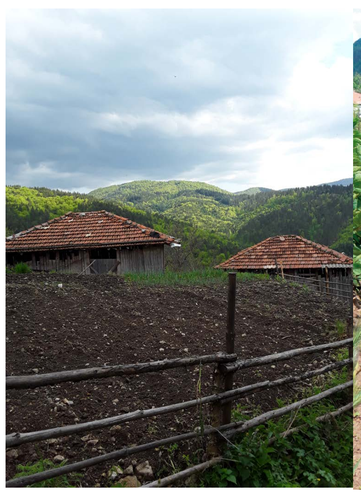
Introduction

Globalization and industrial intensification of agri-food sector in the last 50 years have caused reduction of crop diversity and have suppressed local markets to offer locally grown products with added value. Meanwhile the increasing quest for ecological sustainability and high quality of live demand for eco-oriented and adaptive agriculture that can provide not only diverse and healthy food but also preventing biodiversity loss and coping with climate changes. Hence, alternative provisions for preservation and development of local plant diversity should be found to attain diversification and resilience of agro-ecosystems and food provision chains. In the near past (1990s) Bulgarian self-subsistent food provision was considered also a cultural phenomenon that provided sustenance especially in the rural areas (Balcombe et al. 1999, Kostov & Lingard 2002). Nowadays, however, the transformation in food provision chains and depopulation in rural areas are changing the maintenance of plant diversity (Dimitrova et al. 2015a,b).



Objectives and methodology

Our project addresses the interrelations and interplays between the plant and the social worlds. Its main objective is to study how particular plant species/landraces grown in home gardens affect social relations and cultural patterns and how cultural orientations, local knowledge and experience, social relations and cultural practices affect plant diversity, influence selection, modification or loss of plant genetic resources. We assume that Bulgarian bio-cultural heritage related to plant diversity and associated traditional knowledge is preserved in home gardens and small market-oriented production plots in the rural regions. Thus, we have assessed this plant biodiversity and related traditional ecological knowledge preserved in village and small urban settlements (35) in four provinces in South and North-West Bulgaria. We present data on food, medicinal and aromatic plants, traditionally grown or currently introduced from abroad, as well as garden cultivation of wild plants. randomly selected or specifically recommended respondents were approached by semi-structured interviews. Plant diversity was video documented and herbarium specimens were collected for subsequent identification of taxa. Additionally, when possible, interviews were done during local farmer markets and festivals related to plant diversity.

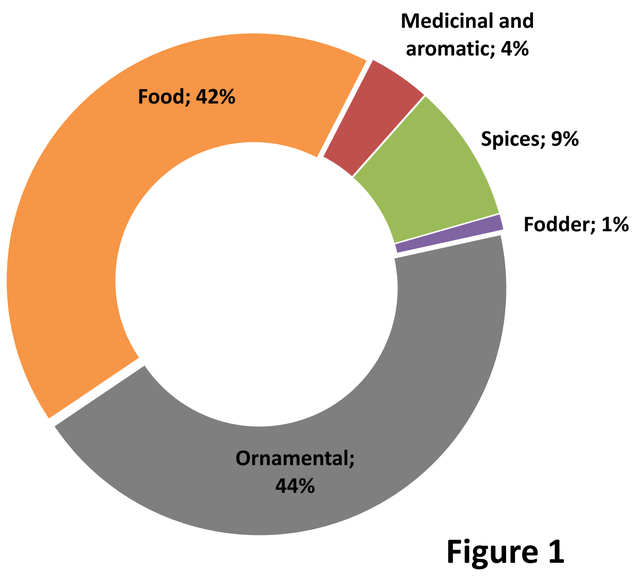


Figure 1

Results and Discussion

Field data demonstrate a prevalence of plant taxa with provisional purposes (food, medicinal and aromatic plants and animal fodder, 56%, **Fig. 1**). Numerous ornamental (44%) and few technical and insecticidal plants complete the typical view of Bulgarian rural garden. Food plants grown in home gardens are from almost all groups except grains. **Food plants (42%)** occupy main place not only as a taxonomic diversity but also spatially especially in remote areas with insufficient food supply and/or within economically challenged households. Traditionally grown fruit and vegetable varieties are often considered as "own" or "local" especially when seeds or planting material are inherited landraces, personally grown and/or acquired in the near past. The latter include both favoured varieties acquired from the former state-owned cooperatives (i.e. Berkovska yagoda – several *Fragaria* x *ananassa* varieties imported in the 1950s) and imported varieties distributed non-commercially by relatives and friends.

These "owned" varieties are considered of higher (even cultural) value by local people. However, their unavailability on the market and the very limited local knowledge about them pose concerns for their maintenance and/or threat from extinction of some of them. Hence, the economic potential of these varieties/landraces has been either neglected or undervalued. Only few notable exceptions with geographical reference (i.e. Smilyanski fasul (*Phaseolus vulgaris/Ph. multiflorus*), Kurtovski rozov domat (*Solanum lycopersicum),* Reseleshki luk (*Allium cepa*) have been marketed as such and at a higher price**. Over 30 species of medicinal and aromatic plants** are documented in rural gardens (Table 1). Medicinal plants and related knowledge were preserved for emergency purposes even though the owner does not always rely on traditional/alternative medicine practices. Spices were often shared in the community and seeds were purchased only if local origin is lost. In such cases we found that international, foreign and Bulgarian varieties (not only of spices) were not particularly well discerned. There is a common notion that state and/or at least academia have already preserved plant diversity among the participants. Such misconception, especially among senior people, we can explain with the former state-managed collection programs. However, expeditions made in the past covered limited crop diversity, while in the last 30 years such work has not been carried out. Moreover, many of the past collections are not accompanied by information about related traditional knowledge and thus, fail to contribute to engagement of local communities or interested individuals in preservational practices *in situ*.

Species	Medicinal plant	Aromatic and/or Spice plant
Allium cepa L..	1	
Allium sativum L.	1	1
Allium ursinum L.	1	1
Anethum graveolens L.		1
Apium graveolens L.		1
Armoracia rusticana P.Gaertn., B.Mey. & Scherb.	1	
Artemisia sp. L.		1
Calendula officinalis L.	1	
Chelidonium majus L.	1	
Echinacea sp. Moench	1	
Foeniculum vulgare Mill.		1
Geranium macrorrhizum L.	1	
Laurus nobilis L.		1
Levisticum officinale W.D.J.Koch	1	1
Melissa officinalis L.	1	
Mentha × *piperita* L.	1	
Mentha spicata L.		1
Ocimum basilicum L.		1
Origanum vulgare subsp. *hirtum* (Link) Ietsw.		1
Pelargonium graveolens L'Hér.		1
Petroselinum crispum (Mill.) Fuss		1
Plantago major L.	1	
Ruta graveolens L.	1	
Satureja hortensis/S. pilosa		1
Salvia officinalis L.	1	
Sambucus nigra L.	1	
Sideritis scardica Griseb.	1	
Tagetes patula L.	1	
Thymus sp.	1	1
Tilia tomentosa Moench	1	
Trigonella foenum-graecum L.		1
Urtica dioica L.	1	

Table 1. Medicinal and aromatic plants grown in rural home gardens in Bulgaria

Conclusions

Home gardens in rural Bulgaria still provide substantially for the subsistence of local communities and could be regarded as *in situ* gene pool for local plant genetic resources and as reservoirs of traditional ecological knowledge. However, lack of consistent national policy for *in situ* and/or *ex situ* plant conservation, globalized seed market, prevalence of industrial agriculture and food production, together with some gaps in agricultural entrepreneurship during and after Communist era and inconsistent implementation of EU regulations are among major drivers of (agro)biodiverisity loss n Bulgaria.

- Further collection programs and studies of the cultural value of plant diversity in home gardens are needed in order to preserve local plant genetic diversity, to raise awareness to food sovereignty among broader public and to motivate local communities to cooperate for the preservation of plant genetic resources.
- Grass-root initiatives and eco-oriented (social) entrepreneurship could contribute to preservation of local bio-cultural heritage through promotion of *in situ* and *ex situ* conservation practices and further valorization of these resources.
- New regional and/or national policies supporting micro-entrepreneurship are needed to facilitate cooperation among producers, to reduce administrative load and achieve more sustainable results in the preservation and utilization of local genetic resources.
- Well established local plant varieties and land races that are fundamental for the local communities have the potential for PDO/PGIs to achieve fair price and market sustainability

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ALEXANDRU IOAN CUZA UNIVERSITY of IAŞI

AN OVERVIEW ON *THYMUS DACICUS* BORBÁS.: DISTRIBUTION, ANATOMY AND CHEMICAL COMPOSITION OF ESSENTIAL OILS

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Introduction

Thymus species are known to have medicinal properties due, in particular, to essential oils with numerous antimicrobial effects. *Thymus dacicus* is a perennial herbaceous plant that grows on rocky pastures and sands in Romania, and NE of Serbia [1]. The purpose of this paper is to make a synthesis of the accomplishment studies carried out on *Thymus dacicus*, regarding the distribution, the structure and the composition of the essential oils, known that in this genus exist a high variability of populations (with respect to morphological and the composition of essential oils).

