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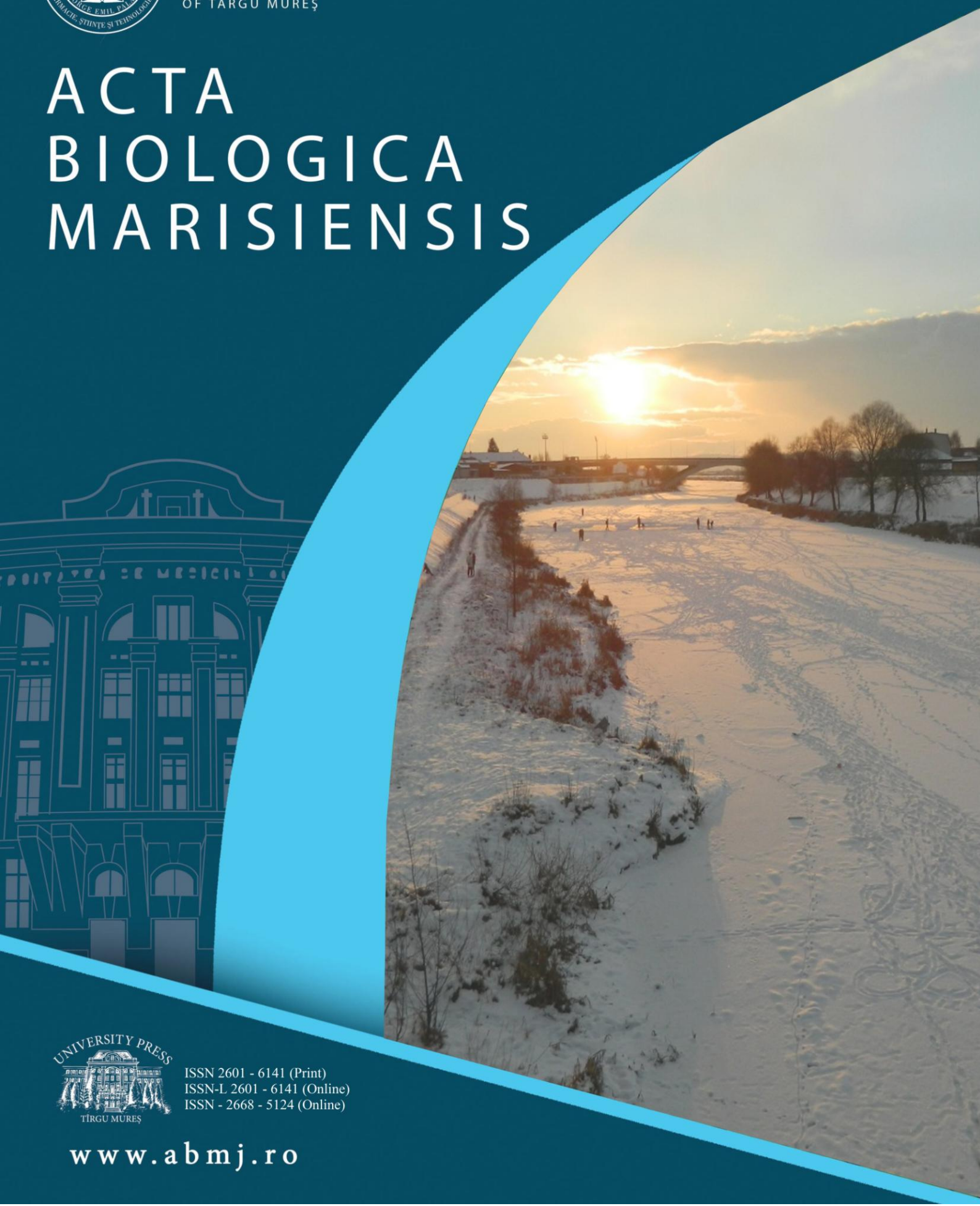

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COMPARISON OF DIFFERENT ULTRAFILTRATION DEVICES FOR THE STUDY OF PLASMA PROTEIN BINDING OF CARVEDILOL

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Abstract: The aim of the present study was to assess the suitability of different Amicon Ultra and Centrifree ultrafiltration devices for the study of the plasma protein binding process in the case of carvedilol, a highly protein-bound and lipophilic beta-blocking agent. Samples at different levels of concentration were prepared in both proteic and non-proteic matrices (human plasma, 5% human serum albumin solution and saline solution) and subjected to the classical ultrafiltration method using the different devices considered. Furthermore, an attempt to apply a previously described modified ultrafiltration method was also made. The analysis and quantification was achieved using a validated LC-MS/MS method. For the Centrifree devices, the determined unbound fractions of carvedilol and the corresponding binding degree were in accordance to literature data, while for the Amicon Ultra devices a great degree of carvedilol adsorbtion to the sample reservoir was observed, the analyte not being detected in the ultrafiltrate samples. Thus, it was further demonstrated that the type of ultrafiltration device used has a significant influence on the outcome of a plasma protein binding study. In the case of carvedilol, the evaluation of the protein binding interaction could be achieved using the Centrifree ultrafiltration devices, but not the Amicon Ultra devices.

Keywords: ultrafiltration, protein binding, carvedilol, Amicon Ultra, Centrifree

Introduction

The process of plasma protein binding (PPB) of drugs greatly influences both their pharmacokinetic and pharmacodynamic properties, being a key parameter which should be always evaluated for the comprehensive characterization of any compound (Bohnert and Gan, 2013; Yuan et al, 2020; Seyfinejad et al., 2021). Based on the importance of the PPB process, there is an increased interest in developing study methods for the accurate assessment of the binding degree of drugs. Among the different study approaches proposed over time for the assessment of PPB, the classical ultrafiltration (UF) method is still

widely accepted based on its main advantages which include: accuracy, short analysis time and ease of implementation (Howard et al., 2010; Vuignier et al., 2010). However, the method also has some limitations, non-specific binding (NSB) being the most important, but there are a lot of possibilities described in literature as ways to overcome and suppress them (Toma et al., 2021). Several ways to reduce NSB of studied drugs to the UF devices include the following: pre-treatment with different surfactant solutions, determination of NSB using phosphate buffer saline solution and the use of a correction factor, blocking of the NSB sites in the presence of plasma or proteic matrices, using different approaches and modifications of the classical UF technique.

The UF method implies the physical separation of the free and protein-bound fractions of drug through a semipermeable membrane, using the centrifugal force (Howard et al., 2010). The separation is achieved in an UF device which consists of two different compartments delimited by the semipermeable membrane with different molecular weight cut-off. After centrifugation, the ultrafiltrate containing only the free drug fraction can be quantified using an appropriate analytical technique.

It has been demonstrated that the experimental conditions, including the type of the UF device used, can greatly influence the accuracy of the results obtained in a PPB study (Kratzer et al., 2014; Dorn et al., 2018; Toma et al., 2021). Regarding the UF device, the factors that may play a role in their suitability for a particular study, are the type of the semipermeable membrane and also the material from which the other constituent components of the device are made since they can provide NSB sites (Lee et al., 2003; Kratzer et al., 2016).

The UF devices designed and commonly used for PPB studies are represented by the Millipore Centrifree filters, but in some studies, other devices initially designed for concentration of different constituents in biological samples (proteins, RNA, antigens, antibodies, enzymes) have also been successfully used (Du et al., 2014; Downing et al., 2017; Catalani et al., 2018). Furthermore, some authors suggest a validation of these other UF devices to the Centrifree ones, which are considered as reference, since differences in the results obtained based on the UF devices used have been frequently reported (Vogeser et al., 2007; Jensen et al., 2011; Larsen et al., 2011; Arellano et al., 2012; Ciobotaru et al., 2022).

The aim of this study was to assess the suitability of different ultrafiltration devices from the same manufacturer for the study of plasma protein binding of carvedilol (CVD), a highly protein-bound and lipophilic beta-blocking agent, using a validated LC-MS/MS method for quantification.

2. Materials and methods

Chemicals, reagents, and solvents

Pharmaceutical secondary standard of carvedilol was acquired from Sigma-Aldrich (Saint Louis, USA) and metoprolol succinate was purchased from Moehs (Barcelona, Spain). Acetonitrile (Honeywell, Muskegon, USA) and ammonium formate (VWR Chemicals, Radnor, USA) of LC-MS grade were used as solvents or components of the mobile phase. Human Albumin, as proteic matrix, was purched in the form of 200 g/L solution for infusion from Baxalta Innovations GmbH (Wien, Austria), while human plasma was obtained from The Regional Blood Transfusion Center Targu Mures (Romania). Saline solution was purchased from STADA Hemofarm (Timisoara, Romania) and ultrapure water was obtained with the aid of a Millipore Direct-Q 3 (Milford, USA).

LC-MS/MS analysis

A validated LC-MS/MS method previously published was used (Toma et al., 2023). The characteristics of the equipments were: a Perkin Elmer Flexar FX-10 UHPLC (Waltham, USA) and a Sciex QTOF 4600 mass spectrometer (Framingham, USA). The isocratic chromatographic separation was performed on a Phenomenex Luna C18 column 125 × 4 mm, 5 µm (Torrance, USA) with a mobile phase composition of 53% (v/v) 20mM ammonium formate at pH 4.4 and 47% (v/v) acetonitrile. The pump delivered the mobile phase with a flow rate of 0.4 mL/min, the column temperature was set at 25 °C and the injection volume was 4 µL. Metoprolol (MTP) was used as internal standard.

The MS detection was achieved after positive electrospray ionization, in MRM mode and the monitored transitions were the following: for CVD m/z 407.29 → 100.10, 222.15, 224.18, 283.22 and for the internal standard MTP m/z 268.23 → 116.12, 121.08, 133.08, 159.10, 191.14. The ion source parameters were set as follows: electrospray voltage +3300 V, source temperature 500˚C, nebulizing gas 30, drying gas 25, curtain gas 30 and collision energies of 32 for CVD and 24 for MTP (values in arbitrary units).

Preparation of solutions

Stock solutions. The stock solution of 10 µg/mL CVD was obtained by appropriate dilution with ultrapure water of a 1 mg/mL CVD solution prepared in acetonitrile, resulting in a 1% (v/v) acetonitrile concentration in the final stock solution. For the internal standard, the solvent used consisted only of acetonitrile and the final stock solution of 1 µg/mL MTP was obtained by an appropriate dilution of a 500 µg/mL MTP solution.

Standard solutions. Ten standard solutions for the calibration curve over the concentration range of 2.5 – 500 ng/mL CVD were obtained by spiking 150 µL of matrix with 50 µL aliquotes of corresponding intermediate working solutions. Three different matrices were considered: saline solution, human plasma and 5% (w/v) human serum albumin (HSA), prepared by appropriate dilution with saline of the 20% infusion solution.

Sample solutions. The sample solutions with concentrations of 25, 75, 125 and 500 ng/mL CVD were prepared in the three matrices considered following the same protocol applied for the standard solutions.

Experimental ultrafiltration protocol

Classical ultrafiltration method. Different UF devices from the same manufacturer were used: Centrifree® Ultrafiltration Centrifugal Filters (Ultracel® PL Regenerated Cellulose, 30 kDa MWCO, 1 mL), Amicon Ultra-2 and Amicon Ultra-0.5 centrifugal filter units (Ultracel-10K regenerated cellulose membrane, 2 mL and 0.5 mL) from Merck Millipore (Cork, Ireland).

For the determination of the total CVD concentration, a volume of 200 µL of each sample solution was separately added to an Eppendorf microcentrifuge tube, while, for the determination of the free/unbound fraction, 400 µL were added to the UF devices. In order to allow the establishment of the protein-binding equilibrium, all UF devices and microcentrifuge tubes containing CVD samples were incubated at 37°C for 30 minutes. After the incubation period, the 200 µL sample solution aliquotes were immediately processed for analysis, while the UF devices were centrifuged with the aid of an Eppendorf Centrifuge 5430R at room temperature for 15 min. Following the manufacturer's recommendations for each UF device, the following centrifugal forces were applied: 1877 x g for the Centrifree devices, 5214 x g for Amicon Ultra-2 and 4829 x g for Amicon Ultra-0.5. After centrifugation, 200 µL of the

ultrafiltrate obtained were processed for analysis in order to determine the unbound concentration of CVD.

Modified ultrafiltration method. An attempt to apply a modified version of the ultrafiltration method previously described by Taylor and Harker was also made (Taylor and Harker, 2006). For this method, the Amicon Ultra-0.5 centrifugal filter units were used since from all the UF devices considered in the present study, only these were suitable based on their mode of construction. For the modified UF method, for each UF device containing 400 µL CVD sample solution, a partner UF device containing 400 µL control matrix was also prepared. After incubation at 37°C for 30 minutes, all devices were centrifuged at room temperature for 15 minutes, applying a centrifugal force of 4829 x g. Following this first centrifugation, the upper compartments of the UF devices containing the retentate were inverted and placed on the ultrafiltrate collection compartments of their partner UF device. The devices were then centrifuged again for 10 minutes. 200 µL aliquotes of each reconstituted sample obtained were then removed and processed for quantification.

Processing of samples for LC-MS/MS analysis

To all sample and standard solutions, 100 µL aliquotes of 1 µg/mL MTP internal standard solution were added. The solutions were further deproteinized with acetonitrile (1:3 ratio), vortexed for 30 seconds and centrifuged for 10 minutes at 10000 rpm. The supernatants was subjected to the LC-MS/MS analysis.

Statistical analysis

The data sets obtained for determinations made using Centrifree devices were statistically evaluated in terms of normality of distribution using the Kolmogorov-Smirnov test, in terms of homogeneity of variances using the Cochran's C test and in terms of mean difference using the ANOVA single factor test. The statistical tests were applied considering a significance level of 0.05.

3. Results and discussion

Quality parameters of the analytical method

An already validated LC-MS/MS method for quantification was used (Toma et al., 2023). Specificity/selectivity, accuracy, precision and linearity of the method were tested and proved to be suitable. CVD and MTP were separated at retention times of 4.36 (±0.03) min and 2.51 (±0.01) min, respectively, demonstrating selectivity. The method presented good linearity over the concentration range 2.5-500 ng/mL CVD, with correlation coefficients greater than 0.995. Values of accuracy (relative error, Er%) and precision (relative standard deviation, RSD%) were within the acceptance limits according to the EMA Guidelines on bioanalytical method validation (Er% and RSD% < 15%).

Classical ultrafiltration method

The accuracy and relevance of PPB study results using the UF method is greatly influenced by the experimental conditions. Besides pH and temperature, which should be in accordance to the physiological values, a great attention should be paid to the type of UF device used. In the present study, two different types of ultrafiltration devices from the same manufacturer were tested regarding their suitability for the study of CVD binding to proteins. Both types of UF devices present a regenerated cellulose semipermeable membrane, but with different molecular weight cut-off: 30 kDa in the case of Centrifree devices and 10 kDa in the case of Amicon Ultra devices. Other differences between the devices considered, are related to the materials

used for the sample reservoirs and collection tubes. In the case of the Centrifree devices, the sample reservoir is made of styrene/acrylonitrile and the collection tube of polyethylene, whereas in the case of the Amicon Ultra devices, the materials used were styrene/butadiene and polypropylene, respectively.

According to the data sheet of the product, only the Centrifree devices were specifically designed for separating free from bound microsolutes in biological samples, but other PPB studies report good results also obtained using the Amicon Ultra devices, initially designed for concentration of different components of biological samples (antigens, antibodies, enzymes, nucleic acids, microorganisms), protein extraction and purification (Du et al., 2014; Imre et al., 2021).

For the classical ultrafiltration method, samples with concentrations of 25, 75, 125, and 500 ng/mL CVD prepared in human plasma, 5% HSA and saline solution were subjected to ultrafiltration using Centrifree and Amicon Ultra-2 devices. Additionally, Amicon-Ultra 0.5 devices were used for the ultrafiltration of 125 ng/mL CVD samples in the three matrices considered. The results obtained in terms of determined unbound CVD fraction (%) are presented in **Table 1**.

In the case of determinations made in human plasma using Centrifree devices, the chromatographic CVD signal observed for the samples after UF, although present, was bellow the lower limit of quantification of the LC-MS/MS method used (LLOQ – 2.5 ng/mL), thus not allowing further assessments and calculations of the unbound faction. In the case of determinations made using Amicon Ultra devices, no noticeable CVD signal was observed in the chromatograms of samples after UF. Representative chromatograms of samples before and after UF are presented in **Figures 1-3**.

From the results obtained, we could conclude that for the considered analyte, CVD, which is a highly lipophilic compound, Amicon Ultra devices are not suitable for the purpose of plasma protein binding assessments, compared to Centrifree, since no significant presence of the analyte in the ultrafiltrate was detected, not even for the higher concentration samples. In the case of the Centrifree devices, the results obtained for the determinations of CVD in human plasma could indicate a very high degree of binding to plasma proteins, which would be in accordance to literature data sustaining a more than 95% protein bound fraction (Book, 2002).