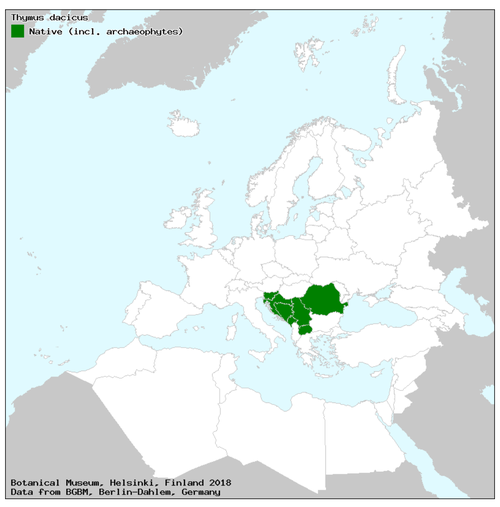
Material

The plant material is represented by *Thymus dacicus* Borb., a perennial plant with initial vigorous recumbent stems, then ascendents, very branched. The leaves are elliptic or prolonged, green in color, both faces are covered with hairs, nervures little proeminent (Guşuleac M., 1961). This plant was earlier treated as an hybrid between *Thymus kosteleckyanus* Opiz and *Thymus pulegioides* L. (Jalas, 1972), however according to some authors it is closely related to *Thymus longicaulis* C. Presl. (Diklić & Vasić, 2000).

Thymus dacicus Borb. (original photo)

Discussions

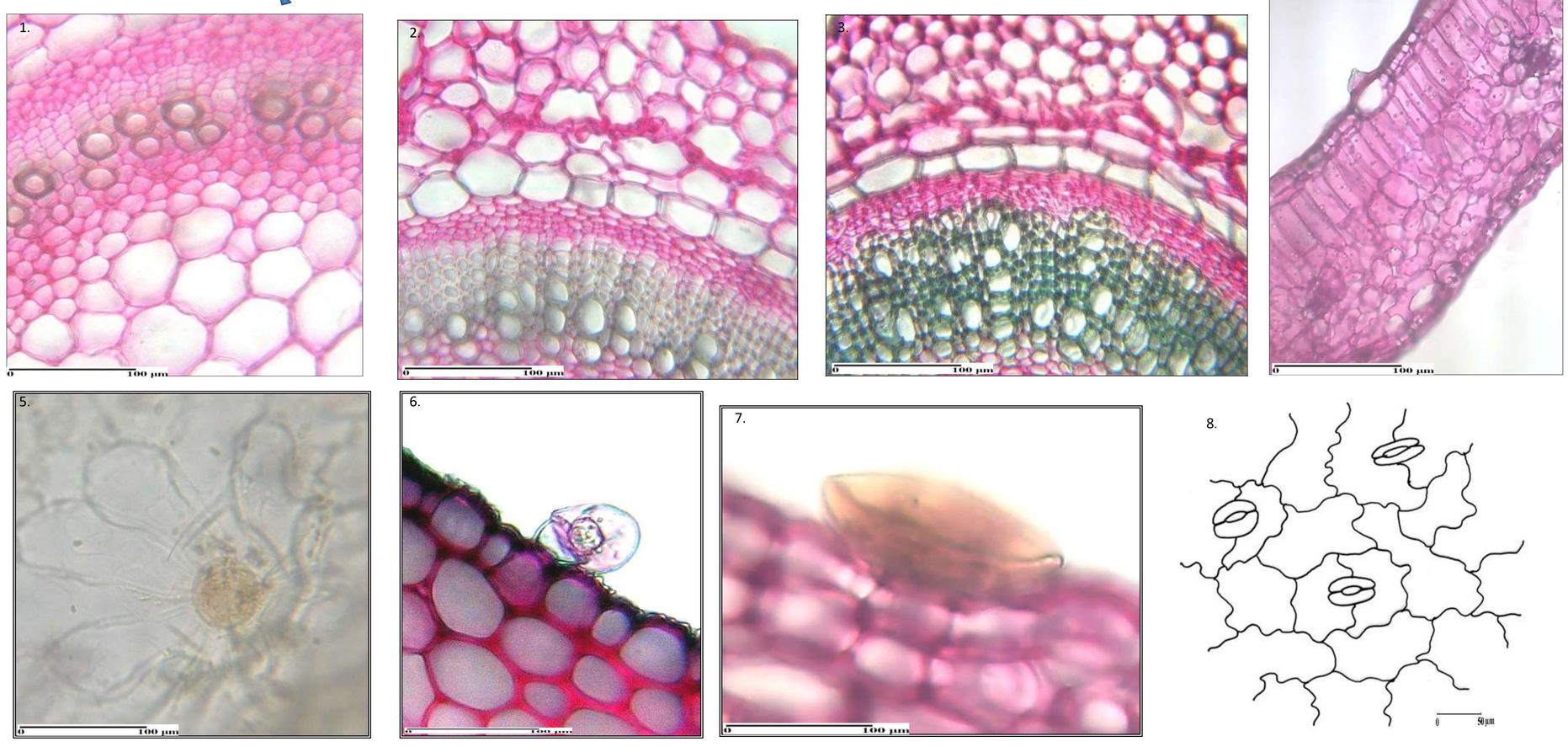
Distribution



http://euromed.luomus.fi/euromed_map.php?taxon=512152&size=medium

From histo-anatomical point of view it can be mention that: passing to the secondary structure of the stems takes place early do to cambium activity; the endoderm is of Casparian type (specific to Lamiaceae); secretory hairs are always multicellular, being formed by a unicellular base, a unicellular pedicel and a single or multicellular gland (Berciu and Toma, 2008).

Anatomy



Structure of vegetative organs of Thymus dacicus: 1. **Cross-section of stem (superior level); 2. Cross-section of stem (median level); 3. Cross-section of stem (inferior level); 4. Cross-section of foliar blade; 5- 7 secretoy hairs; 8. stomata (Berciu and Toma, 2008)**

Chemical composition of the essential oils

Tabel 1. Chemical composition of the essential oils of *Thymus dacicus*, collected in various phenophases in two consecutive years (2013-2014) from Novaci, jud. Gorj (% area) (Boz et al., 2017)

Compound	RT, min	Vegetative stage 2013	Vegetative stage 2014	Anthesis stage 2013	Anthesis stage 2014	Fruiting stage 2013	Fruiting stage 2014
α-Pinene	13.640	-	0.53	-	-	0.809	-
Camphene	14.332	-	0.842	-	-	0.361	-
Octen-3-ol	15.863	-	0.395	-	0.445	0.808	0.577
Myrcene	16.435	-	2.276	-	-	-	-
β-Pinene	16.463	-	-	-	-	1.145	0.188
p-Cymene	17.904	-	7.466	-	4.698	-	10.832
Limonene	18.109	-	0.328	-	0.25	0.236	-
Eucalyptol	18.207	0.661	0.527	-	0.492	-	0.623
cis-β-ocimene	19.012	-	0.372	-	-	-	-
γ-Terpinene	19.452	-	0.278	-	1.313	0.923	2.119
cis-Sabinene hydrate	19.795	2.022	1.824	-	-	2.057	-
Linalool	21.201	-	0.702	11.44	1.928	11.667	0.359
Linalyl acetate	21.157	-	-	1.822	-	-	-
Borneol	23.876	0.842	2.632	2.636	0.598	1.95	1.043
Terpinen-4-ol	24.333	1.642	0.487	1.197	-	6.206	-
α-Terpineol	24.859	-	3.819	9.879	0.248	3.568	-
Nerol	26.271	2.568	2.628	1.305	1.489	1.051	1.893
Methyl thymol	26.717	2.14	5.407	-	3.057	0.401	4.78
Neral	26.860	0.811	0.349	0.665	0.456	1.213	1.015
Geraniol	27.254	5.696	18.376	2.724	33.025	1.39	28.81
Geranial	27.666	1.974	-	1.162	-	1.891	0.769
Citral	27.808	-	-	-	0.76	-	-
Bornyl acetate	27.837	1.798	7.894	1.741	-	5.106	-
Thymol	28.454	2.314	5.397	-	4.124	-	4.697
Carvacrol	28.934	6.025	0.365	-	12.477	-	16.045
α-Terpinyl acetate	30.660	-	-	-	7.904	-	0.174
Neril acetate	31.106	-	1.543	0.383	-	1.39	-
Lavandulol acetate	31.581	-	-	1.027	-	-	-
Geranyl acetate	31.763	1.589	18.489	-	12.683	1.126	11.044
β-Cariophyllene	33.186	1.299	5.333	2.518	2.49	-	1.788
Alloaromadendren	34.347	-	-	1.047	-	1.275	-
α-Cariophyllene	34.541	-	1.435	-	-	0.584	-
Germacrene D	35.141	-	2.166	0.808	3.065	1.125	-
γ-Elemene	35.678	-	1.546	0.537	0.586	3.355	0.729
β-Bisabolene	35.953	3.136	1.377	2.052	5.283	-	4.972
γ-Cadinene	36.010	1.05	0.968	-	0.449	1.017	-
β-Cadinene	36.204	-	-	0.879	-	-	0.625
τ-Cadinene	36.262	0.772	2.394	-	-	1.624	-
Elemol	37.045	0.85	0.388	0.875	-	0.362	-
Trans-Nerolidol	37.599	0.367	3.081	0.304	0.28	0.456	0.317
Spathulenol	37.919	-	-	9.079	-	9.572	0.32
Farnesene	38.148	0.811	0.33	-	-	-	-
Caryophyllene oxide	38.324	2.334	10.531	2.525	0.511	3.171	0.883
Leden	38.776	-	-	0.845	-	-	-
τ-Cadinol	39.845	-	-	2.563	-	2.261	-
τ-Muurulol	40.228	-	9.698	5.456	-	3.845	-
γ-Cadinol	41.342	5.23	-	18.807	-	7.834	-
Aromadendrene epoxyde	41.645	-	-	2.453	-	1.118	0.285
Total		84.228	87.879	87.281	99.128	93.346	97.145

Analyzing the chemical composition of essential oils a number of qualitative and quantitative differences have been observed, depending on phenophase and harvest year (Boz et al., 2018). The main chemical components identified was geraniol, linalool, geranyl acetate, γ-cadinol and caryophyllene oxide (Boz et al., 2018). Petrovic et al., 2017, mentions that the oil of *Thymus dacicus* was mainly characterized by oxygenated monoterpenes , with geranial (trans-citral or citral A) and neral (cis-citral or citral B) being the most abundant.

CONCLUSION

➢The factors determining the composition and quantity of volatile oils are numerous, sometimes is difficult to differentiate between them, because many are interdependent. These factors include seasonal and maturation variations, genetic variations, geographical origin, growth phases and plant parts that are used.

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ALEXANDRU IOAN CUZA UNIVERSITY of IAŞI

SALICORNIA EUROPAEA L.: PRELIMINARY ASPECTS ON MORPHOMETRY, ANATOMY AND BIOCHEMISTRY

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Introduction

Salicornia europaea L., a succulent halophyte (Chenopodiaceae family), is widely distributed in coastal and inland salt marshes [1]. Many studies on halophytes have indicate that these species have a high content of polyunsaturated fatty acids, carotenoids, vitamins, sterols, essential oils, polysaccharides, glycosides, and phenolic compounds [2,3], being potential candidates in food and pharmaceutical industry. The purpose of this paper is to bring new data on morphometry, anatomy and biochemistry of *Salicornia europaea*, a species collected from 4 different areas of the Danube Delta.

Material and Methods

The vegetal material is represented by individuals of *Salicornia europaea* L., collected from 4 different locations in the Danube Delta (Murighiol, Sarinasuf, Plopu, Enisala) in 2017 (Fig. 6 - 9).

Study sites The investigation were carried at the low, flat and flooded area in Danube Delta Biosphere Reserve in different habitats such as: 1310 - communities of *Salicornia* and other annuals colonising mud and sand; 1530* Pannonic salt steppes and salt marshes, according to Natura 2000 Annex I, Habitats. Analysed soils belonged to Gleyc Solonceak (Sarinasuf and Enisala) and Sali-sodic Gleysols (Plopu and Murighiol). For each individual specific measurement was made. The structure were analysed through classical techniques of histo–anatomy. The photosynthetic pigments (chlorophyll a, chlorophyll b, total carotenoids) and sugars leaf fraction (monosaccharides, disaccharides and polysaccharides) were analysed by spectrophotometric methods.

Results and Discussions

Tabel 1. Morphometric measurements at *Salicornia europaea*, collected from 4 different locations

Location	*Murighiol*	*Sarinof*	*Plopu*	*Enisala*
Height of the plant (cm)	$16,20^{a}\pm2,16$	$13,06^{b}\pm3,75$	$29,20^{c}\pm3,32$	$15,13^{a,b}\pm2,66$
Height from root to first branching (cm)	$1,48^{a}\pm0,50$	$1,43^{a}\pm0,89$	$2,34^{c}\pm0,59$	$1,20^{a}\pm0,39$
No. of internodes	$16,73^{a}\pm1,90$	$14,46^{a}\pm5,09$	$26,86^{c}\pm3,73$	$15,00^{a}\pm3,13$
Internode 1 length (cm)	$0,91^{a}\pm0,31$	$0,80^{a}\pm0,23$	$1,66^{c}\pm0,17$	$1,00^{a}\pm0,18$
Internode 2 length (cm)	$1,02^{a,b}\pm0,28$	$0,76^{a,b}\pm0,13$	$2,71^{a,c}\pm3,68$	$1,04^{a,b}\pm0,25$
Intermediate internode length (cm)	$0,48^{a,b}\pm0,11$	$0,41^{a,b}\pm0,12$	$0,54^{a,c}\pm0,14$	$0,42^{a,b}\pm0,11$
Last internode length (cm)	$0,36^{a}\pm0,08$	$0,31^{a}\pm0,08$	$0,50^{c}\pm0,11$	$0,36^{a}\pm0,10$
Fertile segment length (cm)	$0,33^{a}\pm0,08$	$0,62^{a,c}\pm0,70$	$0,96^{c}\pm0,25$	$0,57^{a}\pm0,14$

In order to identify the morphological variations induced by the living environment, a number of specific characteristics were observed (plant height, height from root to first branching, number of internodes, internodium lengths, stem branching) (Table 1). To highlight significant statistical differences, the Tukey HSD test ($p<0.001$) was applied.

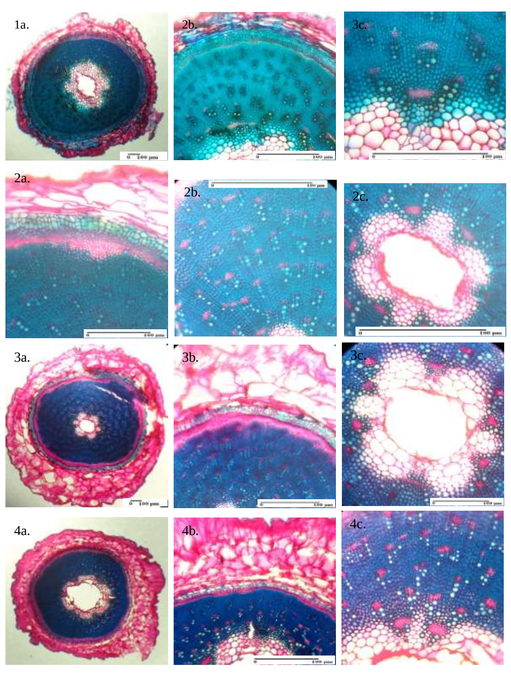

Fig. 1. Cross sections of *Salicornia europaea* stem collected from: 1. Murighiol; 2. Sarinasuf; 3. Plopu; 4. Enisala; a. Overview; b-c. details.

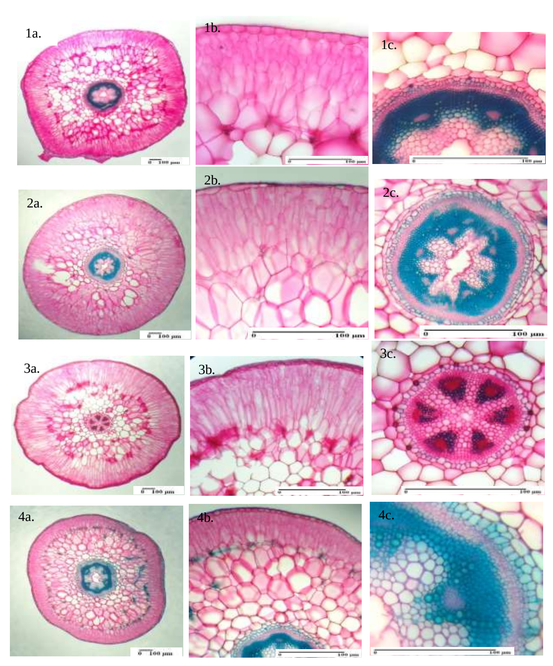

Fig. 2. Cross sections of the *Salicornia europaea* fleshy stem segments collected from: 1. Murighiol; 2. Sarinasuf; 3. Plopu; 4. Enisala; a. Overview; b-c. details.