Table 1. Determined unbound fraction of CVD (%) using different ultrafiltration devices

Type of UF device	c (ng/mL)	Unbound fraction % mean (standard deviation)		
		Human plasma	5% HSA	Saline solution
Centrifree*	25	N/A	7.87 (±0.51)	64.76 (±3.94)
	75	N/A	7.64 (±0.79)	60.09 (±3.26)
	125	N/A	7.57 (±0.75)	66.43 (±2.64)
	500	N/A	8.72 (±0.54)	64.46 (±2.70)
Amicon Ultra-2*	25	N/A	N/A	N/A
	75	N/A	N/A	N/A
	125	N/A	N/A	N/A
	500	N/A	N/A	N/A
Amicon Ultra-0.5**	125	N/A	N/A	N/A

*n=3 ; **n=1 ; N/A – data not available

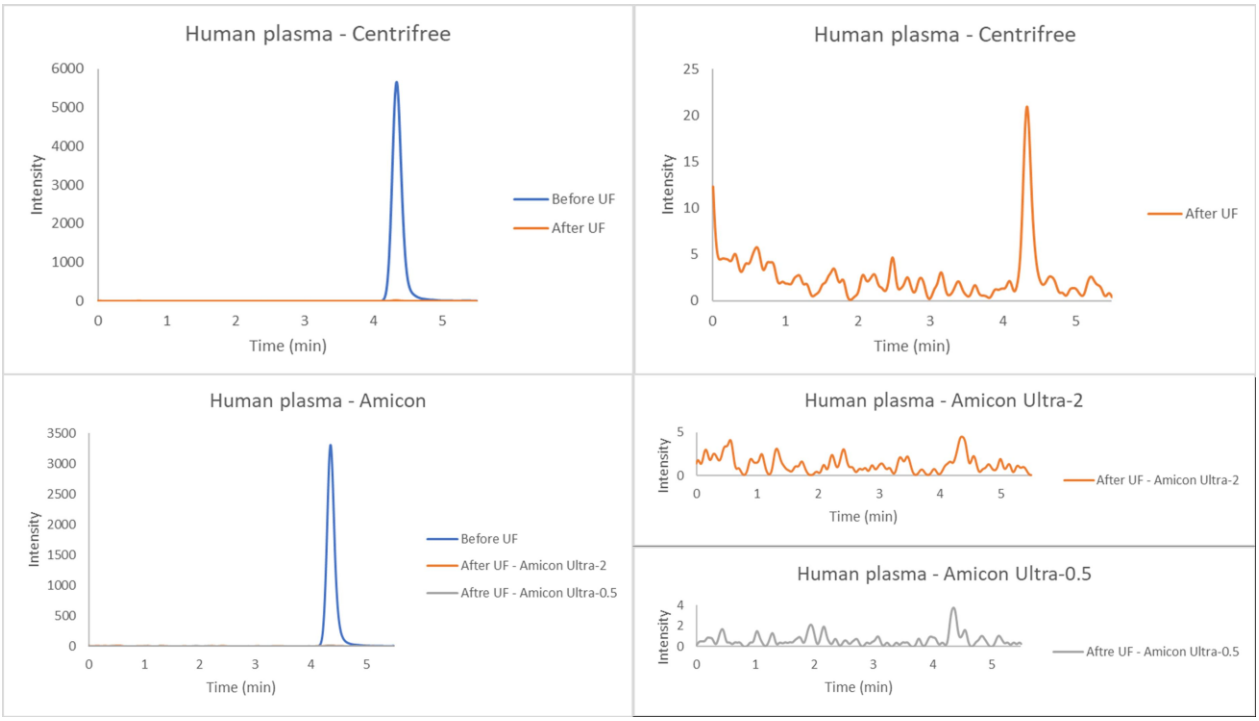

Fig. 1. Representative chromatograms of a 125 ng/mL CVD sample in human plasma before and after ultrafiltration (UF) using Centrifree vs. Amicon Ultra devices

Fig. 2. Representative chromatograms of a 125 ng/mL CVD sample in 5% HSA before and after ultrafiltration (UF) using Centrifree vs. Amicon Ultra devices

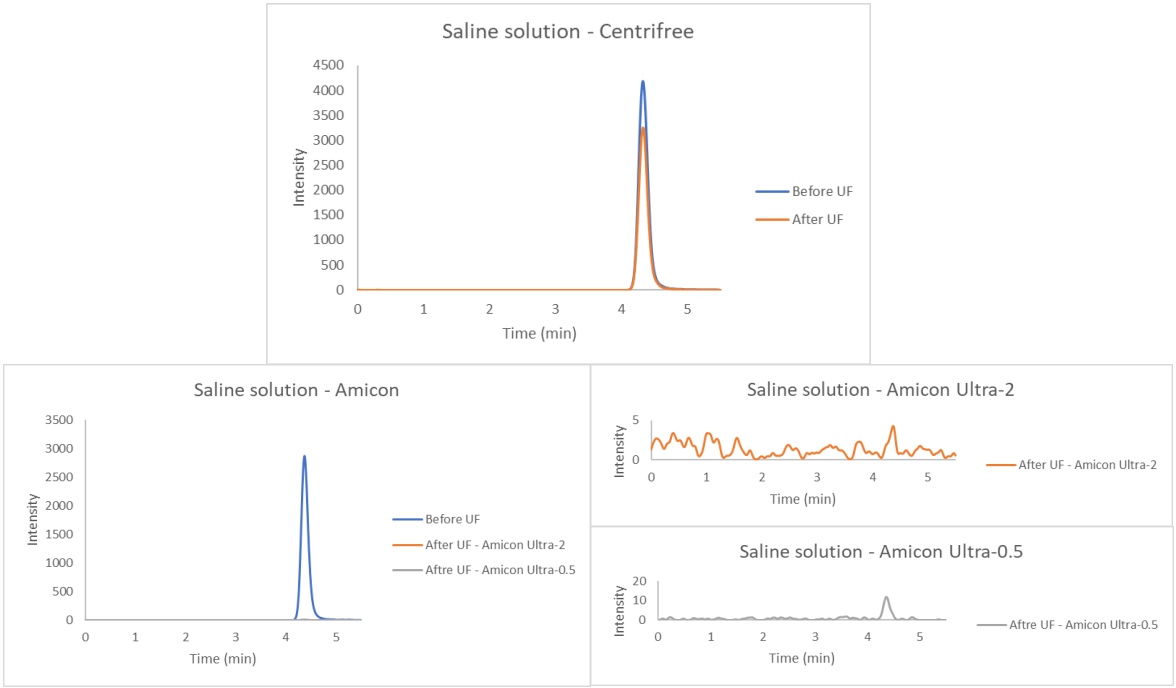

Fig. 3. Representative chromatograms of a 125 ng/mL CVD sample in saline solution before and after ultrafiltration (UF) using Centrifree vs. Amicon Ultra devices

Furthermore, in our experimental conditions, determinations made in HSA solution revealed an overall unbound fraction mean for CVD of 7.95% (±0.053), which implies a protein binding degree of more than 92%, results also in accordance to other literature data (Morgan, 1994). Compared to human plasma, which is a very complex matrix, containing a variety of proteins (albumin, alpha-1-acid glycoprotein, globulins, lipoproteins), the 5% HSA solution was chosen as a simple proteic matrix for the present study in order to also emphasize the difference in the binding behavior of CVD, and the possible influence of the proteic matrix complexity on the results obtained for the different UF devices.

The purpose of the determinations made in saline solution, were to allow an assessment of the possibility of NSB occurrence in the absence of proteins from the matrix. In a previous study, Wang S and Williams NS have shown that NSB is greatly reduced, even in the case of compounds with high lipophilicity, when samples containing protein environments are incubated in the UF device because proteins present a protective effect of blocking the NSB sites (Wang and Williams, 2013). Furthermore, the NSB and adsorption from proteic matrices can also be expected to be significantly lower, as the protein-bound drug fraction cannot be adsorbed.

The results obtained for CVD samples in saline solution indicate that, in the absence of proteins from the matrix, in the case of both types of UF devices considered, a different degree of NSB occurs. While for the Amicon Ultra devices, because of the lack of analyte in the ultrafiltrate, we could conclude that the NSB degree is maximum, in the case of the Centrifree devices an overall mean of less than 35% NSB was observed.

The separate statistical analysis of the data sets obtained for the Centrifree devices, regarding the two matrices considered (5% HSA and saline solution), revealed no significant statistical difference in terms of normality of distribution, variance and mean

free fraction of CVD ($p > 0.05$). These results indicate that, for the considered concentration range (25-500 ng/mL), the binding behavior of CVD to HSA and the adsorbtion which takes place in saline solution, respectively, are not influenced by concentration.

The very poor results obtained for the Amicon Ultra devices could be related to the materials from which the sample reservoir and collection tube are made, leading to a great adsorbtion of CVD, this being the main difference from the Centrifree devices. The difference in the molecular weight cut-off of the semipermeable membrane (10 kDa vs. 30 kDa) should not have an influence on the diffusion of CVD, taking into account its much lower molecular weight (406.5 g/mol). Furthermore, the volume of the sample reservoir in the case of Amicon Ultra devices (2 mL vs. 0.5 mL) seems to not have an influence on the result.

Modified ultrafiltration method

The modified ultrafiltration method described by Taylor and Harker (Taylor and Harker, 2006) was used for assessments regarding samples containing 125 ng/mL CVD in both human plasma and 5% HSA solution. For this method Amicon Ultra-0.5 devices were selected and samples were analyzed in singlicate. In the case of both matrices, a very high recovery of the analyte was observed in the retentate reconstituted samples (111.83% for samples in human plasma and 98.38% for samples in 5% HSA), while no presence of the analyte was detected in the filtrate reconstituted samples.

These results further sustain the very high degree of CVD adsorbtion in the sample reservoir of the Amicon Ultra devices. Even though, in the mentioned study, the research was also focused on highly lipophilic compounds (corticosteroids), the much better results using the modified UF method could be related to the different UF devices used (Microcon).

Conclusions

When studying PPB of drugs using the UF method, a very close attention should be paid to the implied UF protocol and to the UF devices used. The Centrifree filter devices, which were specifically designed for evaluations of PPB, have also proven to be suitable for the study of the protein binding process in the case of the lipophilic compound CVD, in comparison to the Amicon Ultra devices for which very poor results were obtained. The different materials used for the components of the Amicon Ultra devices, compared to Centrifree, seemed to result in a great adsorbtion of the analyte to the sample reservoir, making the devices impractical for use in the desired study approach.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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UNVEILING DECEPTIVE CLAIMS: A CROSS-SECTIONAL OBSERVATIONAL ASSESSMENT OF DIETARY SUPPLEMENT ADVERTISEMENTS FROM THREE NEWS CHANNELS IN ROMANIA

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Abstract: Dietary supplements can provide the necessary nutrients for groups of people who need them, but their use comes with a few risks, such as excessive or unwarranted utilization, side effects, unwanted interactions, and the promotion of unhealthy behaviors or neglecting professional health care. This cross-sectional study analyzed 49 TV advertisements on three major news channels in Romania regarding their compliance with European and national laws concerning the advertisement of dietary supplements. A list of criteria was extracted from the current laws in Romania and was used to check the video promotional materials' compliance systematically. Despite more than half of the commercials complying with the technical criteria, it was found that a concerning amount of advertisements targeted people with various or specific pathologies, while a third of the ads included non-compliant words or representations associated with pathologies and medical professionals or institutions. As a practical implication, the study suggests a need for more consistent and closer surveillance of dietary supplement commercials broadcasted in the Romanian media.

Keywords: dietary supplements, nutrivigilance, advertisement, compliance, legal regulations

Introduction

The consumption of dietary supplements among adult populations, particularly in the USA and Europe, has experienced substantial growth over the last few decades (Starr, 2015). The two main reasons for people using dietary supplements are to protect themselves from potential future illnesses proactively, and to seek healing for their existing health conditions (Lam et al., 2022). An analysis of the data about dietary supplement utilization collected from the participants in the European Prospective Investigation into Cancer and Nutrition study has shown a wide variation across nations, from 2.0% in Greece to 51.0% in Denmark (Skeie et al., 2009). A survey recently conducted in city located in central Romania has shown that half of the respondents used dietary supplements. Vitamins, protein preparations, and minerals were the most popular dietary supplements (Fagaras et al., 2023).

Various forms of media, including television, are recognized as potent forces shaping an individual's choice to consume

nutritional supplements (El Khoury and Antoine-Jonville, 2012). According to some observations, the impact of media advertisement on dietary supplement utilization may have increased during the COVID-19 pandemic (Adams et al., 2020). In Romania, television stands out as the most prevalent information source, effectively reaching nearly 100% of the population (Crețu, 2017).

According to Romanian laws, more specifically, Ministry of Public Health Order No. 1069 of 19 July 2007 for the approval of the Norms regarding dietary supplements, Ministry of Public Health, Official Monitor No. 455 of 5 July 2007, and European Parliament and Council Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements, dietary supplements fall into the food products category whose intended purpose is to complement a regular diet by providing a proper intake of nutrients and can be used to correct a deficiency or to sustain physiological functions. However, there is no universal consensus regarding how the dietary supplements should be defined (Dwyer et al., 2018).

In theory, dietary supplements are helpful by providing beneficial effects to the organism, but several systematic reviews suggest that for most of the products on the market, there is no evidence to support their preventive or curative properties (Huang et al., 2006; Fortmann et al., 2013; Wierzejska, 2021). Moreover, there are numerous risks related to the use of these products, such as adverse reactions and unwanted interactions with other supplements or drugs administered together (Morgovan et al., 2019). As they can be obtained and administered without medical recommendations, there are additional risks such as excessive administration, dangerous interactions with other medications or supplements, and most alarmingly, consuming counterfeited dietary supplements available on the market, which may contain toxic ingredients (Marcus, 2016). Professionals and regulators are increasingly aware of the need for stricter regulations to detect, monitor, and record adverse events associated with dietary supplements (Malve and Fernandes, 2023).

In 2002, the European Union Directive 2002/46/EC has regulated dietary supplements under food laws, noting that products containing concentrated nutrients or other types of substances with nutritive or physiological effects alone or in combination can be considered dietary supplements, with the note that only vitamins and minerals fall under the nutrients category. In addition, official guidelines in Romania state that dietary supplements are to be used in certain groups of people, more specifically those who may need to complete their daily intake of nutrients (elderly, kids, teenagers, for restrictive diets, or periods of pregnancy/ breastfeeding) (Garban and Florescu, 2013).

Dietary supplements can only be sold or advertised if they meet all the requirements provided by laws specific to each country. In Romania, according to Order No. 1069 of the Ministry of Health of 19th of June 2007, all dietary supplements require an authorization notice from the Public Health Ministry, and any advertisement can only be done after receiving approval from the same organization. One of the requirements for dietary supplements is for the label, presentation, and advertisement of the product not to include the following claims: prevention properties, capability to heal or prevent any human pathology or induce the idea that a balanced diet, in general, cannot provide the required nutrients.

Although some governmental agencies and authors pointed out that deceptive or questionable marketing and sales practices for

dietary supplements and exposure to distorted messages could potentially lead to detrimental impacts on consumers, including adoption or continuation of unhealthy behaviors and excessive intake of supplements, there has not been much research done on whether or not promotional materials meet all the legal criteria implemented by institutions (United States, Government Accountability Office, 2010).

The aim of this study was to investigate whether TV commercials on dietary supplements broadcasted on Romanian news channels meet all the necessary criteria under the current legislation. The study also sought to identify and analyze messages presented in promotional materials that would interfere with consuming dietary supplements responsibly or undermine the importance of a healthy lifestyle in favor of consuming these products.

2. Materials and methods

The main part of the research was designed as a quantitative, observational, cross-sectional study but also included a few qualitative assessments of the language of the health claims associated with dietary supplement advertisements. The most representative TV channels in Romania, namely, ProTV, Antena 1, and Kanal D, were selected based on audience statistics (Asociația Română pentru Măsurarea Audiențelor [Romanian Association for Audience Measurement], 2018). Each media channel was monitored for a whole day between January 1 and May 31, 2019, and the commercials were recorded using Bandicam Screen Recorder (Bandicam Company).

The study sample consisted of 49 dietary supplement advertisements that underwent content and formal analysis. Product names were anonymized and classified according to basic attributes like product type, presentation form, intended consumer demographic, and duration of the video content.

The advertisements' compliance with the specific regulations was checked based on a list of requirements extracted from European and Romanian legislation and good practice guides in the field:

1. European Parliament and Council. Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements.
2. Ministry of Public Health. Order No. 1069 of 19 July 2007 for the approval of the Norms regarding dietary supplements, Ministry of Public Health, Official Monitor No. 455 of 5 July 2007.
3. National Audiovisual Council DECISION No. 220/2011 of February 24, 2011, regarding the Audiovisual Content Regulatory Code.
4. REGULATION (EU) NO. 432/2012 OF THE COMMISSION of May 16, 2012, establishing a list of permitted health claims written on food products other than those referring to the reduction of the risk of illness and the development and health of children.
5. National Audiovisual Council DECISION No. 614/11.06.2019.