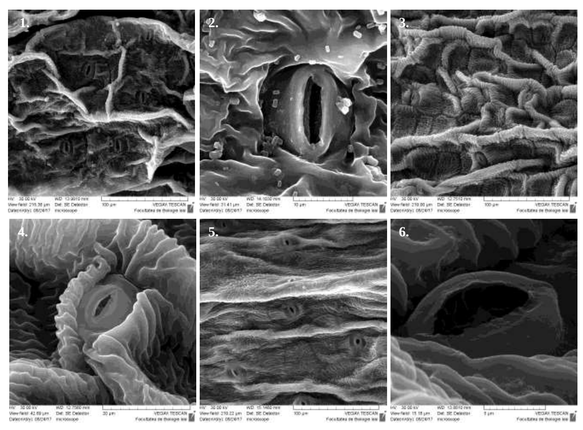

Fig. 3. Epidemics with stomata from the *Salicornia europaea* fleshy stem segment (SEM images): 1-2 Murighiol, 3-4 Sarinasuf; 5 Ploplu; 6 Enisala

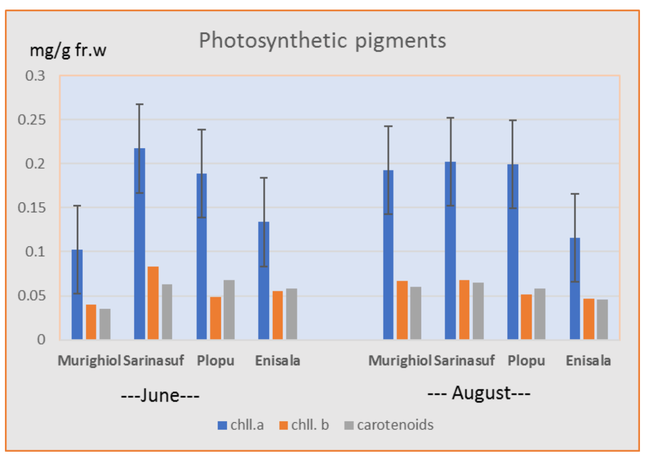

Fig. 4 Dynamics of photosynthetic pigments in *Salicornia europaea* from saline habitats from Danube Delta ($p \le 0.05$)

The chlorophylls biosynthesis showed a higher amount of chlorophyll a in *Salicornia europaea,* being arround 60 % of total photosynthetic pigments and has increasing during vegetation stage, until June to August. Chlorophyll b and total carotenoids registered small values and also small variation in analysed period (Fig. 4).

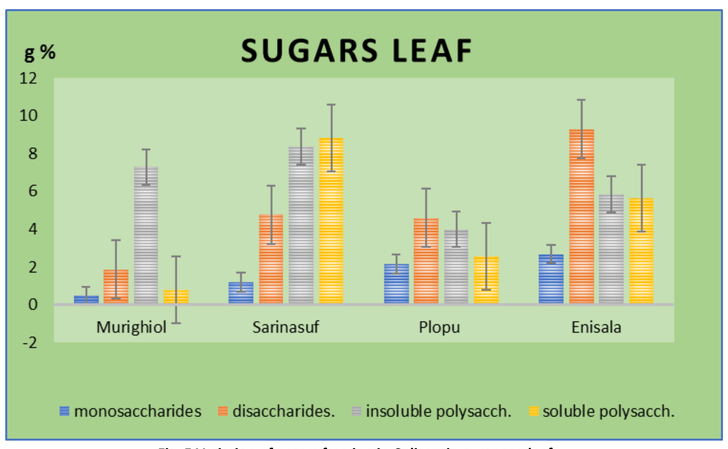

Fig. 5 Variation of sugars fraction in *Salicornia europaea* leaf

Among the carbohydrate fractions, the disaccharides, important in maintaining cell osmotic pressure, are increased in individuals from Plopu and Enisala plots, where *Salicornietum prostratae* phytocoenosis layed on the large surfaces. Monosaccharides have registered lower values between 0.4 % (in Murighiol plot) until 2 % (Enisala). In that individuals polysaccharides were also higher. Disaccharides compounds with an important role in maintaining osmotic pressure registered higher values especially on August followed by the polysaccharides. Sugars leaf fractions were reduced in Plopu where *Salicornia europaea* is formed a monocoenosis on a large surface.

Highest total values of carbohydrates fraction were recorded in plots with higher saline concentration, such as Sarinasuf and Enisala, being around of 23 % (Fig. 5).

Fig.6 Enisala - *Salicornia europaea*

Fig. 7 Plopu - Monocoenosis with *Salicornia europaea*

Fig. 8 Sarinasuf – *Salicornia europaea*

Fig.9 Murighiol – *Salicornia europaea*

CONCLUSIONS

- **From a morphological point of view, the tracking of specific characters (height of the plant, height from root to first branching, number of internodes, lengths of internodes, stem branching) highlights the fact that the living environment has a significant influence on the growth and development of plants.**
- **Biochemical analyzes showed that chlorophyll a and disaccharide content of *Salicornia europaea* in Enisalaand Plopu plots with higher soil salt content (GleycSolonceak) were correlated with the morphometric parameters observed in these plants.**
- **All samples taken in the study highlight the presence of supernumerary cambium, active over the vegetation period, cambium specific to halophyta chenopodiaceae.**
- **At the level of the fleshy stem segment (large internodes) it is observed that mesophyll from the sample collected from Enisala has thinner palisade tissue than the other analyzed samples, and the spongy tissue much thicker. The thickness of the spongy tissue is in close connection with the plant's succulence and can be an adaptation to the living environment.**
- **At the SEM, we see visible elongated epidermal cells and isodiametric cells, all with straight side walls and covered with a finely striped cuticle. From place to place, stomata are observed.**

Acknowledgements

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SAMBUCUS NIGRA L.: BIOACTIVE COMPOUNDS WITH MEDICINAL AND NUTRITIONAL POTENTIAL

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INTRODUCTION

According to the information resource for Euro-Mediterranean plant diversity (http://www.emplantbase.org/home.html) *Sambucus nigra* (european elderberry) belongs to family Viburnaceae. In Romania the species is frequent in forests (margins and gaps), abandoned fields, from the plain area up to the inferior mountain area (Pârvu, 2006; Sârbu et al., 2013).

Currently, in many European countries, including Romania, there are preoccupations regarding the cultivation, research and valuing the fruits and the flowers from the elderberry, for nutritional and therapeutic purposes.

Studies have shown that fruits and flowers of *Sambucus nigra* are a potential source of bioactive compounds (in particular phenolic compounds) with medicinal and nutritional potential.

Epidemiological studies also show that high consumption of foods rich in polyphenol compounds reduces the risk of developing degenerative diseases (cardiovascular disease, inflammatory disease, cancer, diabetes) (Gardini et al. 2000; Yoon et al. 2005). Recent studies have highlighted that *Sambucus nigra* fruits and / or flowers extracts have immunomodulatory, antiviral, antimicrobial, antioxidant effects (http://www.ema.europa.eu/; Barros et al. 2011; Duymuș et al. 2014, Cioch et al. 2017), cardioprotective effect (Ciocoiu et al. 2012).

The purpose of this paper is to provide a synthesis of knowledge about the bioactive compounds identified in different organs, their biological activities, the environmental significance of some compounds and the ways of using this species.

Biological characteristics

Sambucus nigra in the urban area

- the flowers are grouped into coriformiform cymes inflorescences with a diameter of 12-25 cm
- it blooms from mid-May to July.

➢ the fruits are spheric berries, violet-black, 6-8 mm, ripening in August – September.

Seed of elderberry from frozen fruits (Novex binocular magnifier, 40x)

➢the leaves are pinnate compound with 3-7 leaflets.

- shrub sometimes reaching to forms tree (7-8-10m)
- it easily multiplied by seeds, cuttings, marcots and grafts
- a plant produces 8 -10 (15) kg of fruits

Ecological characteristics: is a mesophilous-mesohydrophilous, heliophylous- heliosciophylous and eutrophic species (Pârvu, 2006; Sârbu et al., 2013); can also grow on soils with poor fertility.

Geografical distribution

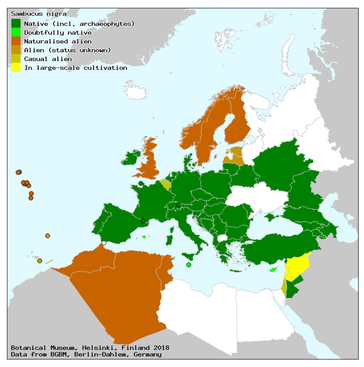

Fig. 1 Geografical distribution (http://euromed.luomus.fi/euromed_map.php?taxon=349466&size=medium)

Sambucus nigra grows spontaneous in Asia, Europe, Nord Africa and SUA (Veberic et al., 2009) and also is begin cultivating in some countries from Europe (Austria, Denmark, Germany, Hungary, Romania, etc).

Chemical composition

Chemical composition (leaves)
- ➢soluble glucids
- ➢lipids
- ➢phenolic acids (behenic, caffeic, ferulic, shikimic acids)
- ➢tannins
- ➢sterols: sitosterol, campesterol
- ➢vitamins: ascorbic acid, riboflavin
- ➢cyanogenic glycoside
- ➢volatile oils
- ➢anthocianins
- ➢triterpens: cycloartenol, ursolic acid, oleanolic acid

(Burzo et al. 2005, Burzo 2015)

Chemical composition (flowers)
- ➢**glucids**: glucose, fructose, sucrose, mucilage, pectin; total sugars (83,6mg/g DW: Baros et al. 2011)
- ➢tannins,
- ➢fatty acids; sterols (sitosterol, campesterol, stigmasterol)
- ➢**tocopherol**: total (324 μg/ gDW), α-topherol, β-tocopherol, γ-tocopherol (Barros et al. 2011)
- ➢**phenolic compounds: flavonol glycosides** (Quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, Isorhamnetin-3-rutinoside, in larger quantities etc), **phenolic acids** (caffeic, ferulic, chlorogenic, neochlorogenic)
- ➢**vitamins**: B2, B6, ascorbic acid, folic acid, biotin, panthotenic acid, etc
- ➢**β-carotene, lycopene**
- ➢**Triterpens:** α- amirină, β-amirină, ursolic acid, oleanolic acid, cicloartenol.
- ➢**essential oils:** 0,03 - 0,14 % (Burzo, 2015);
- ➢cyanogenic glycosides: lower amount

(Christensen et al. 2008, Barros et al. 2011 Ferreira et al. 2012, Burzo 2015)

Biological activity of bioactive compounds, health and economic benefits

polyphenols

➢biological actions: antioxidant, anti-inflammatory, antibiotic (Yoon et al. 2005; Christensen et al. 2008; Ho et al. 2017)

polyphenols, polyglucides, peptides, proteins, lipid derivatives, triterpenoids

➢can have a potential modulation effect on immunological and inflammatory processes (Tzianabos 2000; Ho et al. 2017)

pentacyclic triterpenes (ursolic acid, oleanoic acid, α-amirin, β-amirin)

➢ have potential anti-inflammatory, antibacterial, antiviral, anti-tumoral and wound healing properties (Jäger et al. 2009)

➢fruits have nutritional value due to the content of sugars, fibers, minerals, vitamins, organic acid and high content of polyphenols

➢citric and malic acids (present in large quantities in fruits) are important parameters of fruit processing (Kan, 2019).

➢seeds / seed meal is a potential source of beneficial compounds (polyunsaturated fatty acids, phenolic compounds with significant antioxidant capacity) and valuable raw material for the food industry (Fazio et al. 2013; Dulf et al. 2013)

Tabele 1 The content of mineral elements in fruits

Sambucus nigra L. wild		
S. nigra (Poland)	N (0.69 -1.49%DM), P (0.32-0.87), K (2.38-3.8), Mg (0.22-0.28), Ca (0.25-0.58), Na (0.010-0.029)	(Ważbinńska et al. (2007)
S. nigra L. cultivars		
S. nigra L. (Czech Republic	K (2953 – 5494mg /kg FW); P (735-1337); Ca (574-1528); Mg (396-739); Na (13-146); Fe (12.4-84.7); Zn (1.9-11.3); Mn (3.6- 9.5); Cu (1.7-2.9)	Diviš et al. (2015)
S. nigra L. (Serbia)	K (391.33mg/100g), P (54), Ca (28.06), Mg (25.99), Na (217), Fe (1.86), Zn (0.36), Mn (0.27), Cu (0.14)	Vulić et al. (2008)

Chemical composition (fruits)
- ➢ **glucids** (4,7- 5,8%): glucose, fructose, sucrose, pectin, acid pectic, protopectin, Ca - pectate, cellulose, dietary fibre
- ➢ **proteins** (2.48 g%): aminoacids
- ➢ **organic acids:** citric, malic, tartric, fumaric, shikimic acids
- ➢**phenolic compounds:** phenolic acids, flavonoids, anthocianins (the main ones are: cyanidin -3 glucoside, cyanidin- 3– sambubioside)
- ➢ **mineral elements (table 1)**:
- ➢ **essential oils**
- ➢**vitamins:** B2, B6, ascorbic acid, folic acid, biotin, panthotenic acid, etc
- ➢**triperpenoids**: ursolic acid
- ➢**carotenoid pigments**: beta carotene, flavoxanthin, lutein, neoxanthin
- ➢**cyanogenic glycosides**: lowest amount; are potentially toxic and are degraded by heat treatment

(Veberic et al., 2009; Vulić et al., 2008; Anton et al. 2013; Burzo, 2015, Kan, 2019; www.ema.europa.eu/en)

➢some polyphenolic compounds give color to flowers and fruits (anthocyanic and flavonoid pigments), helping to attract animals for pollination and seed propagation and protect plants against viral, bacterial or fungal infections.

Chemical composition (seeds)
- ➢ **oils** content (15,9 %: Fazio et al. 2013); α and δ tocopherols;
- ➢ **oil content**: 22.4 g/100g seeds (Dulf et al. 2013)
- ➢**fatty acids**: linoleic, oleic, linolenic, stearic, arachidic, palmioleic, behenic, myristic, palmitoleic, erucic, lauric, tricosanoic, etc.
- ➢ **phenolic compounds:** cyanidin-3-*O*-sambubioside-5-glucoside; pelargonidin 3-rutinoside; quercetin 3 rutinoside; quercetin 3 glucoside
- ➢ **total phenolic content:** 54.2mg GAE/g extract
- ➢ elagic acid

(Fazio et al. 2013; Dulf et a. 2013)

Uses

➢preparation of soft drinks, syrups, juices, wines

➢flavoring beverages or other products

➢obtaining of phytopharmaceuticals, cosmetics

➢due to phenolic compounds, fruits are important in food industry as beverage dyes / other products and as antioxidants

➢obtaining of phytopharmaceuticals, cosmetics

➢production of wine, syrups, juices, soft drinks, jam, jelly, cakes, candy, sweets, and other products

Food and medicicinal products containing extracts of flowers and fruits of elderberry

Medicinal uses:

➢**Flowers**: sudorific, diuretic, emollient properties; in the treatment of respiratory diseases.
➢**Fruits**: laxative, sudorific, depurative, expectorant properties.
(Pârvu, 2006; Muntean et al. 2007)

CONCLUSIONS

➢Selected data from the literature show that leaves, flowers, fruits and seeds contain a wide range of chemical compounds with multiple biological actions.

➢Flowers and fruits are a natural, easily accessible, important source of substances useful to the human body and a valuable raw material for the food and phytopharmaceutical industry.

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The photos were performed by Stratu A., Boz I. and Ene R.

BIOPROSPECTING MEDICINAL FLORA FROM PIETRICICA PEAK, COUNTY BACAU (ROMANIA)

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INTRODUCTION

❑ This paper analyzes the medicinal flora of the Pietricica Peak, Bacau County (Romania).
❑ This area is located in the Subcarpathian Region of Moldova (Eastern Carpathian District) and has an area of over 1000 Km².

❑ From a geological and morphological point of view, the Pietricica Peak is composed predominantly of miocene rocks. In the central area, where heights are higher, burdigal conglomerates and harder sandstones have been preserved.

❑ The relief is fragmented, with a general orientation of interfluves from north to south.

❑ The area is crossed by the Tazlau River, which has the largest intake of surface water in the area. Groundwater is at a depth of 0.5 m - 1.5 m on the valley bottom, 3 – 5 m in the meadows, over 10 m on the terraces and 40 m on the slopes.

❑ In the habitats of the Pietricica Peak there is an high floristic diversity, accounting for more than 900 plant species, representing 24,3 % of Romanian vascular Flora. This floral diversity is explained by: the variety of climatic, geographic and ecological factors, the existence of specific local microclimates, the multiple influences (Central European, Mediterranean and Pontic), as well as the traditional practices of vegetal resources valorisation (agriculture and shepherding).

MATERIAL AND METHOD

❑ The inventory of the species was carried out between 2015 and 2016 on the itinerary and their determination was made using the determinant elaborated by Sârbu I. et al (2013).

❑ Based on the specialty bibliography (Alexan et al., 1988, 1989; Ciulei et al., 1993; Tiţă, 2003; Munteanu et al, 2007; Bojor 2003; Burzo I., 2015) species with medicinal potential, have been selected.