The criteria were operationalized in multiple-choice questions:

1. Health claims: If the commercial includes health claims, does it comply with legal regulations? a. Yes, fully; b. Yes, only for some of the ingredients; c. No; d. It does not include health claims; e. It does not include explicit mentions but suggests an effect on health. [National Audiovisual Council. Decision No. 220 of February 24, 2011, regarding the Audiovisual Content Regulation Code. Article 120 (2); Regulation (EC) No. 1924/2006 of the European Parliament and of

the Council of December 20, 2006, on nutrition and health claims on food products.]

2. Audio and visual warnings: Does the advertisement include the warning "This is a dietary supplement. Read the leaflet/information on the package carefully." in audio format for a minimum of 3 seconds? a. Yes; b. No." Does the advertisement include the warning "This is a dietary supplement. Read the leaflet/package information carefully." in visual format? a. Yes, visible and readable; b. Yes, but it is hard to read because the letters are too small; c. Yes, visible, but the display duration is insufficient for reading it fully; d. Absent. [National Audiovisual Council. Decision No. 220 of February 24, 2011, regarding the Audiovisual Content Regulation Code. Article 131; Art. 133]

3. Food intake: Does the commercial suggest that a varied and balanced diet cannot provide the daily requirements? a. Yes; b. No. [Directive 2002/46/EC the European Parliament and of the Council of June 10, 2002 on the approximation of the laws of the Member States relating to food supplements of June 10, 2002 Art. 7]

4. Prohibited words: Does the advertisement include prohibited words that indicate one of the following terms: "medical", "sick", "disease", "remedy", "medicine", "treatment", as well as their translations, synonyms or words that come from their lexical family (except for some warnings), names of diseases, the names or the representation of symptoms of diseases or sick people? a. Yes; b. No. [Ministry of Health, National Institute of Public Health. Food supplements - Guide, p.29; National Audiovisual Council. Decision No. 220 of February 24, 2011, regarding the Audiovisual Content Regulation Code. Art. 128 (a-f)]

5. Personalities, doctors, medical associations: Does the commercial include visual or audio messages stating or implying that the dietary supplement is recommended by public figures, doctors or pharmacists, or medical associations recommending dietary supplements? a. Yes, public figures; b. Yes, health professionals; c. Health-related groups; d. No. [National Audiovisual Council. Decision No. 220 of February 24, 2011, regarding the Audiovisual Content Regulation Code. Art. 126 (1), (2), (3)]

6. Preventive, therapeutic, curative effect: Does the advertisement include information that attributes or suggests that the food supplement presented has properties to prevent, treat and cure human diseases? a. Yes, prevention; b. Yes, treatment; c. No. [National Audiovisual Council. Decision No. 220 of February 24, 2011, regarding the Audiovisual Content Regulation Code. Art. 120 (1); Directive 2002/46/EC of June 10, 2002 Art. 6 (2).]

7. Lifestyle: Does the commercial suggest that the dietary supplement could counteract the effects of an unhealthy lifestyle, overeating, alcohol abuse, etc.? a. Yes; b. No. [National Audiovisual Council. Decision No. 220 of February 24, 2011, regarding the Audiovisual Content Regulation Code. Art. 93 - (1)]

8. Sales, discounts: Does the advertisement include information that could encourage the unwarranted use of the dietary supplement by offering the product at reduced prices? a. Yes; b. No. [National Audiovisual Council. Decision No. 220 of February 24, 2011, regarding the Audiovisual Content Regulation Code. Art. 93 - (1)]

Descriptive statistics were calculated for each collected variable.

3. Results and discussion

The distribution of commercials based on the type of ingredients contained in the advertised dietary supplements is displayed in **Figure 1**.

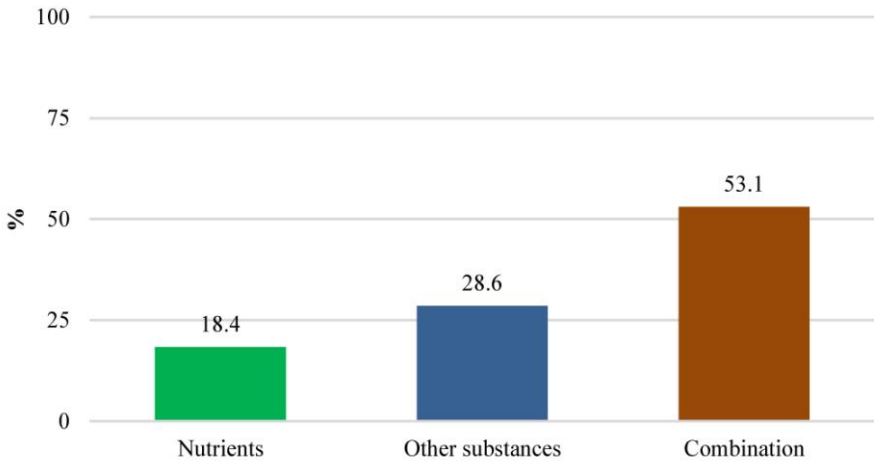

Fig. 1. Percentage of commercials according to the type of ingredients.

Most of the food supplements studied fell into the category of combined supplements, which contain other substances besides vitamins or minerals. This observation draws attention to a problem pointed out by specialists, namely, that the ingredients of food supplements also contain other substances (especially phytochemicals of plant origin), which have no role in supplementing the diet or possible deficiencies in healthy people but can lead to adverse reactions in combination with the medication prescribed by the doctor (for example St. John's wort, ginseng, goldenseal, garlic) (Ronis et al., 2018).

Regarding the pharmaceutical presentation, the following forms were found: tablets – 22 (of which 15 were simple tablets, two film-coated tablets, three effervescent tablets, and two chewable tablets); capsules – 17 (of which 14 were simple capsules and three soft capsules); powders – 4 (of which 1 was simple powder ad three powders for oral solution); syrups – two; oral solutions – one; oro-dispersible granules – one; soft gummy jellies – one, and lozenges – one. Market research data indicates that even on the global scene, tablets are the predominant form of presentation (Persistence Market Research, 2017).

The distribution of dietary supplement commercials depending on the intended consumer group is shown in **Figure 2**.

The analysis of the intended target groups also highlighted the orientation of the messages in the advertising materials to people other than healthy ones. Practically, only one out of five advertisements unequivocally had healthy people as a target group. The rest of the ads focused on a wide range of people affected by various diseases or symptoms, from liver and psychiatric conditions to eye disorders and hypercholesterolemia. The most frequently encountered advertising messages were addressed to people with weakened immunity, urological conditions, respiratory system conditions, and musculoskeletal conditions (each of them with more than 10% of the total ads).

There were 47 advertisements that included health claims. Of these, 16 (34.0%) fully complied with the EU regulations on the matter, two (4.3%) showed partial compliance, and 29 (61.7%) did not comply at all. A comprehensive examination of supplement advertisements published in the USA from 2003 to 2009 reported that they encompassed a wide variety of claims from common to very severe diseases (Avery et al., 2017).

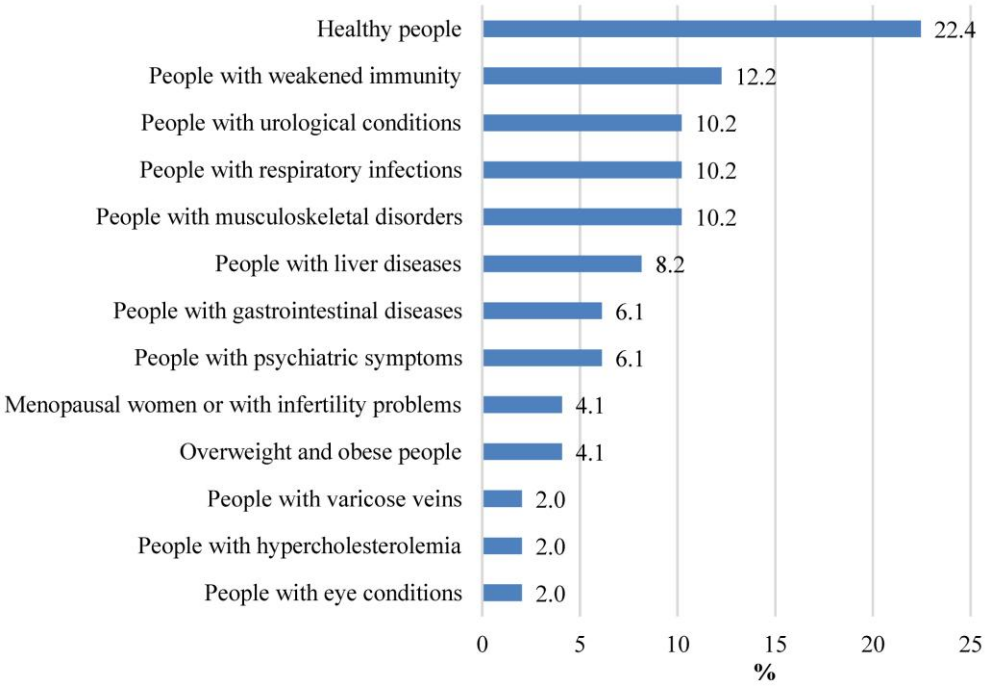

Fig. 2. Proportion of commercials by type of ingredients.

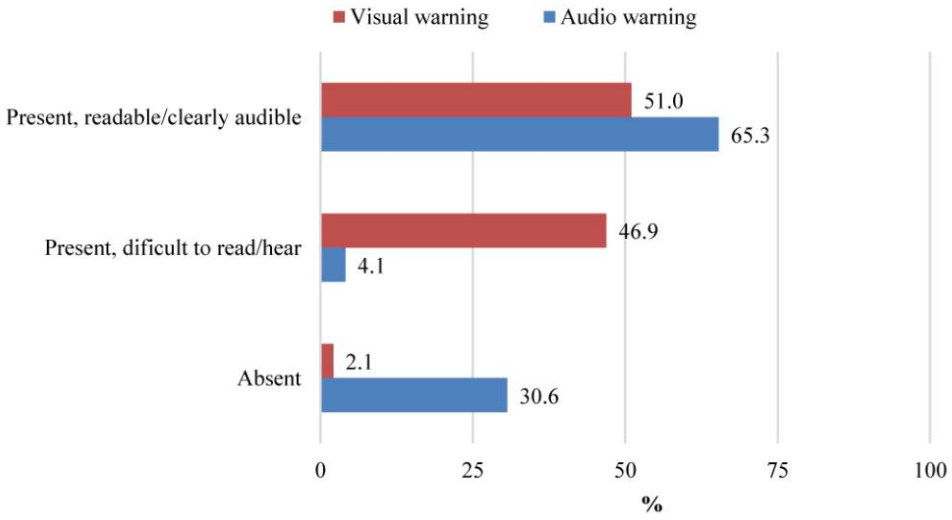

Fig. 3. The presence of visual and audio warnings in the advertisement of dietary supplements ("This is a dietary supplement. Read the leaflet/information on the package carefully").

Another earlier investigation conducted in San Francisco and Los Angeles, California, USA, revealed that advertisements for dietary supplements were more prevalent on non-English media outlets compared to English ones and a significant proportion of them did not comply with the regulations and disseminated unsubstantiated claims (Lee et al., 2015). In a study conducted in Poland, the authors found that approximately 30% of the promoted dietary supplements made claims about their effectiveness in various health situations, such as overweight and obesity, without reliable proof to back up those claims (Wierzejska, 2022).

European and national legislation mandates that all commercials of dietary supplements should specifically warn potential

consumers that "This is a dietary supplement. Read the information on the leaflet/package carefully." The assessment results regarding mandatory visual and audio warnings in the TV advertisement of dietary supplements are presented in **Figure 3**.

Only slightly more than half of the advertisements included the mandatory visual and audio warnings. Of further concern is the complete absence of audio warnings in almost a third of the sampled advertisements. Also, in almost half of the examined videos, the visual warnings were difficult or impossible to read because of the small size of the fonts or the short display time. The mean duration of video ads in the sample was 19 seconds (SD= 7.4 seconds, minimum 9 seconds, maximum 30 seconds). Non-compliance to requirements regarding visual and audio warnings is better understood considering these constraints imposed by costs of air time and the pressure to use the spots to convey persuasive information that increases sales.

Among the 49 evaluated commercials, one (2.0%) subtly suggested that a particular dietary supplement is needed besides physical activity and diet to provide adequate nutrients to the joints. Dietary supplement marketers must strictly refrain from stating or implying that a balanced or varied diet cannot provide appropriate quantities of nutrients or from conveying the idea that the population at large is at risk of vitamin or mineral deficiency. Our examination suggests that the marketers in Romania at the time of the study had almost fully complied with this critical requirement.

Prohibited words such as "medical", "sick", "disease", "remedy", "medicine", "treatment", or terms referring to names of diseases, representations of symptoms, or sick people were detected in 17 (34.7%) of the monitored advertisements. In comparison, 32 (65.3%) did not incorporate any of the unaccepted or related words. These deviations from the regulations in the field also seem surprising, taking into account that detecting illegal words does not raise technical difficulties. However, this phenomenon is not unique to Romania as authors from the USA also observed a significant number of banned verbs in health and function claims, which hinted toward therapeutic effects (Avery et al., 2017). This type of non-compliance may interfere with the standard of objectively informing the consumers, potentially leading to a misunderstanding regarding the purpose of food supplements, and possibly to the decision to buy supplements in the hope of improving symptoms or healing from certain conditions.

Another aspect regulated by the legislation regarding the publicity of dietary supplements refers to visual or auditory representations that evoke medical professions (through the clothing, equipment, or emblems presented) or recommendations, prescriptions, certificates, or statements of medical approval. The proportion of dietary supplement advertisements that integrated in a more or less direct or explicit way various unacceptable recommendations or endorsements is shown in **Figure 4**.

Detecting more or less direct, explicit, or subtle non-compliance to regulation in almost a fifth of advertisements is also alarming. These messages might manipulate the consumer, inducing the idea that medical experts endorse the promoted supplement. These observations are similar to those reported by researchers from Poland, where some advertisements elicited the medical profession's authority to support the claimed effects (Wierzejska, 2016). Furthermore, an investigation conducted in Spain on dietary supplement advertisements on the radio showed that unauthorized endorsers, including healthcare practitioners, everyday consumers, and celebrities, were featured in 40% of the promotional audio spots (Muela-Molina et al., 2020).

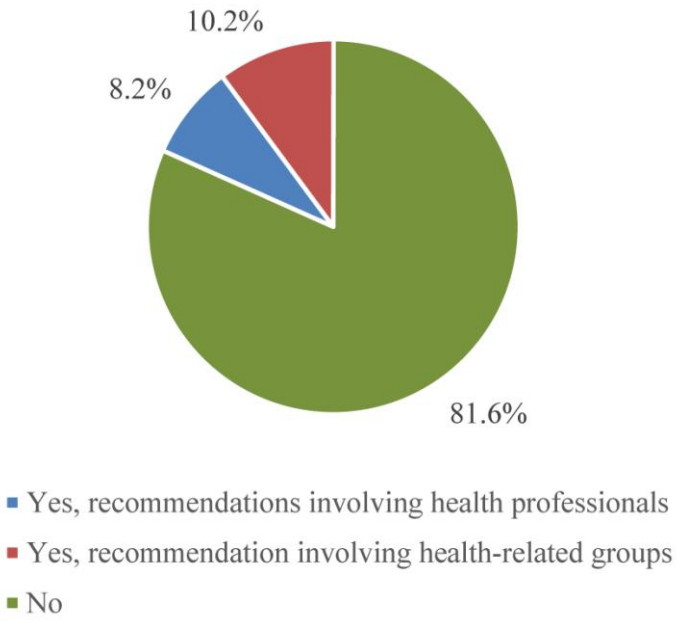

Fig. 4. The proportion of dietary supplement advertisements integrating unacceptable recommendations.

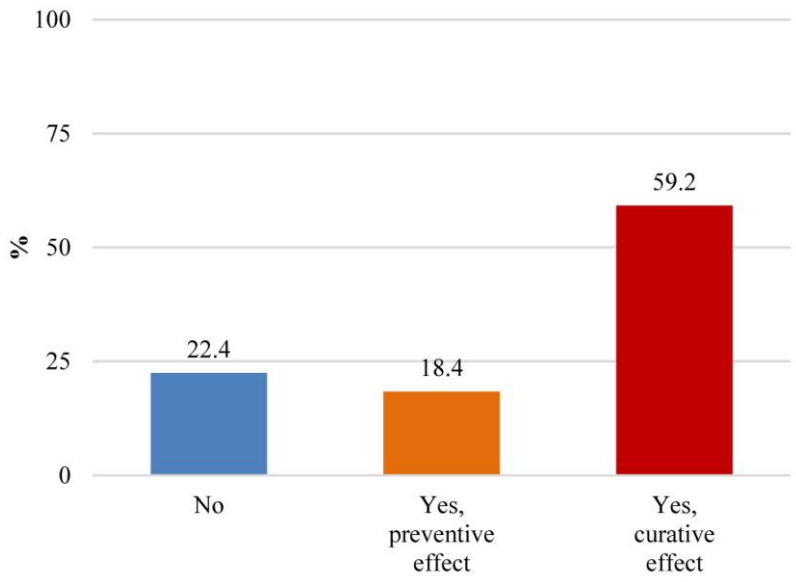

Fig. 5. The prevalence of advertisements with information suggesting that dietary supplements have preventive or curative properties.