❑ For each species, the organ / parts of the plant used for medicinal purposes were mentioned; the main bioactive compounds and indications and pharmacologic activities.

RESULTS AND DISCUSSIONS

Check list of medicinal plants from the Pietricica Peak, Bacau County

Species	Botanical families	The vegetal organ used / the chemical composition	Pharmalogical effects/uses	References
1. Equisetum arvense	Equisetaceae:	Plant – alkaloids, carbohydrate, proteins and amino acids, phytosterols, saponins, ascorbic acid, silicic acid, phenol, tannin flavonoids	Diuretic, hemostatic.	Alexan et. al. 1989 Tiţa 2003
2. Papaver rhoeas	Papaveraceae:	Petalele - alkaloids, anthocyanins, flavonols, mucilage, potassium, sodium, calcium.	Sedative, antioxidante, antimicrobial	Alexan et. al., 1989 Muntean et al. 2007 Kostic et al. 2010
3. Humulus lupulus	Cannabaceae:	Female flowers – Resins, polyphenols, tannin Essential oil	Anaphrodisiac/ anorexia, anxiety, insomnia,	Craciun et. al., 1976 -1977 Muntean et al. 2007
4. Betula pendula	Betulaceae	Leaves – resins, Polyhenols, triterpenic compounds, vitamin C	Diuretic, bacteriostatic	Alexan M. et. al. 1989
5. Polygonum aviculare	Polygonaceae:	Plant/aerial part – hydroxycinnamic and phenolcarboxylic acids, flavonoids, essential oils, carotene, vitamin C, vitamin K_1, sugars, saponins, coumarins, slime, and anthraquinone glycosides	antitussive, astringent, antimicrobial, diuretic/ mineralizing, adjuvant in pulmonary tuberculosis	Ciulei et al 1993 Munteanu et al. 2007; Hediat, 2010 Burzo 2015
6. Hypericum perforatum	Hypericaceae	Plant - tannin, proanthocyanidins, flavonoids, fenolic acid, essential oil, vitamin C, vitamin A, hypericin, hyperoside, rutin, quercetin.	Antidepressant, anti-septic, anti-inflammatory, antioxidant, analgesic/ Colitis, cholecistopathy, eczema, burns	Asgarpanah, 2012 Burzo, 2015
7. Viola odorata	Violaceae	Flowers - essential oil, resins, sugars, viola-quercetin, violamine, salicylic acid, mucilages. leaves - essential oil Root and rhizomes - saponosides, odoranthin, essential oil, etc.	Expectorant, anti-inflammatory, emollient, diuretic	Muntean et al. 2007, Burzo, 2015
8.Viola tricolor	Violaceae:	Plant to flowering - flavonoids, anthocyanoside, essential oil, carotenoids, vitamin C, tannin, terpenoids, .saponins, phenolic acids, flavonoids	Antialergic, diuretic, depurativ, antimicrobial/ Allergy, dermatoses.	Craciun Fl. et. colab., 1976 -1977 Witkwoska- Banaszczak et al. 2005 Muntean et al. 2007

Species	Botanical families	The vegetal organ used / the chemical composition	Pharmalogical effects/uses	References
9.Capsella bursa-pastoris	Brasicaceae	Plant - alkaloids, tyramine, choline, acetylcholine, histamine, tannin, diosmin, saponides, potassium oxide, flavonoids, vitamin C	Haemostatic, hypotensive antimicrobial, antioxidante, antinflammatory, anti cancer, sedative	Alexan et. al. 1989 Muntean et al. 2007 Al-Snafi, 2015
10. Berberis vulgaris	Berberidaceae	Root - alkaloids (berberine, berberubine) Bark - tannins, chelidonic acid, resins, s.a Fruits - glucose, fructose, malic acid, pectins, vitamin C	Cardiovascular, hepatic disorders	Alexan et. al., 1989 Muntean et al. 2007
11. Populus nigra	Salicaceae	Buds - essential oil, flavonoids, flavonols, flavonones, salicin, salicortin, benzoic derivatives, resins, alkanes.	Antiseptic, anti-inflammatory, analgesic	Bojor , 2003
12. Salix alba	Salicaeae	Bark of young branches - phenolic glycosides, tannins, flavonoids.	Analgesic, antipyretic, anti-inflammatory, sedative/ rheumatism, insomnia, uterine pain.	Bojor, 2003 Muntean et al. 2007
13.Primula veris	Primulaceae	Rhizomes and roots - triterpene saponosides, sugars, tannins, flavonoids, starch. Flowers - flavonosides, saponosides Leaves - vitamin C, beta - carotene.	Bronchitis, treatment of contusions	Parvu, 2006
14. Althaea officinalis	Malvaceae	Root - polysaccharide mucilages, flavonoid glycosides, camphorol, quercetin, phenolic acids, sugars, amines, coumarins, sterols	Emollient, expectorant , anti-inflammatory/ Mucosal inflammation of the respiratory and digestive tract	Tiţă 2003 Parvu C., 2006 Muntean et al. 2007
15.Malva sylvestris	Malvaceae	Flowers - anthocyanosides, mucilages Leaves - mucilages, provitamin A, vitamin. B1, vitamin B2, vitamin C.	Antioxidante, anti-inflammatory,anticancer, emollient/ Tonsillitis, pharyngitis, inflammation of the mouth, eye, vaginal, rectal	Craciun et. al., 1976 -1977 Tiţă 2003 Cleverson et. al. 2011
16 Tilia tomentosa	Tiliaceae	Flowers - mucilages, condensed tannins, flavonoids, phenolic acids, essential oil.	Sedative, expectorant / insomnia, bronchitis.	Alexan et. al., 1989 Muntean et al. 2007

Species	Botanical families	The vegetal organ used / the chemical composition	Pharmalogical effects/uses	References
17. Crataegus monogyna	Rosaceae	Flowers and leaves - oligomeric procyanidins, flavonoids, amines, catechins, phenols, carboxylic acids, purines, sterols, triterpene acids, pectins, tannins. Fruit - tannins, vitamin B1, vitamin C, anthocyanins, flavonoids, acids, glucose, fructose, pectins	Sedative, antispasmodic, coronary vasodilator / Paroxysmal tachycardia, hypertension, arteriosclerosis	Alexan et.al. 1989 Muntean et al. 2007
18.Potentilla anserina	Rosaceae	Plant - tannin, bitter substances, mucilages, essential oil, flavonoids	Enterocolitis, diarrhea, metrorrhagia, gingivitis	Bojor, 2003
19 .Agrimonia eupatoria	Rosaceae	Plant - tannin, flavonosides, vitamin C, vitamin K ursolic acid, pentacyclic triterpenes	Chronic diseases of the stomach, rheumatic diseases, diarrhea, virosis.	Popescu 1984; Grigorescu et al. 1986; Muntean et. al., 2007
20. Geum urbanum	Rosaceae	Rhizome and root - tannins, essential oil, geese, geese, bitter substances, starch, sucrose, raffinose, mineral salts.	Diarrhea, local haemostatic, antiseptic, calming	Bojor, 2003
21. Prunus spinosa	Rosaceae	Flowers - flavonoids, organic acids, quercetin, kamferol, potassium and magnesium salts, glycosides. Fruits - sugars, organic acids, anthocyanins, polyphenols, tannins, vitamin C, calcium and magnesium salts	Flowers -hypertension, fruit - diarrhea, kidney disease and biliary dyskinesia.	Alexan et. al. 1989 Parvu 2006
22. Fragaria vesca	Rosaceae	Leaves - tannin, fragarol, quercetin, salicylic acid, essential oil, sugars, vitamin C Fruits - salicylic acid, vitamin C, vitamin A, vitamin B1, vitamin B2.	Astringent, depurative hypotensive/ Diarrhea, diuretic, diabetic diet, liver disease, eczema,	Bojor, 2003 Muntean et al. 2007
23.Sorbus aucuparia	Rosaceae	Fruits - sugars, tannin, vit. C, provitamin A, vitamins B1, B2, B3, PP, E, malic acid	Fortifies SN, reduces cholesterol, increases visual acuity, slows down aging.	Bojor, 2003
24. Glycyrrhiza echinata	Fabaceae	Roots - triterpene saponosides, flavonoids, amines, asparagine, betaine, choline, amino acids, glucose, sucrose, beta-sitosterol, polysaccharides, coumarins,.	Antioxidant, emollient and expectorant in cough and bronchial inflammation, gastric and duodenal ulcer, dyspepsia, rheumatism, arthritis, tuberculosis, adenocorticoid insufficiency.	Alexan et. al., 1989 Çakmak et al. 2012