One of the essential requirements regulated by legislation concerning labeling and advertising of dietary supplements concerns the claim or suggestion of preventive and therapeutic effects. The results of screening the TV publicity of dietary supplements on the Romanian news channels for information that attributes or suggests the product has preventive or curative properties in human diseases are reported in **Figure 5**.

Only one out of five advertisements in the studied sample fully complied with the rules in force. Almost 20% of the ads suggested some preventive effects, and worse, almost 60% suggested curative effects. For example, supplement X1 prevented viral infections in

children; supplement X2 prevented diseases (unspecified) in children; commercial for supplement X3 suggested preventing cardiovascular diseases; supplement X4 prevented complications associated with prostate dysfunctions; supplement X5 treated "all types of coughs"; supplement X6 hinted it could treat menopausal symptoms; supplement X7 cured urinary incontinence; supplement X8 solved infertility in women; and supplement X9 inferred it treats varicose veins. The qualitative analysis of the content of the advertisements suggests that these messages could persuade consumers to purchase and use dietary supplements for a purpose foreign to the very definition and destination of such products established by the relevant legislative and professional bodies. These findings suggest that many dietary supplements may be bought and administered for their preventive-curative properties, specifically for properties distinctive to pharmacological drugs.

These findings align with those reported in 2014 by authors from Poland. A sample of 27 dietary supplement advertisements broadcast on TV and radio were analyzed, and 23 of them stated that the products would improve organ functions using expressions like "prevents," "treats," and "maintains." The investigators concluded that supplements are publicized in disregard of regulations for dietary supplements, thus contributing to further wrong opinions regarding the properties of these products (Wierzejska, 2016).

One last critical aspect of the research resides in analyzing food supplement advertisements in terms of their potential influence on health behaviors. The assessment found that 11 (22.4%) of the commercials more or less directly suggested that the respective dietary supplements could offset or mitigate the undesired effects of some detrimental behaviors. Finally, seven (14.3%) advertisements included information that might encourage the unwarranted use of the dietary supplement by offering reduced prices. Apparently, almost a quarter of dietary supplement ads incorporated messages that could undesirably influence the lifestyle of the target consumers by encouraging excessive food and alcohol intake or irresponsible use of pharmacological medications. Many advertisements implied that the unwanted consequences of behavioral risk factors, such as physical or mental overstrain, can be offset by administering food supplements, a view that may interfere with health education and promotion efforts. For example, the advertisement for supplement Y1 depicted appealingly unhealthy foods and drinks, after which it mentioned that "in case of burns and gastric discomfort, it [the supplement] calms and keeps the digestive system healthy," suggesting that the unpleasant consequences associated with unhealthy eating were canceled by using the dietary supplement. Likewise, the ads for supplement Y2 ("Say stop to fatigue!"), supplement Y3 ("Helps reduce fatigue") and supplement Y4 ("Increases resistance to stress") conveyed the notion that dietary supplements may be an easy solution to fatigue and that recovery may be achieved without physiological rest. Another example with serious implications would be the advertisement for supplement Z1, which suggested that administering it counteracted the effects of an unhealthy lifestyle, including smoking and alcohol abuse, by "naturally restoring the liver cell membrane." Another commercial with troublesome implications was the one for supplement Z2, which urged consumers to "naturally protect their liver" with the advertised product "in the case of excessive medicinal drugs consumption," suggesting that in this way, the liver will no longer be affected by the possible adverse effects of drugs.

Authors from Japan also investigated this type of impact of a dietary supplement on

individual lifestyles. They found that the most prevalent dietary supplement ads were in the category of so-called "Exemption ads". This term means that using the supplement frees the individual from the necessity to abstain from certain unhealthy behaviors. The unspelled but implied message of these types of advertisements is "No need to resist the desire for binge eating if the product is consumed" (Iye et al., 2021).

Regarding the study's limitations, despite monitoring the television channels with the largest audience, the findings may not be representative for all news channels in Romania. Also, certain aspects of the evaluation were influenced, at least in part, by the subjectivity of the evaluator. Future research should address these issues to ensure more reliable conclusions.

Conclusions

Although, by definition, dietary supplements should be aimed at healthy people, most of the studied advertisements targeted people with various diseases or symptoms.

Only about half of the analyzed advertising materials exhibited the visual and audio warnings required by regulations.

Most advertisements incorporated non-compliant health claims, and more than a third included words or visual and acoustic representations not permitted by the regulations.

More than three-quarters of the advertisements claimed or suggested preventive or curative effects expressly prohibited by the relevant legislation, and almost a quarter of the advertisements included messages that could undesirably influence the consumers' lifestyle.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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PLANT SPECIES IMPORTANT FOR POLLINATING INSECTS. CASE STUDY: BĂICENI LOCALITY (BOTOŞANI COUNTY)

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Abstract: The aim of this paper is to point out plant species that are of benefit to pollinators, from the territory of the Băiceni (Botoșani county, NE region of Romania). The plant species were analyzed on the basis of the specialized literature as follows: bioform, flowering period, flower grouping, flower color, floral resources, melliferous potential. A number of 106 plant species belonging to 31 botanical families were identified; 41.50% are woody species and 58.50% are herbaceous species. The representative botanical families are: Rosaceae (19.81%), Fabaceae (14.15%) and Asteraceae (14.15%). The majority of the species recorded in the area of study have flowers grouped in inflorescences, blooming in spring and summer and are nectar-polleniferous. The color of the flowers varies from white, yellow, yellow-green to red, blue, purple. These species are an essential resource for pollinators (mainly for the honeybee) and thus contribute to keeping the ecological equilibrium of the ecosystems in the study area and to supporting local beekeeping.

Keywords: forest, meadow, life form, floral resources, melliferous potential

Introduction

Pollination is an important stage for plant fruiting and has benefits for mankind. According to Ollerton et al. (2011), globally, 87.50% of flowering plant species depend on biotic pollination; in temperate ecosystems 78% of plant species depend on animals for sexual reproduction. For species with biotic pollination, pollination is carried out by pollinating insects (honeybees, solitary bees, bumblebees, butterflies, moths, flies, beetles, etc.), hummingbirds, etc. Most pollinators in the temperate zone are insects (Reverté et al., 2016).

In the EU, approximately 84% of plant species and 76% of food production depend on bee pollination (https://www.europarl.europa.eu/news/ro/headlines/economy/); approximately 15 billion of the EU's annual agricultural income is attributed to pollinating insects (https://www.europarl.europa.eu/news/ro/headlines/society/). According to Vancea (2006), the honeybee is considered the most valuable pollinator for agricultural and fruit crops; its contribution to achieving additional yield increases is very high: 30-60% for sunflower; 50-60% for fruit trees per fruit.

Some studies indicate the existence of decline in pollinators, a fact that can affect the pollination service. The decline of pollinators would be due to the complex interaction between several factors such as: the reduction, fragmentation and loss of habitats, intensive agriculture, pesticide treatments, food availability, climate change, pollution, etc. (Kremen et al., 2002; Carvell et al., 2006; Harwood and Dolezal, 2020; https://www.fao.org/; https://www.europarl.europa.eu/news/ro/headlines/society/). According to Venjakob et al. (2016), the reduction of floristic diversity can alter the spatio-temporal resource use of pollinators. Among pollinating insects, butterflies are very sensitive to microclimate conditions and extremely sensitive to changes in the composition and structure of vegetation (Sawchik et al., 2005); are often used as bioindicators of ecosystem health (Bonebrake and Sorto, 2009).

Grasslands, forests, field crops, gardens and orchards provide favourable habitats for pollinators. These offer a variety of food sources such as nectar (for bees, bumblebees, butterflies, etc.), pollen (for bees, bumblebees), leaves (for butterflies' larvae), and also survival and reproduction spaces. Woody species (from forests, orchards and cultivated in gardens) provide food resources (pollen, nectar) for pollinating insects at certain times of the year when food resources are limited (especially in spring). Permanent meadows in Romania are a valuable plant resource for biodiversity, 238 melliferous species have been identified; the melliferous potential of permanent meadows in Romania was estimated at an average of 2-6 kg honey/ha (Motcă, 2010). In Romania there are concerns about the study of melliferous resources considering the importance of the pollination service offered by the honeybee as well as the importance of bee products. Among the studies related to the melliferous flora we list: Ion and Ion (2007); Motcă (2010); Covaliov et al. (2012); Dincă et al. (2014); Antonie (2017); Ion et al. (2018).

The purpose of this paper is to highlight the plant species with importance for pollinating insects (mainly for bees), from the territory of Băiceni (Botoșani county, NE region of Romania).

2. Materials and methods

The village of Băiceni is a component part of the Curtești commune (47°42′59.4″ N and 26°38′44.9″ E), which is located in the SW area of Botoșani county (NE region of Romania). The relief of the Curteşti commune is characteristic of the Moldavian Plain (a geomorphological unit that is part of the Moldavian Plateau), being made up of hills, hillocks and small plateaus with an altitude of less than 200 m. The surface of the administrative territory of the commune is 5783 ha (Plan urbanistic general, comuna Curtești, Județul Botoșani, 2009). The vegetation consists of deciduous forests, meadows, and agricultural crops.

The research was carried out in the vegetation seasons of 2018, 2019. The plant species (from forests, grasslands, agricultural crops, gardens and orchards) were identified using the specialized bibliography (Săvulescu, 1952-1976; Ciocârlan, 2009; Sârbu et al., 2013). For the nomenclature of plant species was used *Plante vasculare din România. Determinator ilustrat de teren* (Sârbu et al., 2013). The species were analyzed based on the specialized literature, taking into account the following aspects: bioform type, flowering period, flower grouping, flower color (Săvulescu, 1952-1976; Kovács, 1979; Pârvu, 2002-2005; Ciocârlan, 2009; Sârbu et al., 2013); the resources offered by flowers (nectar and pollen) and the melliferous potential (Cîrnu, 1980; Pop, 1982; Pîrvu, 2002-2005; Karácsonyi, 2009-2010; Grozeva, 2011; Jarić

et al., 2013; Mačukanović-Jocić and Jarić, 2016; Güneș Özcan et al., 2016). Some observations were also made regarding the pollinating insects in aestival season of 2018. In the paper only a few common species of pollinating insects were mentioned, which were determined by Associate Professor Ion Cojocaru (Faculty of Biology, "Alexandru Ioan Cuza" University of Iași). The species were determined on the basis of specialized literature (Niculescu 1961, 1963, 1965; Stănoiu et al. 1979; Chinery 1988).

3. Results and discussion

Studies about the flora and vegetation of Botoșani county were published by Mihai (1970, 1971); Mititelu and Chifu (1994); Huțanu (2004); Tanase (2013). The study area has been little researched, Șchiopu et al. (2020) mentioned a list of meadows plant species in Băiceni (Botoșani county) and their economic importance.

In the flora of the study area 106 species belonging to 80 genera and 31 botanical families were identified (**Table 1.**). The botanical families with a large number of species are: Rosaceae with 21 species (19.81%), Fabaceae with 15 species (14.15%) and Asteraceae with 15 species (14.15%). The Lamiaceae family includes 7 species (6.60%). The other families are represented by a small number of species.

Of the 106 species, 44 are woody species (41.50%) and 62 are herbaceous (58.50%). 25 species (23.58% of the total) were identified in the forest ecosystem, 43 species (40.57%) in the meadow ecosystem and 38 species (35.85%) are cultivated (in fields or gardens). Regarding the spectrum of bioforms, a significant share of phanerophytes (42.45%) and hemicryptophytes (31.13%) was found. Next in descending order are therophytes (13.21%), geophytes (6.60%), hemitherophytes (4.72%) and chamaephytes (1.89%). Woody species, as well as herbaceous perennials provide food resources for pollinating insects over a long period of time.

Among the species of pollinating insects identified, we list: *Apis melifera* Linnaeus, 1758 (Ord. Hymenoptera, Fam. Apidae); 5 species of butterflies (ord. Lepidoptera: *Argynis paphia* Linnaeus, 1758 - Fam. Nymphalidae; *Colias croceus* Fourcroy, 1785 - Fam. Pieridae; *Pieris brassicae* Linnaeus, 1758 - Fam. Pieridae; *Iphiclides podalirius* Linnaeus, 1758 - Fam. Papilionidae; *Vanessa atalanta* Linnaeus, 1758 - Fam. Nymphalidae); *Eristalis tenax* Linnaeus, 1758 (Ord. Diptera, Fam. Syrphidae). Plants are essential food sources for butterflies (some species provide food resources for larvae, other species provide nectar for adults), but they also provide support and a suitable microclimate for their survival and reproduction (Sawchik et al., 2005).

Table 1. Representative botanical families and genera

Families	Genera	%	Species	%
Rosaceae	11	13.75	21	19.81
Asteraceae	14	17.50	15	14.15
Fabaceae	10	12.50	15	14.15
Lamiaceae	6	7.50	7	6.61
Liliaceae	4	5.00	4	3.77
Poaceae	4	5.00	4	3.77
Caprifoliaceae	3	3.75	4	3.77
Aceraceae	1	1.25	4	3.77
Others (20)	27	33.75	32	30.20
Total	80	100	106	100

Table 2. Flowering period of the species (%)

The flowering period	Cultivated species	Grassland species	Forest Species	Participation (%) of total species
Spring	34.21	0	64	27.36
Spring-Summer	23.68	11.63	20	17.92
Summer	10.53	48.84	16	27.36
Summer-Autumn	28.95	13.95	0	16.04
Spring finale-Autumn	2.63	25.58	0	11.32

In the case of the species identified in the study area, the host plants of the larvae can be: specimens of *Crataegus monogyna*, *Prunus spinosa*, *Urtica dioica*, etc. (for *Argynis paphia*); *Lotus corniculatus*, *Medicago falcata*, *Medicago sativa*, *Coronilla varia*, *Trifolium* sp., etc. (for *Colias croceus*); cabbage and other species of cultivated or wild brassicas (for *Pieris brassicae*); *Prunus spinosa*, *Prunus avium*, *Prunus cerasus*, *Pyrus communis*, etc. (for *Iphiclides podalirius*); *Urtica dioica* and other species (for *Vanessa atalanta*) (Niculescu, 1961; 1963, 1965). The adult specimens of *Argynis paphia* settle on the flowers of the species *Achillea millefolium*, *Carduus acanthoides*, *Ligustrum vulgare*, *Prunella vulgaris*, etc.; those of *Iphiclides podalirius* visit plants such as *Prunus spinosa*, *Carduus acanthoides*, *Medicago sativa*, etc. The *Colias croceus* species is found on cultivated land, meadows; *Pieris brassicae* occurs in vegetable gardens etc.; *Vanessa atalanta* is found in gardens, orchards, parks, etc and can visit flowers of *Carduus acanthoides*, *Sambucus* sp., *Ligustrum* sp. (Niculescu, 1961, 1963, 1965; http://www.eurobutterflies.com). *Eristalis tenax* is a cosmopolitan species; the adults are pollinators for some cultivated species (onion, soybean, carrot, sweet pepper, etc.) (Howlett and Gee, 2019).

The flowering period of identified plant species is long; it starts in early spring in the case of some forest species (*Cornus mas*, *Corylus avellana*) or fruit trees (*Armeniaca vulgaris* Lam. var. *communis*, *Armeniaca vulgaris* Lam. var. *amarella*) and ends in autumn (*Dahlia* sp., *Satureja hortensis*, *Symphyotrichum novi-belgii*, etc.). It was found that the largest number of species bloom in the spring (29 species; 27.36%) (*Chaenomeles japonica*, *Prunus avium*, *Ribes aureum*, most forest species) and in the summer (29 species) (*Helianthus annuus*; most meadow species such as *Echium vulgare*, *Onobrychis viciifolia*, *Prunella vulgaris*, etc.). The species that bloom in the spring-summer period (*Robinia pseudoacacia*, *Rosa canina*, *Tilia cordata*, etc.) and those that bloom in the summer-autumn period (*Calendula officinalis*, *Carduus acanthoides*, etc.) have a significant share (17.92% and 16.03% respectively). A smaller number of species (12 species; 11.32%) bloom staggered from May to September (October): *Convolvulus arvensis*, *Lotus corniculatus*, *Medicago lupulina*, *Trifolium pratense*, etc. (**Table 2.**; **Tables 4-6. of the Supplementary Material**). In our opinion, this high flowering period means diversified food resources (nectar, pollen) for different groups of pollinators.

Characteristics of flowers. The flower, through its characteristics (color, smell, nectar secretion, pollen production, arrangement, shape, size) has a main role in attracting pollinating insects. In 12 species (11%) the flowers are solitary (*Convolvulus arvensis*, *Cucurbita pepo*, *Cydonia oblonga*, *Prunus spinosa*, etc.) and in 94 species (89%) the flowers are grouped in inflorescences such as raceme, corymb, umbel, anthodium, capitulum,

panicle, ament, spike, cyme (**Tables 4-6. of the Supplementary Material**).

Some inflorescences are looser (raceme, panicle), while others are well structured, compact (Asteraceae). Concering the *flowers' color* (shades of the main colors) of the species identified in the study area, the dominant color is white (33.96%) followed by yellow (28.30%) and red (12.26%). Green (10.37%) and purple (3.77%) colors are less represented (**Table 3.**). Pollinating insects have a certain visual system of color perception. It has been shown that there are differences in the color of flowers perceived by humans and that perceived by pollinating insects: the yellow color perceived by humans can be perceived as green by bees; white color (which reflects all the radiation of the visible spectrum) can be perceived by bees as blue-green (Chittka et al., 1994). Studies by different authors have found that some pollinator species have innate preferences for certain colors: bees prefer blue, flies prefer yellow and white flowers, lepidoptera prefer pink and red flowers, beetles white and cream and wasps prefer brown and yellow flowers. The pollinator can use color as a signal of floral reward (pollen, nectar) (Reverté et al., 2016).

Regarding the food resources offered to pollinating insects, in this paper only pollen and nectar were considered, although some species (*Acer* sp., *Corylus avellana*, *Tilia* sp.) provide bees with other and other products (manna). Most of the identified species are nectar-polliniferous (**Tables 4-6. of the Supplementary Material**). Nectar is an important food resource for many pollinators, being the main source of carbohydrates, but it also contains amino acids in variable proportions; minerals and fatty acids (in low amounts). It is considered the most important floral reward for attracting pollinators (Venjakob et al., 2022). Nectar production varies depending on several factors: species, position of flowers on the plant, flowering stage, external factors, etc. (Cîrnu, 1980; Jabłoński and Kołtowski, 2005). According to Venjakob et al. (2022), total carbohydrate content in nectar is high in *Trifolium campestre* and *Lotus corniculatus*; the content of amino acids in the nectar is high in the species *Centaurea jacea*, *Taraxacum officinale*; the essential amino acids in the nectar are in large quantities in *Prunella vulgaris* and in small quantities in *Trifolium campestre*, *Trifolium repens*, *Trifolium pratense*, *Vicia cracca*. Pollen contains protein, lipids, carbohydrates, minerals, vitamins and is a food source especially for the honeybee.

The melliferous potential. For the area under study, a large number of melliferous species (98 species) that provide nectar and pollen for the honeybee was highlighted. Species with medium melliferous potential are representative (44 species; 44.83 %) (*Echium vulgare*, *Cydonia oblonga*, *Cucumis sativus*, *Malus domestica*, *Salvia nemorosa*, *Zea mays*, etc.).

Tabel 3. Flowers' color of identified species

The flowers color	Shade of the main color	Number of species	Participation (%)
White	white pink, white yellow, greenish white	36	33.96
Blue	blue gray, deep blue, violet blue	7	6.60
Yellow	creamy yellow, yellowish, greenish yellow	30	28.30
Pink, red	purplish pink, bright red, purple	13	12.26
Green	Greenish, yellowish green	11	10.38
Violet	light purple	4	3.78
Multicolored		5	4.72

Following in descending order are the species with a small melliferous potential (23 species; 23.4%) (*Lonicera caprifolium*, *Lotus corniculatus*, *Medicago lupulina*, *Rosa canina*, *Satureja hortensis*, etc.) and those that provide food for bees (nectar, pollen) sporadically and for a short time (20 species; 20.40%) (*Centaurea jacea*, *Calendula officinalis*, *Dahlia* sp., etc). The species with high melliferous potential (*Brassica rapa*, *Onobrychis viciifolia*, *Acer campestre*, *Acer tataricum*, *Tilia cordata*, *Tilia platyphyllos*, *Trifolium repens*) and with very high melliferous potential (*Helianthus annuus*, *Rubus idaeus*, *Robinia pseudoacacia*, *Tilia tomentosa*) represent respectively 7.14% and 4.08%. Among the melliferous species identified in the study area that are characterized by a high polliniferous potential, the following are listed: *Corylus avellana*, *Prunus avium*, *Prunus cerasus*, *Pyrus communis*, *Prunus spinosa*, *Malus domestica*, *Taraxacum officinale*, *Acer tataricum*, *Onobrychis viciifolia*, *Trifolium repens*, *Rubus caesius*, *Rubus idaeus*, *Dahlia* sp., *Rosa canina*, *Zea mays*.

Regarding the botanical families with the most representatives identified in the study area, the following aspects can be specified. The species of the Rosaceae family are mostly woody plants, both cultivated (trees and fruit-bearing shrubs) and forest species, which bloom in spring or spring-summer and show white or white-pink flowers. These species are valuable because they provide nectar and pollen in the early period necessary for the reproduction and development of bee families and for production pickings. The species of the Asteraceae family are spontaneous as well as cultivated, with variously colored flowers (white, yellow, purple, blue), grouped in inflorescences, in some cases of large size (*Helianthus annuus*, *Dahlia* sp., *Tagetes erecta*, *Zinnia elegans*) and with long flowering period (summer, summer-autumn). They attract a wide variety of pollinating insects (bees, bumblebees, butterflies, hoverflies, etc.) (Jabłoński and Kołtowski, 2005; Rolling and Gouson, 2019; Michelot-Antalik et al., 2021). From the point of view of the melliferous potential among the cultivated species, the *Helianthus annuus* species stands out, cultivated on a large area in the study area. Among the spontaneous, valuable species are *Taraxacum officinale*, *Carduus acanthoides*, *Inula britannica*, *Cichorium intybus*, *Centaurea jacea* (Cîrnu, 1980, Jarić et al., 2013; Mačukanović-Jocić and Jarić, 2016). From the Fabaceae family, most of the species identified in the study area are specific to the meadow ecosystem, 66% are hemicryptophytes. It stands out for its long flowering period (**Tables 4-6. of the Supplementary Material**), with variously colored flowers (white, yellow, pink, red, purple) which represent valuable sources of nectar and pollen. Studies have shown that they attract different pollinating insects (bees, bumblebees, butterflies, etc.) (Jabłoński and Kołtowski, 2005; Venjakob et al., 2016; Michelot-Antalik et al., 2021). The species *Onobrychis viciifolia*, *Trifolium repens*, *Trifolium pratense*, *Vicia craca*, *Lotus corniculatus*, *Medicago falcata*, *Medicago lupulina* are considered very good plants that produce nectar and pollen (Cîrnu, 1980; Jarić et al., 2013; Mačukanović-Jocić and Jarić, 2016). *Robinia pseudoacacia* is known as a good nectariferous species; the estimated nectar production was between 1.6-3.7mg nectar/flower/day with a sugar concentration of 34-67% (Papadopoulou et al., 2018).

Conclusions

In the study area, 106 plant species were identified that provide food for pollinating insects. Of these, 41.50% are woody species

and 58.50% are herbaceous species. Most of the species identified in the study area have flowers grouped in inflorescence. All species bloom in the active season for pollinating insects. The flowers show a variety of colors (white, yellow, red, greenish, blue, purple and their shades), which is very attractive to pollinating insects (especially bees). The identified species represent an important resource for pollinators and thus contribute to maintaining the ecological balance of the ecosystems in the study area. They also support beekeeping in the area.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Material

Table 4. Plant species identified in meadows

Scientific name / Family	Period of blooms	Flower grouping	Flower color	Floral resource
Achillea millefolium L. / Asteraceae	VI - VIII	anthodium	white	NP
Achillea setacea Waldst. et Kit. / Asteraceae	VI - VIII	anthodium	white	NP
Agrimonia eupatoria L. subsp. *eupatoria* / Rosaceae	VI - VIII	raceme	yellow	N
Anthyllis vulneraria L. subsp. *polyphylla* (DC.) Nyman / Fabaceae	V - VIII	capitulum	yellow	NP
Arctium lappa L. / Asteraceae	VII - VIII	anthodium	purple	PN
Carduus acanthoides L. / Asteraceae	VI - IX	anthodium	red purple	NP
Centaurea jacea L. / Asteraceae	VI - IX	anthodium	pink	N
Chelidonium majus L. / Papaveraceae	V - IX	umbel	yellow	PN
Cichorium intybus L. / Asteraceae	VII - IX	solitary	blue	NP
Convolvulus arvensis L. / Convolvulaceae	V - IX	solitary	white	PN
Coronilla varia L. / Fabaceae	VI - VIII	umbel	white pink	PN
Dactylis glomerata L. / Poaceae	VI - VII	panicle	greenish	P
Echium vulgare L. / Boraginaceae	VI - VIII	cyme	blue	NP
Eryngium campestre L. / Apiaceae	VII - VIII	capitulum	greenish white	N
Eryngium planum L. / Apiaceae	VII - VIII	capitulum	blue gray	NP
Galium verum L. / Rubiaceae	VI - VIII	cyme	yellow	PN
Inula britannica L. / Asteraceae	VII - IX	anthodium	yellow	P
Lathyrus tuberosus L. / Fabaceae	VI - VIII	raceme	red	N
Lavatera thuringiaca L. / Malvaceae	VI - VIII	solitary	pink	PN
Linaria vulgaris Mill. / Scrophulariaceae	VI - IX	raceme	yellow	NP
Lolium perenne L. / Poaceae	V - IX	spike	greenish	P
Lotus corniculatus L. / Fabaceae	V - IX	umbele	yellow	NP
Medicago falcata L. / Fabaceae	V - IX	capitulum	yellow	NP
Medicago lupulina L. / Fabaceae	V - IX	capitulum	yellow	NP

Onobrychis viciifolia Scop. / Fabaceae	VI - VIII	raceme	pink	NP
Papaver rhoeas L. / Papaveraceae	V - VI	solitary	bright red	PN
Phleum pratense L. subsp. *pratense* / Poaceae	VI - VIII	panicle	greenish	P
Polygonum aviculare L. / Polygonaceae	VI - X	flowers: in fascicles	white pink	NP
Potentilla argentea L. subsp. *argentea* / Rosaceae	VI - VII	biparous cyme	yellow	P
Prunella vulgaris L. / Lamiaceae	VI - VIII	cyme	purple	NP
Rubus caesius L. / Rosaceae	V - IX	corymbe	white	NP
Salvia nemorosa L. subsp. *nemorosa* / Lamiaceae	VI - VIII	cyme	deep blue	NP
Salvia pratensis L. subsp. *pratensis* / Lamiaceae	V - VII	cyme	blue purple	NP
Scabiosa ochroleuca L. / Dipsacaceae	VI - VIII	capitulum	creamy yellow	PN
Medicago sativa L. / Fabaceae	V - IX	raceme	purple	NP
Taraxacum officinale Weber / Asteraceae	IV - VI	anthodium	yellow	NP
Teucrium chamaedrys L. / Lamiaceae	VI - VIII	cyme	purplish pink	NP
Thymus pannonicus All. subsp. *auctus* (Lyka) Soó / Lamiaceae	V - VIII	cyme	purplish pink	NP
Trifolium campestre Schreb. / Fabaceae	V - IX	capitulum	yellow	NP
Trifolium pannonicum Jacq. / Fabaceae	VI - VIII	capitulum	white	NP
Trifolium pratense L. subsp. *pratense* / Fabaceae	V - IX	capitulum	red	NP
Trifolium repens L. subsp. *repens* / Fabaceae	V - IX	capitulum	yellow	NP
Vicia cracca L. / Fabaceae	VI - VIII	raceme	purple	NP
Legend: N= nectar, P= pollen				

Table 5. Cultivated plant species

Scientific name / Family	Period of blooms	Flower grouping	Flower color	Floral resources
Armeniaca vulgaris Lam. var. *communis* Schübl. et Mart. / Rosaceae	III - IV	corymb/umbel	white	NP
Armeniaca vulgaris Lam. var. *amarella* (Rchb.) Buia / Rosaceae	III - IV	corymb/umbel	white	NP

Brassica rapa L. / Brassicaceae	V - VI	raceme	creamy yellow	NP
Calendula officinalis L. / Asteraceae	VI - X	anthodium	yellow	NP
Callistephus chinensis (L.) Nees / Asteraceae	VII - X	anthodium	multicolored	P
Chaenomeles japonica (Thunb.) Lindl. ex Spach / Rosaceae	IV	solitary	red	NP
Cucumis sativus L. / Cucurbitaceae	VI - IX	male flowers - in fascicles; female flowers are solitary	yellow	NP
Cucurbita pepo L. / Cucurbitaceae	V - IX	solitary	yellow	NP
Cydonia oblonga Mill. / Rosaceae	V - VI	solitary	white pink	NP
Dahlia sp. / Asteraceae	VII - X	anthodium	multicolored	NP
Gladiolus x *hybridus* C.Morren / Iridaceae	VII - X	raceme	multicolored	PN
Gleditsia triachanthos L. / Fabaceae	VI	raceme	greenish	NP
Helianthus annuus L. / Asteraceae	VII	anthodium	yellow	NP
Hemerocallis fulva (L.) L. / Liliaceae	V - VIII	raceme	orange	PN
Hosta plantaginea (Lam.) Asch. / Liliaceae	VII - IX	raceme	white	N
Hyacinthus orientalis L. / Liliaceae	III - IV	raceme	multicolored	NP
Lilium candidum L. / Liliaceae	V - VI	raceme	white	NP
Lonicera caprifolium L. / Caprifoliaceae	V - VI	cyme	white yellow	NP
Malus domestica (Suckow) Borkh. / Rosaceae	IV - V	raceme	pinkish white	NP
Ocimum basilicum L. / Lamiaceae	VI - X	cyme	white	NP
Paeonia officinalis L. / Paeoniaceae	IV - V	solitary	pink	P
Philadelphus coronarius L. / Hydrangeaceae	V - VI	raceme	white	NP
Prunus avium (L.) L. / Rosaceae	IV	corymb/umbel	white	NP
Prunus cerasus L. / Rosaceae	IV - V	corymb/umbel	white	NP
Prunus cerasifera Ehrh. / Rosaceae	IV	corymb/umbel	white	NP
Prunus domestica L. / Rosaceae	IV	corymb/umbel	white	NP
Pyrus communis L. / Rosaceae	IV - V	corymb/umbel	white	NP
Ribes aureum Pursh / Grossulariaceae	IV - V	raceme	yellow	NP
Rosa sp. / Rosaceae	VI - VIII	solitary	multicolored	NP

Rubus idaeus L. / Rosaceae	VI - VII	raceme	white	NP
Satureja hortensis L. / Lamiaceae	VII - X	cyme	white pink	NP
Symphyotrichum novi-belgii (L.) G.L.Nesom / Asteraceae	VIII - X	anthodium	violet blue	NP
Syringa vulgaris L. / Oleaceae	IV - V	raceme	light purple	NP
Tagetes erecta L. / Asteraceae	V - VIII	anthodium	yellow	NP
Vitis vinifera L. / Vitaceae	V - VII	biparous cyme	greenish yellow	NP
Viburnum opulus L. f. *roseum* (L.) Nyár. / Caprifoliaceae	V - VI	cyme	white	NP
Zea mays L. / Poaceae	VI - X	panicle	yellowish	P
Zinnia elegans Jacq. / Asteraceae	VII - X	anthodium	multicolored	PN
Legend: N= nectar, P= pollen				

Table 6. Plant species identified in forest

Scientific name / Family	Period of blooms	Flower grouping	Flower color	Floral resource
Acer campestre L. / Aceraceae	IV - V	corymb	greenish	NP
Acer platanoides L. / Aceraceae	IV - V	corymb	greenish yellow	NP
Acer pseudoplatanus L. / Aceraceae	IV - V	panicle	yellowish green	NP
Acer tataricum L. / Aceraceae	IV - V	panicle	yellowish white	NP
Cornus mas L. / Cornaceae	II - III	umbel	yellow	NP
Corylus avellana L. / Corylaceae	II - III	male flowers- catkins; female flowers - fascicles	yellow	P
Crataegus monogyna Jacq. / Rosaceae	V - VI	corymb	white pink	NP
Fagus sylvatica L. / Fagaceae	IV - V	male flowers- capitulum; female flowers - in pairs	greenish	NP
Fraxinus excelsior L. / Oleaceae	IV - V	panicle	greenish yellow	P
Ligustrum vulgare L. / Oleaceae	VI - VII	panicle	white	NP
Malus sylvestris (L.) Mill. / Rosaceae	V	solitary	white pink	NP
Populus alba L. / Salicaceae	III - IV	catkin	greenish	P

Prunus avium (L.) L. var. *avium* / Rosaceae	IV	corymb	white	NP
Prunus spinosa L. / Rosaceae	IV	solitary	white	NP
Pyrus pyraster (L.) Medik. / Rosaceae	IV - V	corymb	white	NP
Quercus petraea (Matt.) Liebl. / Fagaceae	V	male flowers- catkins; female flowers-spike	yellowish green	P
Quercus robur L. / Fagaceae	IV - V	male flowers- catkins; female flowers-spike	yellowish green	P
Robinia pseudoacacia L. / Fabaceae	V - VI	raceme	white	NP
Rosa canina L. / Rosaceae	V - VI	solitary	pink	NP
Sambucus nigra L. / Caprifoliaceae	V - VI	cyme	white	NP
Tilia cordata Mill. / Tiliaceae	VI - VII	cyme	pale yellow	NP
Tilia platyphyllos Scop. / Tiliaceae	VI - VII	cyme	pale yellow	NP
Tilia tomentosa Mnch. / Tiliaceae	VI - VII	cyme	pale yellow	NP
Ulmus glabra Huds. / Ulmaceae	III - IV	fascicle	greenish	P
Viburnum lantana L. / Caprifoliaceae	V - VI	cyme	white	NP
Legend: N= nectar, P= pollen				

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REDISCOVERING THE HISTORICAL GARDENS IN THE BANAT COUNTY

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Abstract: The study of non-historical monument gardens aimed to inventory and assess their current state compared to the original. These gardens were researched in detail, following the steps used for historical parks and gardens. Unclassified gardens were categorized using newly established criteria based on collected information. While there's less data about these gardens compared to historical ones, the primary goal of redevelopment is to recreate their era's ambiance while preserving existing landscape, dendrological, and architectural values.