Species	Botanical families	The vegetal organ used / the chemical composition	Pharmalogical effects/uses	References
25. Robinia pseudacacia	Fabaceae	Flowers - robinina, acacia, essential oil. Bark - robin and pheasant, silingin, tannins.	Cough, gastritis	Bojor, 2003
26. Melilotus officinalis	Fabaceae	Plant - coumarin derivatives, pentacyclic triterpenic saponosides, flavonosides, allantoin, alantonic acid, choline, polyholosides, phenolic acids.	Diuretic, astringent, anti-inflammatory, antispasmodic, anticoagulant, hepatorotector.	Bojor, 2003 Muntean et al. 2007
27.Eryngium planum	Apiaceae	Plant - saponoside, eryngium-sapogenines D, F, G, H, sucrose, ferulol.	Coughing (traditional Romanian remedy), diuretic	Alexan et. al. 1989 Muntean et al. 2007
28. Carum carvi	Apiaceae	Fruits - carvone, limonene, pinen, felandrene, lipids, minerals, starch, carbohydrates, resins, tannins, mineral salts.	Carminative, antihistaminic, antimicrobial, antiseptic, astringent, diuretic, galactogog, vermifug	Pârvu, 2006 Muntean et al. 2007
29.Fraxinus excelsior	Oleaceae	Leaves - mannitol, inositol, quercitrine, malic acid, ursolic acid, gums, essential oil. Bark - oxicumarin derivatives.	Diuretic, diaphoretic, laxative, articular rheumatism	Bojor, 2003
30.Hippophae rhamnoides	Elaeagnaceae	Fruits - sugars, organic acids, flavonoids, pectins, tannins, fatty oil, microelements, liposoluble vitamins, water-soluble vitamins, essential fatty acids, lycopene, tocopherols, phytosterols. Seeds - unsaturated fatty acids Leaves and sitosterol bark, tocopherol	Polyvitamins, antioxidants/ dermatoses, gastric and duodenal ulcers, hepatitis, anemias.	Bojor, 2003
31.Sambucus nigra	Caprifoliaceae	Flowers - rutosid, essential oil, sugars, mucilages, cyanosene heterosides, tannin, saponosides, vitamin. C, vitamins. from complex B, chlorogenic acid, anthocyanins, amino acids, Fruits can substitute blueberries	Antioxidante, diuretic, emollient, diuretic, depurative/ Respiratory viruses, flu.	Craciun et. al., 1976 -1977 Pârvu 2006 Anton et al. 2013
32. Valeriana officinalis	Valerianaceae	Rhizomes and roots - essential oil (in particular with boronil acetate, valenyl isovalerianate, valeric acid, s.a.), tannins, lignans, flavonoids, triterpenes, phenols, ketones, oxilactones, glucose, lipase	Insomnia, nervousness, motor and sensory hyperexcitability, cardiac neurosis, mild antispastic	Bojor, 2003

Species	Botanical families	The vegetal organ used / the chemical composition	Pharmalogical effects/uses	References
33. Galium odoratum	Rubiaceae	Plant - cumarine, hydrolysed derivatives, furanocumarins, monotropitozide, bitter substances, tannin, fatty oil, phytonutrients, mineral salts.	Anticoagulant, diuretic, sedative/ soothing in insomnia, wine flavoring	Bojor, 2003
34. Pulmonaria officinalis	Boraginaceae	The plant /leaves- saponoside, mucilagia, silicic acid, tanoids, polyphenolic derivatives, flobafen, polyols, stearic acid, palmitic acid, mineral salts, resins, carotenoids, vit. C.	Expectorant, astringent Easy diuretic, vitamin C intake, provitamin A	Crăciun et. al., 1994 Muntean et al. 2007
35. -37 Plantgo lanceolata, Plantgo media, Plantgo major	Plantaginaceae	Leaves - polysaccharide mucilages, tannins, iridoid glycosides, silicic acid, carboxyl phenolic acids, flavonoids, mineral salts	Antiinflammatory, analgesic, Biting states of the respiratory tract, inflammation of the pharyngeal mucosa, fresh leaves are haemostatic, healing, antiinfectious	Crăciun et al., 1994 Parvu 2006 Núñez Guillén et al. 2016
38.Verbascum phlomoides	Scrophulariaceae	Flowers - polysaccharide mucilages, flavonoids, derivatives of caffeic, felic, protocate and verbascoside acids, iridoid monoterpins, specific saponosides, sterols, etc.	Inflammation of the airways (cough, sore throat, dry cough, respiratory congestion)	Bojor, 2003
39.Linaria vulgaris	Scrophulariaceae	Plant - flavonoids, flavonol-glycosides, alkaloids, organic acids, sugars, pectins, mineral salts	Diuretic, sudorific, laxative	Pârvu, 2006
40.Marrubium vulgare	Lamiaceae	Plant/aerial part - diterpenic bitter substances, marubic acid, tannins, flavonosides, essential oil, derivatives of caffeic, chlorogenic, rosmarinic acids, triterpene compounds, pectins, resins, waxes, choline, vit. C, mineral salts	Emollient, expectorant, febrifuge, choleretic	Pârvu, 2006 Muntean et al. 2007
41. Nepeta cataria	Lamiaceae	Plant - essential oil, timol, pulegone, mineral salts	Stomach cramps, insomnia, antispasmodic, sedative, tonic, bitter	Bojor, 2003
42. Origanum vulgare	Lamiaceae	The plant/aerial part - essential oil, flavonoids, anthocyanins, triterpene, ursolic acid, caffeic acid.	Respiratory and stomach infections, bronchitis, tracheitis, colitis, diuretic, sedative, anaesthetized gastritis	Bojor, 2003 Muntean et al. 2007

Species	Botanical families	The vegetal organ used / the chemical composition	Pharmalogical effects/uses	References
43.Inula helenium	Asteraceae	Rhizome - inulin, essential oil, sesquiterpenic derivatives, antibiotic substances, frideline, stigmasterin. Leaves - alantopicrin, vit. C, mineral salts. Flowers - helenin.	Diuretic, anti-inflammatory in hepatic congestion, cholecystitis, biliary and renal arthritis, arthritis, oliguria, cough reliever, collagogue, anthelmintic	Crăciun et. al., 1994; Burzo, 2015
44. Matricaria chamomilla .	Asteraceae	Flowers - flavonoids, essential oil, lactone sesquiterpenes, mucilages, amino acids, fatty acids, phenolic acids, choline, coumarins	Anti-inflammatory, antispasmodic, antiseptic, anesthetic	Burzo, 2015
45.Tussilago farfara	Asteraceae	Leaves - mucilages, tannins, bitter substances, carotenoids, inulin, gallic acid, stearic acid, palmitic acid, phytosterols, mineral salts. Flowers - mucilages, tannins, arnidiol, faradiol, rutoside, hyperin, xanthophil, pyrosilidinic alkaloids, phytosterols, volatile oil	Chronic pulmonary disorders, chronic pulmonary emphysema, silicosis, asthma	Burzo, 2015
46. Artemisia absinthium	Asteraceae	Plant - essential oil, absinthe, absinthe acid, anabsintina, astabsintina, astabsina, artametina, succinic acid, tannins, resins, starch, malates and potassium nitrates	Anorexia, atonia, chronic inflammation of the digestive system, dyspepsia accompanied by constipation	Bojor, 2003 Muntean et al. 2007
47. Achillea millefolium	Asteraceae	Plant - tannins, essential oil, lactone sesquiterpene, amino acids, fatty acids, phenolic acids, vitamins, alkaloids, alkanes, polyacetals, saponins, sterols, sugars, coumarins.	Inflammation, antiseptic, anorexia, bitter tonic, immunostimulant, hemorrhoidal diseases, menorrhagia	Bojor, 2003
48.Taraxacum officinale	Asteraceae	The plant – substanţe amare, triterpene, carotenoide, xantofile, flavonoizi, inulina, săruri minerale.	Hepatobiliary disorders	Crăciun et. al, 1994 Burzo, 2015
49.Cichorium intybus	Asteraceae	Plant - chicory, inulin, arginine, choline, cicloric acid, levulose, mineral substances, bitter triterpenic substances, fructose, choline, tannins, essential oil, resins.	Antioxidante, anti-inflammatory, gastroprotective/ Digestion (facilitates digestion), collagogue, hypoglycemia and antithyroid	Bojor, 2003; Minaiyan et al. 2012 Burzo, 2015;

✓As a result, out of the 900 identified vascular species - 53 species are medicinal, classified in 28 families and 49 genres.

✓Families with the most medicinal species are Rosaceae with 7 species and Asteraceae with 9 species.

✓For medical purposes, only the underground part is used in some species, all or some aerial organisms, or all of the plant for other species.

✓Medicinal species contain bioactive compounds (organic acids, phenolic compounds, tocopherols, carotenoids, volatile oils, glycosides, vitamins, etc.) with various therapeutic actions (antiseptic, astringent, antioxidant, anti-inflammatory, antispasmodic, sedative, bitter tonic etc.).