Keywords: case studies, classified historical gardens, non classified historical gardens, historical garden research

Introduction

In the Banat region, there are various architectural-landscape ensembles, some of which are not categorized as historical gardens but hold local significance due to their architectural elements, vegetation, or historical context. These gardens, despite their smaller scale, contribute to the region's landscape culture and can be considered valuable visual and ecological assets.

In this paper we examine not only gardens listed as historical monument but also castles, mansions, and their associated gardens that may have the potential for monument status. The selection criteria included architectural coherence, condition, historical and stylistic analysis, as well as the natural heritage value. The research involved a comprehensive examination of old documents and family archives, specialized literature, maps, and postcards to piece together the history of these properties (Archives of the National Heritage Institute, 1974; Hungarian National Archives in Budapest; Timiş Real Estate Registration and Advertising Office; Office of Registry and Real

Estate Advertising Arad). Visual materials, including postcards and maps (Horváth H., 2010; Horváth H., 1998), helped provide insights into the historical landscapes. Cadastral records, military topographical maps, and historical maps from the Arcanum Digitheca military surveys, were also invaluable for understanding the development of the sites (Borovsky S., 1896). In total over 40 sites were visited, and 33 mansions/castles and gardens were selected for in-depth study. Among these, 10 were listed as historical monuments, while 23 remained unclassified, spanning across Arad, Timiș, and Caraș Severin counties.

The primary focus of this research was to reveal architectural elements and landscape arrangements that contributed to the historical and cultural significance of these gentry residences, regardless of their official classification. In some cases, valuable information was extracted from the work of Bicsok Zoltán és Orbán Zsolt (Bicsok Z. & Orbán Zs., 2015). During the site visits, a total of 23 gardens related to nobiliary residences were identified, with 16 in Timiș County, 4 in Caraș Severin County, and 3 in Arad County.

2. Materials and methods

After visiting the 23 gardens (**Fig. 1.**) associated with various castles and mansions, whether officially designated as historical monuments or not, we were able to form a comprehensive understanding of their present condition in relation to the historical information and documents (Hegedüs N. M., 2018) we unearthed during our research.

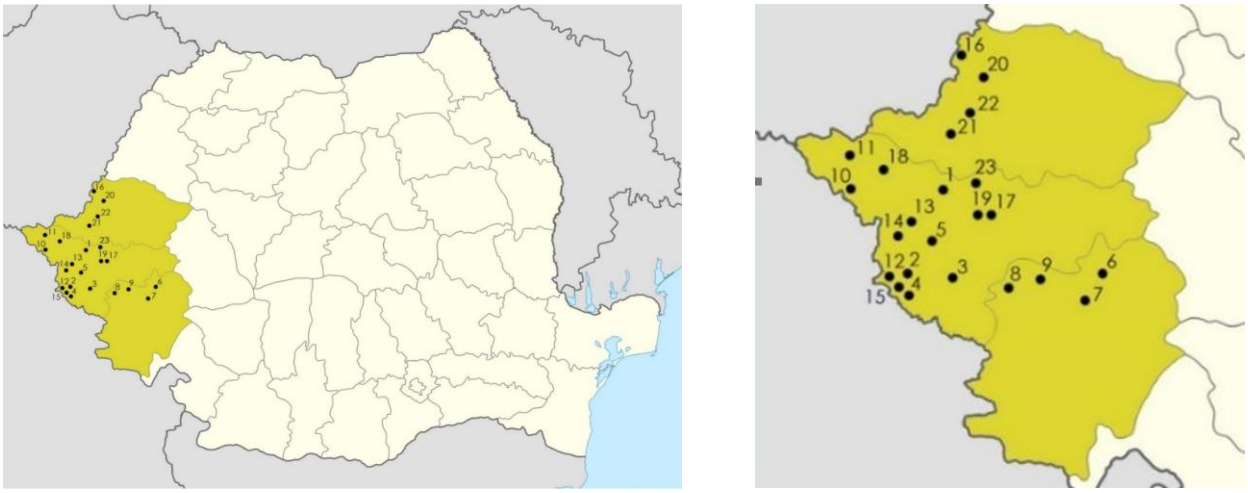

Fig. 1. Gardens not officially classified as historical monuments visited on-site:
(1) Carani-Timiș-Saurau Féger; (2) Rudna–Timiș - Residence Nikolics; (3) Folea–Timiș - Residence Beniczky; (4) Livezile–Timiș - Gyertyánffy Residence; (5) Șag–Timiș-Rónay Residence; (6) Ăgujeni–Caraș Severin - Jakabffy Castle – Juhász; (7) Delinești – Caraș Severin – Bródy Residence; (8) Ghertenis-Caraș Severin-Hollósi Manor Park; (9) Valeapai-Caraș Severin-Athanaszievich Castle Park; (10) Comloșu Mare – Timiș – San Marco Manor Park; (11) Sânnicolau Mare – Timiș – Nákó Manor Park; (12) Foeni –Timiș-Mocsonyi mansion park; (13) Beregsau Mic-Timiș-Damaszkin István Castle Park; (14) Cenei-Timiș-Uzbasich Manor Park; (15) Giera-Timiș- Park of the Gyetyánffy István mansion; (16) Grănicerii-Timiș-Csávossy Castle Park; (17) Izvin-Timiș-Park of the Ottlik Péter mansion; (18) Pesac-Timiș-Zichy Manor Park; (19) Remetea Mare-Timiș-Ambrózy Castle Park; (20) Șimand - Arad - Kintyig Castle Park; (21) New Arad-Arad-Nopcsa Castle Park; (22) Zimandu Nou-Arad-Kintzig Castle Park; (23) Murani-Timiș-Manaszy Barco Manor Park

The primary goal of our study was to highlight historical values that are at risk of disappearing, both in terms of their landscaping and architectural significance. Our aim is to reintegrate these discovered values into the architectural and landscape history of the region, preserving them as historical artifacts

We classified these gardens based on various criteria inspired by a range of sources (Fejérdy T., 2014; Fekete A., 2012; Fekete A., 2004; Marcus R., 1958; Milea A. P., 2088) that considered both their current and historical worth. We compiled an inventory of the gardens, drawing on written and visual information collected over time and cross-referenced this data with their current state. This information was verified through site visits, leading to a photographic documentation that compared their current condition with their historical appearance, reconstructed using historical information, military maps, and historical images.

Classification criteria

Our classification structure was divided into three main categories, each with its own set of subcategories. The classification of properties was made based on the following criteria:

I. General data:
- Current function
- Current owners
- Past owners
- The time of construction

II. Architectural, artistic and urban planning value:
- Valuable buildings, included in the list of historical monuments
- Adopted landscape typology

III. Memorial-symbolic value

Case Study

By understanding the real estate classification criteria used to evaluate architectural and landscape values, we successfully documented the cases we studied. However, owing to a lack of sufficient information and historical documentation to validate all the criteria listed and the fact that some of these gardens no longer exist, our focus shifted away from the original intent of inclusion in the list of historical monuments. Instead, we utilized this methodological framework as a basis for their general classification, adapting it based on the information gathered during our research.

I. General data

In this category we considered the general information about the building, where the accumulated information regarding the current function was taken into account, the identification of past and present owners (**Table 1.**), and a classification of the buildings was carried out according to the criterion of age, which was correlated with the date of the construction of the castle/mansion, because no exact data were found about the start of the landscaping of the gardens. Therefore the age criterion was divided in the following way:

- the building built before 1775 – being considered of exceptional value;
- the building built between 1775-1830 – being considered of very high value;
- the building built between the years 1830-1870 - being considered of high value;
- the building built between the years 1870-1920 - being considered of medium value;
- the building built between the years 1920-1960 - being considered of low value;
- the building built after 1960 – being considered to have zero value (**Table 2.**)

Table 1. General data about the property

	Current function	**Present owners**	**Past owners**
Carani-TM Saurau Féger	No function	Private	Claudius Florimond Mercy; 1780- count Johannes Saurau; 1805 - 1870 the Spanish Lo Presti family from Fontana d'Angioli (1804); 1870 -1874 count János Barinyai; Until 1931 Oskar Feger; It was nationalized and transformed into CAP, operating thus until 1989; After 1990, it was claimed by the descendants
Rudna – TM Nikolics residence	Home	Private Maria and Willie Radermacher	The Nikolics family-János III Nikolics and Todor Ivankovics; János III Nikolics; János IV Nikolics; János VII Nikolics; Peter IV; Fedor I Nikolics; Alexandru Lighezan; The Romanian state
Folea – TM Beniczky residence	No function	Private dr. Maria Goga	George Beniczky
Livezile –TM Gyertyánffy residence	Unknown function	Private Cristian M.	Familia Gyertyánffy-Lukács Gyertyánffy; László Gyertyánffy; Mária, Elisabeta and Gabriela Gyertyánffy; Statul Român; Maria Bogoiu
Șag – TM Rónay residence	No function	Unknown	Ronay family; Mihai Acxel de Zombor and Valentin Watz; Olah Miclos; Mihail Oexel; IAS- farm Olaru
Zăgujeni – CS Jakabffy – Juhász castle	No function	Private	Jakabffy Kristóf; Imre and Gyula Kopal; Jakabffy Elemér; Hermine von der Heydte; Juhász family
Delinești – CS Bródy residence	School	Local admin.	Aristides Manziarli; Emilia Cretin Manziarli; Pia and Aristia Manziarly; Bródy family
Ghertenis-CS Hollósi mansion	No function	Unknown	Hollósi from Gertenyes
Valeapai-CS Athanaszievich castle	No function	Private	Athanaszievich family -Marcel and Emil Athanaszievich; Daughter of Ioana Athanaszievich and Count Baich de Vărădia; Ambrozy Béla; The Riesz family - Petru Riesz
Comloșu Mare – TM San Marco mansion	Town hall	Local admin.	brothers Cristofor and Ciril Nako; count Ioan Nako; Mileva Nákó; The Romanian state
Sânnicolau Mare –TM Nákó mansion	House of culture-Museum	Local admin.	Nákó Kristóf and Cziril; Nákó Kálmán
Foeni –TM Mocsonyi mansion	House of culture	Local admin.	Mocsonyi family
Beregsau Mic-TM Damaszkin István castle	No function	Private Mucsalov family	Damaszkin-Simon family; Iván Mucsalov; The Romanian state
Cenei-TM Uzbasich mansion	Home	Private	Uzbasich family
Giera-TM Gyetyánffy István mansion	No function	Unknown	Gyertyánffy family

Grănicerii-TM Csávossy castle	Non-existent	Unknown	Csávossy family
Izvin-TM Ottlik Peter mansion	Horse stud	Horse stud from Izvin, Under the National Directorate of Forests Romsilva	Ottlik Péter
Pesac-TM Zichy mansion	No function	Unknown	Unknown
Remetea Mare-TM Ambrózy castle	No function	Private	Baronii Ambrózy; Bozsák Francisc; (UJCOOP)Federal Coop
Şimand – AR Kintzig castle	No function	Unknown	Unknown
Aradul Nou-AR Nopcsa castle	School	Local admin.	László Nopcsa; General Berthelot
Zimandu Nou-AR Kintzig castle	Cultural events	Private	Baron Kintzig
Murani-TM Manaszy Barco mansion	Home	Private	Manaszy family

Table 2. The construction age of the building

	Before 1775	**1775-1830**	**1830-1870**	**1870-1920**	**1920-1960**	**After 1960**
Carani-TM Saurau Féger	●					
Rudna – TM Nikolics residence		●				
Folea – TM Beniczky residence				●		
Livezile –TM Gyertyánffy residence		●				
Sag – TM Rónay residence						
Zagujeni – CS Jakabffy – Juhász castle		●				
Delinesti – CS Bródy residence			●			
Ghertenis-CS Hollósi mansion		●				
Valeapai-CS Athanaszievich castle			●			
Comloşu Mare – TM San Marco mansion		●				
Sânnicolau Mare –TM Nákó mansion			●			
Foeni –TM Mocsonyi mansion	●					
Beregsau Mic-TM Damaszkin István castle		●				
Cenei-TM Uzbasich mansion						
Giera-TM Gyetyánffy István mansion			●			
Granicerii-TM Csávossy castle						
Izvin-TM Ottlik Péter mansion				●		
Pesac-TM Zichy mansion						
Remetea Mare-TM Ambrózy castle		●				
Şimand – AR Kintzig castle						
Aradul Nou-AR Nopcsa castle		●				
Zimandu Nou-AR Kintzig castle				●		
Murani-TM Manaszy Barco mansion	●					

Table 3. Architectural and landscape value of the analyzed cases

	Valued buildings, included in the list of historical monuments	**The adopted landscape typology**			
Carani-TM Saurau Féger	TM-II-m-A-06192.				■.□.
Rudna – TM Nikolics residence	TM-II-m-B-06278		◊2.□.		
Folea – TM Beniczky residence				◊1.	
Livezile –TM Gyertyánffy res.					
Sag – TM Rónay residence				◊1.	
Zagujeni – CS Jakabffy Juhász castle	CS-II-m-B-11228		◊2.◊3		
Delinești – CS Bródy residence				◊1.□.	
Ghertenis-CS Hollósi mansion					◊3.
Valeapai-CS Athanaszievich castle	CS-II-m-B-11223				◊3.
Comloșu Mare–TM San Marco mans.	TM-II-m-B-06208			◊3.	
Sânnicolau Mare –TM Nákó mansion	TM-II-m-A-06287				◊3.
Foeni –TM Mocsonyi mansion	TM-II-m-A-06226				■.◊1.□
Beregsau Mic-TM Damaszkin István castle					◊3.
Cenei-TM Uzbasich mansion	TM-II-m-B-06197			◊1.	
Giera-TM Gyetyánffy István mansion					
Grănicerii-TM Csávossy castle					
Izvin-TM Ottlik Péter mansion			◊2.		
Pesac-TM Zichy mansion					
Remetea Mare-TM Ambrózy castle	TM-II-m-A-06276			◊4.□	
Șimand – AR Kintzig castle					
Aradul Nou-AR Nopcsa castle	AR-II-m-B-00568				
Zimandu Nou-AR Kintzig castle		◊4.			
Murani-TM Manaszy mansion	TM-II-m-B-21014.			◊4.□	

The age criteria were correlated following the date of construction of the castle/mansion since no exact data was found about the start of the garden arrangement around the constructions.

II. Architectural, artistic and urban planning value:

The architectural, artistic and urban value of these buildings was determined primarily by considering their presence on the List of Historical Monuments, after which their association with a specific historical era was taken into account, as they are representative of an author or of a specific style (the ■ symbol was used to mark this category). The second subcategory analyzed the landscape typology adopted as follows: valuable landscape components (the symbol □ was used to mark this category) and representativeness within a program or specific typologies (the symbol ◊ was used to mark this category) **(Table 3.)**. This evaluation was realized based on the position of the castle/mansion in relation to the studied land.

III. Memorial-symbolic value

When determining the memorial-symbolic value, the belonging of these buildings to certain personalities of noble rank was taken into account. The classification of these buildings is followed by a photographic documentary that encompasses all the accumulated visual information This collection includes historical maps and contemporary maps, vintage images, and images of the current situation. In some of the studied cases we have the opportunity to observe the evolution over time of these castles/mansions and their related gardens.

These gardens represent the artistic creations of noble families who were the driving force behind the establishment of both the castles and the gardens. In many instances, these nobles played a pivotal role in shaping the development of the localities where they erected their residences.

All the gardens and mansions we've examined hold significant historical and geographical importance. However, in most cases, we cannot discern a coherent landscape plan due to the disappearance of garden features over time. The original garden layout can be reconstructed in some cases through historical maps and vintage images.

Although there's undeniable evidence of a typology for these facilities in relation to the castle, the precise plan of the gardens is no longer discernible. In most cases, the only remnants of the vintage parks are tall vegetation found in specific areas on the site, lacking a clear, logical pattern, serving as a testimonial glimpse into what these landscapes once were.

■ **Representativeness for a historical era, author or style.**

Most of the studied gardens were made during the neoclassical style, belonging to the broader landscape style. However, we also identified gardens from the baroque and neobaroque periods. The classification of gardens in these stylistic periods cannot be achieved by examining the current condition due to their degradation or disappearance. It can only be accomplished through the analysis of photographs, postcards, cadastral maps or the description of these gardens in various specialized articles and books. It is known from the written documents and specialized articles studied that many of these gardens were designed by specialists brought from outside the country, especially by Austrian craftsmen and architects.

□ **Valuable landscaping components**

This criterion was based on the study of valuable visual landmarks, such as vantage points within the garden and outside it. For example: chapels, churches, representative buildings of the locality, etc., neatly located in relation to the position of the castle. It is also important to take into account the position of the construction concerning the surrounding environment, in most cases, these castles and mansions are strategically situated from an urban point of view in dominant positions, thus offering distinctive perspectives to and from the locality or area where they are located.

All these visual landmarks and valuable landscape elements are components of the landscaping. They give a certain character to the landscape, the locality and the place. These castles are visibly positioned at a higher elevation compared to the general built-up background of the locality, such as in the case of the castles from Carani, Delinești, Remetea Mare or Murani. In other cases, they are strategically situated in visual contact related to other architectural elements, like the visual connection between the castle and the village church, or between the castle and the chapel of the noble family. Such examples can be found in the case of the Rudna, Delinești and Foeni castles.

◊ **Representativeness within a program or typologies**

The current planimetric typology was examined, more precisely the nature of the landscaping in accordance with the position of the castle. Thus, we can distinguish the

following typologies of spatial organization:

◊**1**. The approximately central positioning of the castle in relation to the relatively rectangular land, thus having a vegetal surface of a significant size both in front and behind the castle. This typology is observed in the castles/mansions of Delinești, Folea, Cenei, Șag and Foeni.

◊**2.** Positioning the castle/mansion closer to the main boundary of the land, with a vegetal surface in front of the construction and with two or more sectors of landscaping of a different character behind it. The primary rear garden serves as a decorative vegetable garden in the immediate vicinity of the mansion, followed by a garden of a different character and function (agricultural or leisure) with annexed spaces and very little decorative vegetation. This typology is observed in the case of the castles/mansions in Rudna, Zăgujeni and Izvin.

◊**3**. Positioning the castle/mansion very close to the main property boundary, in the immediate vicinity of the street. This arrangement allows a large space for the decorative garden of the mansion, which also serves as the main access to the interior, this being the main facade of the mansion. This typology observed in the case of the castles/mansions in Zăgujeni, Valeapai, Ghertenis, Comloşu Mare, Beregsau Mic and Sânnicolau Mare.

◊**4**. Positioning of the castle/mansion centered on a land area of a larger size than those mentioned earlier, with an irregular planimetric shape. The centrally positioned construction is surrounded by high vegetation in abundance, both in front and behind it and to the side of the construction. This typology is exemplified by the castles/mansions in Remetea Mare, Zimandu Nou and Murani

When granting the qualification based on the criterion related to memorial-symbolic value, the ownership of the buildings by certain personalities of noble rank was considered (**Table 4.**).

Table 4. The memorial-symbolic value of the property (based on Lendvai M., 1911; Hungarian National Pocket Book, 1888; Nagy I., 1858; Reiszig E.,)

Carani-TM-Saurau Féger	Claudius Florimund Mercy, Contele János Saurau, Lo Prești de la Fontana Da Angioli, Groful János Barinyai. (Barinai Kempelen).
Rudna – TM – Nikolics residence	Nikolics noble family
Folea – TM- Beniczky residence	Beniczky noble family
Livezile –TM- Gyertyánffy residence	Gyertyánffy noble family
Șag – TM- Rónay residence	Rónay noble family
Zăgujeni – CS- Jakabffy – Juhász castle	Jakabffy noble family
Delinești – CS- Bródy residence	
Ghertenis-CS- Hollósi mansion	Hollósi de Gertenyes noble family
Valeapai-CS- Athanaszievich castle	Athanaszievich noble family, Ambrózy noble family
Comloşu Mare – TM- San Marco mansion	Nákó noble family
Sânnicolau Mare –TM- Nákó mansion	Nákó noble family
Foeni –TM- Mocsonyi mansion	Mocsonyi noble family
Beregsau Mic-TM- Damaszkin István castle	Damaszkin noble family
Cenei-TM- Uzbasich mansion	
Giera-TM- Gyetyánffy István mansion	Gyertyánffy noble family

Grănicerii-TM- Csávossy castle	Csávossy noble family
Izvin-TM- Ottlik Péter mansion	Ottlik noble family
Pesac-TM- Zichy mansion	Zichy noble family
Remetea Mare-TM- Ambrózy castle	Ambrózy noble family
Şimand – AR- Kintzig castle	Kintzig noble family
Aradul Nou-AR- Nopcsa castle	Nopcsa noble family
Zimandu Nou-AR- Kintzig castle	Kintzig noble family
Murani-TM- Manaszy mansion	Manaszy noble family

3. Results and discussion

The gardens associated with the manor houses we studied, some of which have disappeared entirely, while others still preserve valuable architectural and landscape elements, represent historical treasures with significant documentation sources. These sources include books, articles, and vintage postcards, which offer a somewhat clear representation of the historical landscape designs.

Of the 23 sites we visited, 16 were established between 1775 and 1830, forming a distinct series within a specific historical-geographical region and era. Each estate was once owned by prominent noble families, but today, they are mostly in private ownership, often by individuals unknown to the public or under the stewardship of the Romanian State, and used by local authorities. Through our study of these non-historical monument gardens, it became evident that none of these landscapes have survived entirely in accordance with the garden design of the original period.

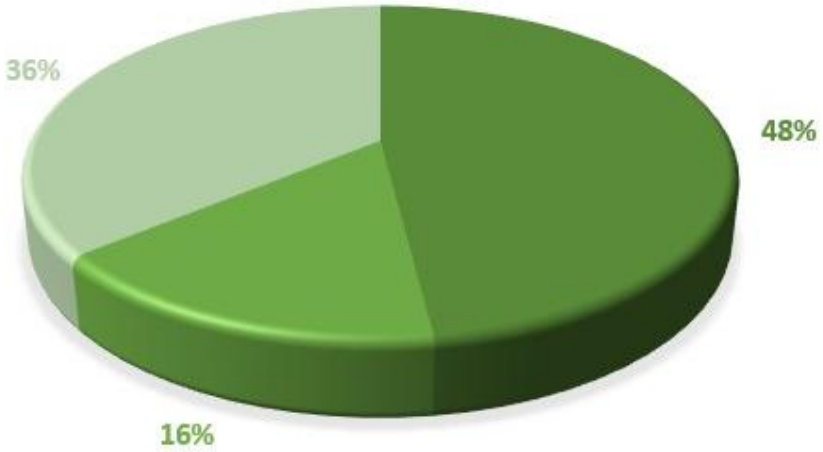

Fig. 2. The original footprint of the historical currently existing garden (Hegedüs N. M., 2018): 48% there are no traces of the landscaping of the original garden; 36% landscaping with plant elements and existing built elements - only tall vegetation positioned in certain areas of the site - less than 50% according to the landscaping model of the original period garden; 16% landscape arrangements with plant elements and existing built elements and currently ~ 50% according to the landscape arrangement model of the original garden; 0% existing landscaping entirely based on the original period garden landscaping model

In a significant number of cases, approximately 48% of the unclassified gardens, there are no visible traces of the original garden. These gardens lack any signs of historical vegetation arrangements, alleys, access pathways, or ancillary constructions that would provide evidence of their original design. Here are some notable examples:

- Gyergyánffy Castle Park in Livezile: The park features a contemporary ornamental rear garden with no signs of historical vegetation or period design. It is laid out in a contemporary style and lacks any vintage architectural elements or vegetation.
- Csávossy Castle Park in Grăniceri: The study couldn't be conducted due to the wild vegetation, indicating the possible loss of this castle.
- Mocsonyi Castle Park in Foeni: The park has been deforested, and there are no traces of its original layout, as indicated on vintage maps.
- Hollósi Castle Park in Ghertenis, Damaszkin Castle Park in Beregsău Mic, and Zichy Mansion in Pesac: These parks have high vegetation, but it is in a wild and neglected state and doesn't necessarily adhere to historical landscape logic.

Another group, approximately 36% of unclassified gardens, still have evidence of the original garden, albeit in an advanced wild state. However, they contain less than 50% of the landscape and plant elements compared to the vintage garden model. Notable examples include:

- Saurau Feger Castle Park in Carani: A Baroque-style park with symmetrical alleys, now in ruins concealed by unkempt vegetation.
- Ronay Castle in Șag: The vintage park has been lost, and the site is overgrown with nettles, with remnants of old trees on the boundaries.
- Bródy Castle Park in Delinești: The park is divided into two areas, one with tall vegetation and another in front of the building, resembling the period image. The latter is used as a school recreation park.
- Athanszievich Castle Park in Valeapai: There are traces of tall, wild vegetation behind the construction and in certain areas of the site.
- Nákó Castle Park in Sânnicolau Mare: The park was intensively restructured and now functions as an open-air theater, with traces of tall vegetation on the site.
- Kintzig Castle Park in Zimandu Nou: The park is spacious, well-maintained, and contains rare tree species, with evidence of tall vegetation reminiscent of the vintage park.
- Ottlik Péter Castle Park in Izvin: The park has new elements and architectural features, along with a field designed for horse riding and various annexes.
- Uzbasich Residence Park in Cena: The park's arrangement differs from vintage images, with an abundance of high and medium vegetation.

A smaller portion, around 16% of the unclassified gardens, still contain plant and architectural elements that resemble the original model. These parks have maintained their site structure according to the historical design. Examples include: Nikolics Castle Park in Rudna, Beniczky Castle Park in Folea, Jakabffy Castle Park in Zagujeni, Ambrózy Castle Park in Remetea Mare, Manaszy Barco Castle Park in Murani. These parks have preserved elements of their original layout and feature original structures like chapels and household annexes.

This segment of the analyses provides an inventory of manor house and castle gardens, both classified and unclassified as historical monuments. The study of unclassified gardens aimed to document their current condition compared to their original state, following a methodology similar to that used for classified historical gardens. However, it's important to note that the quantity of information available for unclassified gardens is significantly less than that available for historical monument gardens.

Conclusions

Throughout history, humans have shown a desire to play the role of the Almighty, attempting to change and improve the environment, which is inherently perfect and not amenable to lasting human-made alterations. The natural environment operates differently; when left undisturbed, it persists and develops naturally according to its own rules. Man-made gardens, structured and restructured, are created based on human concepts, visions, and contemporary fashions. Over time, they may deteriorate due to natural factors and changing trends in landscaping (Vais D., 2008).

This research focuses on the historical gardens associated with castles and manor houses belonging to noble families in the Banat region. The research delves into the evolution of these landscapes over time, examining them from historical, architectural, and socio-cultural perspectives.

The research targets specialists in the field engaged in development and restoration projects for historical monuments. By gathering historical documents and cadastral maps, the study provides these experts with insights into the original historical parks and the possibility of restoring them. The aim of the study is to foster a positive appreciation of historic gardens and monuments and addresses various stakeholders, including cultural heritage preservationists, local and national decision-makers, funding sources, planners, local communities, tourists, cultural institutions, and the commercial sector.

Case studies were conducted to analyze the current state and evolution of historic gardens in the Banat region. These studies followed a specific methodology, encompassing on-site research, archival investigation, and analysis of historical maps. The case studies, although diverse in terms of program and historical period (ranging from the 18th to the 21st century), revealed a relative stability in the state of the historic built heritage. Castles remained structurally unchanged, with only minor modifications to ancillary buildings in some cases.

The state of castles and their parks varies. Some are well-maintained and in constant use, while others have fallen into a state of advanced decay, both in terms of their built heritage and landscape.

Historical documents serve as a reliable source of information for specialists aiming to restore these parks. The study focused on historical gardens, which, although not classified as historical monuments, hold significant historical importance. The available historical and current information, while less extensive than that of listed gardens, is still valuable for understanding their past.

Rehabilitation or restoration of historic gardens should be preceded by thorough historical research. New interventions should complement and enhance the architectural ensemble, and the choice of the stylistic period should be informed by available historical data, ensuring the preservation of elements from different historical periods. The research provides a foundation for the rehabilitation of historic parks, considering historical context, regional trends, and the ensemble's new

functions. Flexibility is crucial, as gardens evolve over time, reflecting the ideas of different owners.

The ultimate goal of redevelopment is to evoke the atmosphere of the era in which the gardens were originally created while preserving existing landscape, dendrological, and architectural values. The techniques for rehabilitating historic gardens represent a complex subject, and the study suggests opportunities for further research and exploration. This research remains an ongoing theme, involving future interweaving in terms of historical documents, authenticity issues, restoration theories, and possibilities for the preservation and rehabilitation of historic gardens.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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THE ECOLOGICAL PERSPECTIVE IN NOWADAYS' URBAN LANDSCAPE PLANNING IN TRANSYLVANIA

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Abstract: Nowadays' (Transylvanian) urban landscape as a complex, built and natural, social and economic environment is undergoing a sudden and accelarated transformation. Urbanization is a defining feature of current spatial/urban metamorphosis in Transylvania, too, yet the current model of urban development profoundly alters the natural environment, often reducing biodiversity and ultimately threatening human wellbeing. Present not only in academic theory, but in a broader sense in the daily public debate for the past decades, ecological perspective has become one of the leading design principles in nowadays' professional practice and theory in urban and spatial planning, a compulsory attribute of the contemporary mindset and (urban) landscape. Proposed research on ecological perspective in current urban (landscape) planning in Transylvania, Romania is focusing specifically on the role ecological perspective can play in understanding, the applying the more general and complex phenomenon of sustainability in the planning and management of landscapes. The idea of sustainability, the ecoregional approach are not only traditional attributes of the historical landscape, but also generators in present day development of cities around Transylvania, Romania. The ecological approach can enhance finding solutions in urgent social and environmental challanges regarding efficient management of resources, sustainable urban and spatial planning.

Keywords: ecological perspective, sustainable city, regulation documentation, General Urban Plan, Local Urban Regulation, biodiversity and ecosystem functions

Introduction

Present not only in academic theory, but in a broader sense in the daily public debate for the past decades, ecological perspective has become one of the leading design principles in nowadays' professional practice and theory in urban and spatial planning, a compulsory attribute of the contemporary mindset and (urban) landscape shaped by concerns about environmental challenges (Fekete et al., 2021), the human's relationship to (natural) environment, climate warming etc. Proposed research on ecological perspective in current urban (landscape) planning in Transylvania, Romania is focusing specifically on the role ecological perspective can play in understanding, the applying the more general and complex phenomenon of sustainability in the planning and management of landscapes.

The idea of sustainability and the ecoregional approach are not only traditional attributes of the historical landscape, but also generators in present day development of cities around Transylvania, Romania (Ványolós, 2020). The (Transylvanian) landscape as a complex, built and natural, social and economic environment is undergoing a sudden and accelerated transformation. The ecological perspective can enhance finding solutions in urgent social and environmental challenges (Ványolós, 2020) regarding efficient management of resources, agricultural production, sustainable urban and spatial planning, public health safety etc. The ever shrinking natural environment, uneven demographic growth altogether with the excessive urbanization, prodigal resource-energy management, multiple pollution are all results of unsustainable development models (Ványolós, 2020). Sustainability, the ecological perspective in landscape architecture, urban and spatial planning are essential in the process of identifying a responsible and coordinated, mutually beneficial coexistence of human and natural habitats.

2. Materials and methods

Current section is structured in two distinct parts: a detailed vocabulary containing a brief, but necessary definition of keywords followed by the proposed methodology, altogether with tools and instruments for current research.

Vocabulary

Setting up a detailed vocabulary for conducting the research and materializing it in the present paper is one of the key elements in facilitating terminological clarity, and a certain objective perspective on the subject.