✓Of the medicinal species, we mention, among others, *Sorbus aucuparia* (Rosaceae) and *Hippophae rhamnoides* (Elaeagnaceae) whose fruits have a strong and healthy influence, with a delay in the aging process.

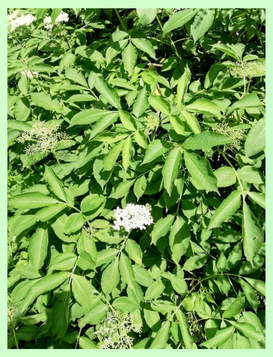
Rosa canina

Robinia pseudacacia

Geum urbanum

Crataegus monogyna

Galium odoratum



Sambucus nigra

CONCLUSIONS

➢The flora of Pietricica Peak and the widespread use of medicinal plants for the treatment of various diseases, constitutes an important local health care resource with significant potential for research and development of phytomedicines.

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Comparison between traditional phytotherapy and scientifically-proven documented phytotherapy: *Oreganum vulgare and O.majurana*

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Abstract

Oreganum species are some of the most recognized plants of the Mediterranean flora with a wide spectrum of application since they can be used as spices and they can be cultivated. Among these species, *Oreganum vulgare* and *Oreganum majurana* attracted great interest about their pharmacological properties. The aim of this study was to highlight the therapeutic potential of these two species via the most recent scientific studies and compare their traditional use as medicinal plants with their scientifically-proven documented phytotherapy. It is obvious that the traditional use of the species is not totally identical with the scientifically proven phytotherapy. There are similarities like its use against diarrhea, but there are also differences concerning their potential use as natural antioxidants or anticancer agents, because of their isolated chemical compounds (1, 2, 3). It is important to emphasize the need to carry out further research studies and phytochemical analysis, so as to isolate more compounds that will justify the pharmacological action that is attributed to extracts or the essential oil of the plants or scientifically prove, or not, the other traditional uses of the plants. All this information may be useful for the promotion of the further use of these plants in food and medicinal products, justifying also their traditional use as beneficial to health.

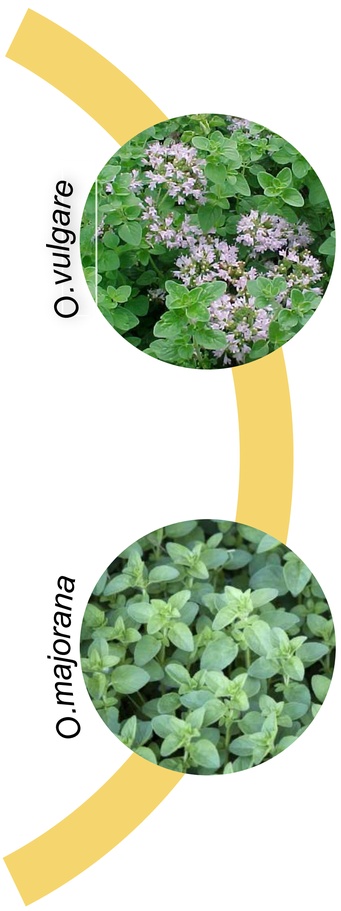
Introduction

Considering that the drug market rates are constantly increasing, scientists are still focusing on the isolation of active compounds from medicinal plants. One such case that attracted their interest is the genus of *Oreganum*. *Oreganum* species have a characteristic smell and flavor due to the composition of its essential oil which is comprised mainly by carvacrol, p-cymene, γ-terpinene and linalool, in various proportions among species. (4,5) Among the species which have attracted such great research interest are the species of *Origanum majorana l., Origanum vulgare l. subsp hirtum* and *Origanum vulgare l. subsp vulgare.* These species have been utilized in the form of decoctions, infusion and for the production of liqueurs, drinks and edible oils. (6) These species are mainly found in areas of Eastern Mediterranean.

Materials and Methods

"*Literature review:* Database: Science Direct, Scopus, Pubmed, Google Scholar / Keywords: *Origanum* species*, Origanum vulgare, Origanum majorana,* characteristics of *Origanum,* ethnobotany, phytochemical properties, folk healing, phytotherapy of *Origanum*, phytopharmacology of *Origanum*, cancer and *Origanum*, antioxidant properties, herbal medicines, / Criteria for selection of articles: Date of articles' s publication, articles focusing on alternative therapies, articles referring to all healing abilities of *origanum"*

Results

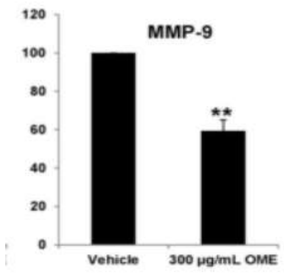


Traditionally, the plant is used in herbal remedies against diarrhea, as an spasmodic agent, as an expectorant, useful in the treatment of menstrual problems, pertussis and cough (3,7). *O.vulgare l. subsp vulgare* is used by local populations of the Greek city of Xanthi as a healing agent against cold, as cardiotonic, and for diabetic patients. *O.majorana* is used for the treatment of stomachache, in vertigo and as an antiemetic, while *O. vulgare l.* subsp *hirtum* is used as an effective agent for indigestion and constipation, for rheumatic diseases and for the sore throat (6). In Turkey, the traditional use of *O.vulgare* is recommended for healing wounds, hemorrhoids, stomachaches, eye diseases and sinusitis (8). In Iran, the plant is used as an antiseptic agent, as spasmolytic, and as a pain reliever effective for toothache (9,10). Moreover, the species *O.vulgare* and *O.majorana* are traditionally used in Armenia, in herbal remedies against gastritis and for skin wounds.

Pharmacological studies referred that *O.vulgare* is effective against diarrhea and it has anticonvulsant properties. The essential oil of *Origanum vulgare* seems to be beneficial for gastrointestinal problems (7,11). Moreover, scientific studies revealed that the ethanolic extract of *O. vulgare*-can be used as a protective agent in the liver toxicity, induced by cyclophosphamide. According to an experiment carried out in mice, their hepatic markers (ALT) were reduced when administered with the ethanolic extract of this species (2). The values of the antioxidant capacity of the essential oil of *Oreganum vulgare* via the scavenging of the stable DPPH radical was almost equal to those of BHT –which was used as a reference standard- thus proving its antioxidant properties. This antioxidant effect was attributed to the high levels of thymol in the extracts that were studied. Several other studies, also confirmed the antioxidant capacity of the essential oil of *Oreganum* vulgare, thus justifying its potential to act as a natural antioxidant that can prevent oxidation and be beneficial to health, while at the same time appears to be safer than synthetic antioxidants (12,13).

Research studies also confirmed the antifungal properties of *Origanum vulgare* and *Origanum majorana* against *Penicillium citrinum* and *Vibrio cholerea,* as well as its antimicrobial capacity against bacteria, such as the antibiotic resistant *Escherichia coli,* due to the presence of carvacrol and thymol (5, 14). It has been proved that the essential oil of *O.vulgare* possess anti-inflammatory and antidiabetic properties, because it contains thymol and carvacrol, which proved to reduce the levels of inflammatory mediators such as NO and increase glucose consumption. Moreover, phytochemical analyses have shown that isolated compounds of the plant can reduce total cholesterol and triglyceride levels in humans, thus being beneficial against atherosclerosis and cardiovascular diseases. (15)

Finally, many pharmacological studies focus on the essential oil of the above *Oreganum* species, concerning their potential to act as anticancer agents. A study has demonstrated the positive correlation between carvacrol and its ability to promote apoptosis on human colorectal cancer cells (LoVo) (16). Additionally, another study highlighted the effect of *O.majorana* ethanol exctract (OME) against breast cancer cells MDA-MB-231, and also via *in vivo* studies which were performed in chicken embryos, where the OME reduced the size of the tumor. OME seems to contribute to the inhibition of MMP-9 proteins, which are associated with the proliferation of cancer cells. Furthermore, OME suppresses the production of NO and of the growth factor VEGF in cancer cells, thus inhibiting angiogenesis (1). There are also scientific reports concerning the essential oil of *O.vulgare* to be effective against other types of cancer such as breast cancer and colorectal adenocarcinoma (15).



Conclusion

In conclusion, it appears that the traditional use of *O. vulgare* and *O. majorana* is not totally identical with the scientifically proven phytotherapy. There are similarities like its use against diarrhea, but there are also differences concerning their potential use as anticancer agents. This study highlighted the great pharmacological potential of these medicinal plants, however it is necessary to carry out further research studies in order to better understand the mechanisms of their pharmacological action and to investigate their pharmaceutical properties that are still not scientifically proven. There is also a necessity for further *in vivo* studies which would assist our knowledge concerning safety and toxicity matters, arising from their use.

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