This vocabulary includes the following main keywords and their brief definitions:

ecological networks and greenways= linear open space established along either a natural corridor, such as a riverfront, stream valley, or ridgeline, or overland along a railroad right-of-way converted to recreational use, a canal, a scenic road, or other route, thus including ecological, recreational and cultural heritage aspects; *ecological compensation* and *ecological stability* of the landscape for human functions, *natural carrying capacity*, *self-purification capacity* are the basis for the ecological networks (Walmsley, 1995)

ecostablisation principle = a concept designed to make landscape suitable for human functions without degrading ecological stability and thus risking environmental quality (Jongman et al., 2004)

green/eco/sustainable city = a city designed as a *complex landscape* (Meurk and Swaffield, 2007), with a thorough consideration for a multiple- social, economic, environmental- impact and resilient habitat, without compromising the ability of future generations to experience the same

strategic/regulation/building documentations = the three basic, different types of technical documentation concerning urban/spatial planning process: the *strategic* character lays in the generic theory, the principles that guide urban/spatial development, the set of specific building and landuse rules are comprised in *regulation* documentations, while the building type of documentations deliver the project for materializing particular construction proposals

General Urban Plan (GUP) = represents the legal ground for any development action proposed, including rules with respect to urban planning matters on short term (delimitation of the buildable area, landuse regulation, delimitation of the area affected by public encumbrances, establishment of the protected zones and historical built areas etc.) and on medium and long term (ex.regulation for the natural risk areas, defining areas of temporary of definitive building interdiction, the list of the

main proposed developments, the delimitation of the areas where urban regeneration projects are intended to be performed)

Local Urban Regulation/Building Code = set of various- landuse, functional and building-regulations, as part of the General Urban Plan

biodiversity and ecosystem functions = the benefits of ecosystem, categorized into four types according to their role: provisioning (ex. providing products such as food), regulating (ex. processes that shape the environment, like the air or water purification), socio-cultural (recreation, spiritual services etc.) and supporting (ex. soil formation and nutrient recycling)

Ecosystem Services (ES) = the entirety of benefits the human population derives, directly or indirectly, from biodiversity and ecosystem functions

ecological perspective = a design/planning approach, a methodology based on the nature focused sustainability principles of the ecological integration theories which propose that natural systems, not designed landscapes should be integrated as support elements within existing urban contexts and processes (Jongman et al., 2004), so that a human environment more aware of nature, of natural resources would be facilitated; there are three main, distinct, but interconnected types of theories in contemporary urban design and planning (Heymans et al., 2019): beside the *ecological integration theories*, the *landscape structure theories* and the *design integration theories*- the first proposing that landscape systems, not the built environment, should be the organizing principle of urban design planning, while the second one proposing that designed landscapes should be integrated into the existing urban context and adapted to the existing urban structure; it is strongly connected to *environmental planning* criteria (Jongman et al., 2004)

network strategy= set of principles designed to conserve and restore *dispersal corridors* and *stepping stones* (habitat islands), which function as habitat structures between core nature areas and facilitate the *biological conductivity* in the landscape

Biologically Vital Areas (BVAs) = an indicator used to assess the environmental value of urban greenery, as well as a planning tool used to recommend its minimum level, referring to zones with ecological functions within cities, generally in green spaces, meant to help to counteract the negative impacts of built-up areas and impermeable structures on urban environments and city dwellers (Kimic et al., 2022)

Methodology

Research methodology consists of two main components: (I) a contextualizing of proposed subject within a review of selected relevant academic/ research literature and current legal framework regarding urban planning in Transylvania, Romania, (II) a general critical analysis of urban planning professional practice, concluded by preliminary findings and later exemplified in the case studies, in specific projects, planning documentations, their implementation and relationing to theoretical fundaments, including legal framework. The first component results in a set of criteria elements that will guide the second component, structuring it and help delivering the desired goals.

A general context for current research of urban planning and corresponding specific documentations is set in component (I), starting from the premise that an ecological approach/ perspective, a natural environment friendly design and planning, also as an organizing principle, is needed in dealing with current problems of the city according to concepts of contemporary new ethics and aesthetics (Mostafavi and Doherty, 2010). It considers the

city and urban landscape planning using multiple instruments and with a holistic worldview that is flexible both in scale and disciplinary focus, thus analyzing urban planning around the core terms "biodiversity and socio-ecosystem functions/ services" and the city from a systemic approach regarding it as dynamic, self-organizing entity (Heymans et al., 2019). The previously defined ecological perspective and related design and planning theories, altogether with the four identified ecosystem functions set up the following criteria elements for the analysis of selected, relevant urban planning projects:

- conservation and protection of existing natural infrastructure, of ecological network and greenway elements (Walmsley, 1995) in urban areas in proposed regulations
- the relationship between built and natural environment, the proposed (shrinking or expanding) urban built/ buildable area
- sustainability of landuse through provided recreational, regulating, provisioning and supporting ecosystem services
- promoting specific urban biodiversity (Swaffield, 2003) functions, increasing the value of BVAs to enhance the role of those areas within urbanized zones (Kimic et al., 2022), as part of a required ecological compensation or ecostabilisation (Jongman et al., 2004),

In component (II), in completion to scientific documents, legal framework represents an important literature resource for current research, scrutinizing urban planning process, in particular how the resulted projects and planning documents are related to legal framework, its theoretical basis, terminology and operational aspects. There are two distinct categories of laws and other similar documents that make up relevant legal framework. The most important elements of the first category are *Law nr.350/2001 of Urban and Spatial Planning* and *Law nr.351/2001 of National Territorial Development Plan*, while the second category contains laws from various different domains, like protection of the environment, of natural resources (*Law of protection of environment nr.137/1995, Law nr.107 /1996 of Water Resources, Law of green open public spaces nr.24/2007, Law nr.46/2008 of the Forestry Code* etc.).

A critical analysis of current urban planning practice in Transylvania, Romania, considering the detailed set of criteria, constitutes the main body of present research. Preliminary general findings then are exemplified in case studies, by focusing on specific project documentations, their implementation and relationing to theoretical fundaments, including legal framework.

Third generation (elaborated after 2010) of GUP documentations were analyzed, the four detailed case studies (Cluj-Napoca, Târgu Mureș, Odorheiu Secuiesc and Miercurea Ciuc) being a representative selection of GUP for cities from the three main categories (large, medium size and smaller city municipalities).

3. Results and discussion

A general analysis of GUP of 25 municipalities (out of the total 33) in counties of Transylvania, Romania, shows some preliminary findigs, common to most municipalities from all four types of cities:

- few clear, recognizible contemporary design and planning theory elements that strategic and regulation proposals are based on;
- insufficient correlation of relevant regulation and strategic documents (of all involved administrative units: the city and the surrounding villages);

- unsustainable landuse that is able to provide only some of all necessary ecosystem services and often is not facilitating enough desired ecological reconstruction of polluted former industrial sites or renaturation process in highly urbanized areas;
- incoherence or/and not enough clarity in applying the general legal framework and planning methodology- ex.lack of differentiation of regulations for the three basic types of urban development interventions and corresponding urban territorial regulation units (maintaining/protection, urbanization and restructuring areas);
- missing or insufficiently correlation between strategic goals and regulations (often no plan concerning the integration of the built urban body into a wider territorial ecosystem through a network of natural areas and other, inner blue and green infrastructure elements).

Above mentioned preliminary results are exemplified in the following in the four selected, relevant case studies:

case study #1:

The GUP of Odorheiu-Secuiesc (**Fig. 1.**, Experiment Proiect, 2016) is a showcase for the postsocialist period in Romania.

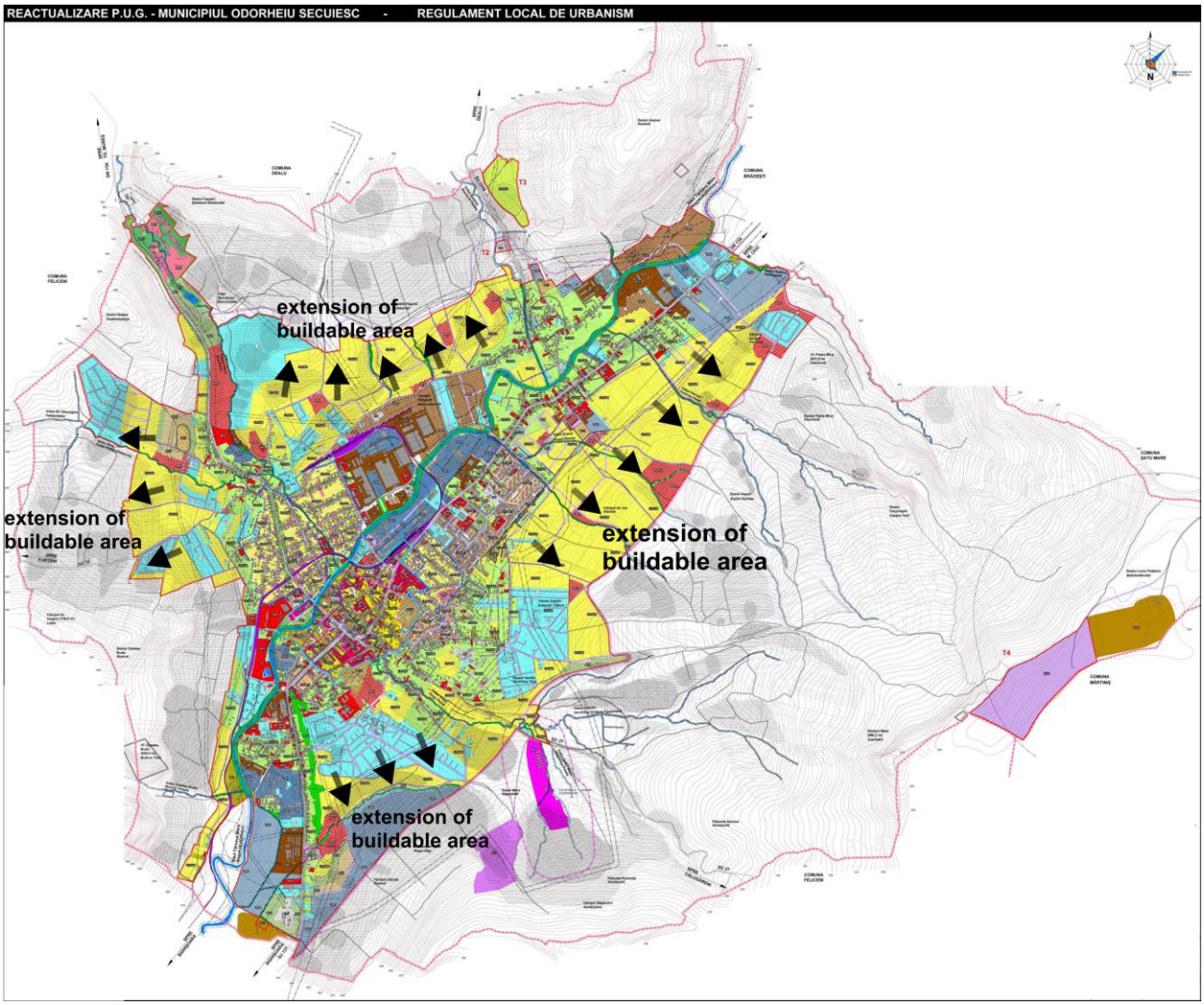

Fig. 1. GUP of Odorheiu-Secuiesc: unsustainable landuse and increase of the buildable area

It contains almost all the symptomatic characteristics of a loosely regulated urban planning simultaneously lacking necessary professional support, detailed legal framework and administrative capacity (Ványolós, 2020).

The proposed increase of the buildable area (with more the 60%, from approx.1085 ha to 1783 ha), in contrast with the demographic decrease and the lack of a significant building pressure, is the most evident proof of an unsustainable land management, that directly affects the wider urban and natural landscape by potentially polluting it not only visually, but in a more complex and lasting way becoming a threat to natural habitats, soil quality, underground water reserves. Insufficient regulation provisions promoting biodiversity (no specific regulations regarding permitted, recommended local species of vegetation or ecological corridors for protected habitats etc.). Furthermore, an expanded built area usually means a more costly and a less efficient infrastructure: road, sewage, water supply, electricity, waste management etc. networks. This excessive increase, combined with an undifferentiated, unphased implementation of the urbanization process lacking essential recreational and provisioning services (new blue and green infrastructure elements, public services etc.), is likely to result in time in a chaotic, unsustainable and ultimately polluting landscape management.

Though lacking overall ecologically rooted urban planning approach, with no clear contemporary design or planning theory fundaments, there are some positive elements, like the proposed green/ecological corridor along the Târnava Mare riverside or regulations for protected natural areas (within the existing legal framework).

case study #2:

Cluj-Napoca has a unique, singular position in the regional context, being the largest city in Transylvania and the one that had undergone probably the most significant and visible landscape changes in the past two decades in Romania, given its special, distinct topography and thus its sprawling expansion on the neighboring hills and along the Someș river valley (Ványolós, 2021). The complex and very much limiting context of its current GUP (**Fig. 2.**, Technical University of Cluj-Napoca, 2014) stems from the spatial planning proposals of the previous similar plan (1998), which set the conditions for the highly extensive and often insufficiently regulated urban development in the following decades, materialized in the previously mentioned urban sprawling.

The proposed increase of the buildable area (approx.10%, from 9867 ha to 10465 ha) is sizeable, but mostly answering existing functional-spatial needs and a building pressure, partially justified by the city's social-economic role on regional and national level. It contains several sustainability planning components shaped by an ecological perspective: it does foresee ecological corridors within the main city body connecting and protecting important natural habitats (but without delivering a list of recommended local vegetation species) also as new elements of the green and blue infrastructure, thus providing some of the required recreational and provisioning services (ex. see Rethinking Someș international design and planning competition, 2017), different measures to counter climate change effects (identifying and limiting heat islands).

Despite having used certain elements of a contemporary ecological planning perspective and design integration theories, the General Urban Plan falls short on considering the larger metropolitan area as an operational landscape unit.

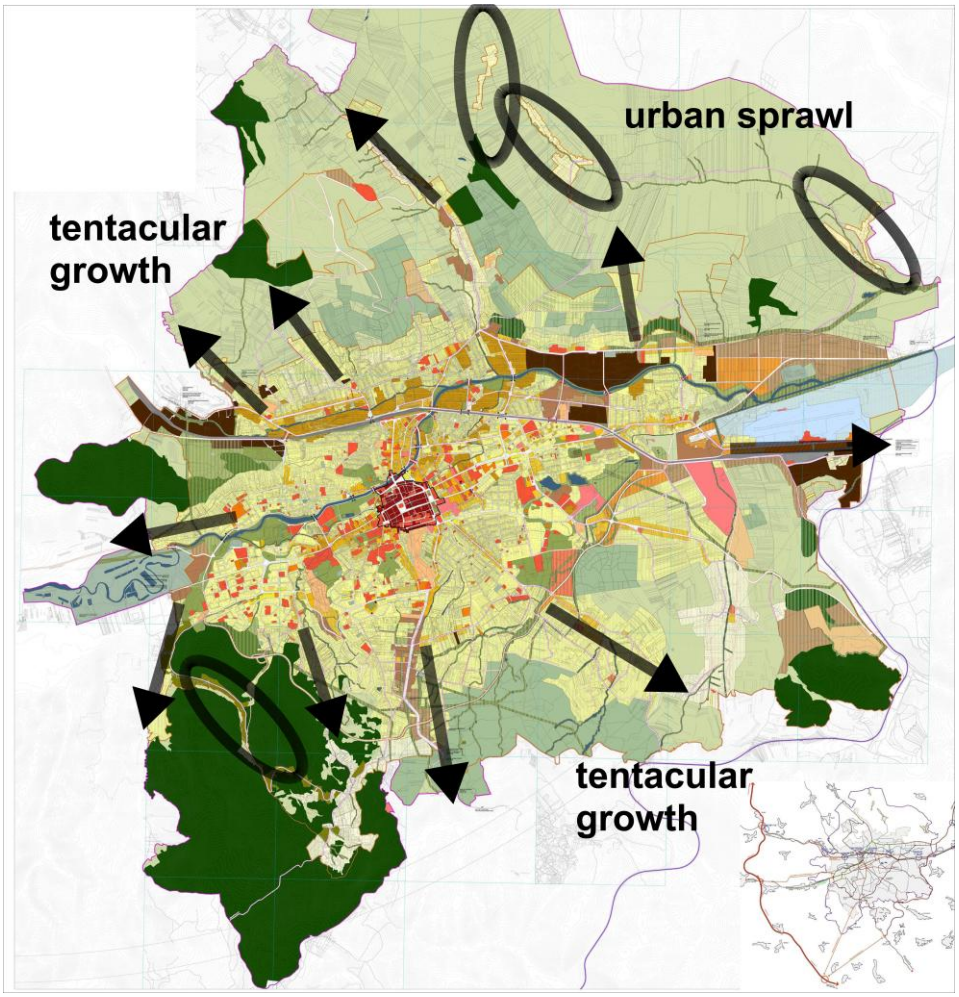

Fig. 2. GUP of Cluj-Napoca: sprawled and tentacular urban development

As a consequence there is no necessary correlation with the development of neighboring settlements (ex. Florești, Apahida etc.), not recognizing and not tackling the risks, the consequences of a generalized increased urbanization process and territorial-spatial polarization phenomenon (continuous overbuilt, urbanized zones vs. shrinking rural areas), and ultimately neglecting the historical landscape of a balanced network of rural and urban settlements.

case study #3:

The case of Târgu Mureș is relevant for medium sized cities in Transylvania, most of them not only administrative centres of their respective counties, but regional urban poles, too. Though the recently approved GUP (**Fig. 3.**, Arhigraf, 2022) keeps the current buildable area almost unchaged (an increase of approx.1%, to 3719 ha), it does confirm the overall increase of approx.15% since 2014. Beyond the unsustainable land increase, almost generally valid for GUPs of most cities in Transylvania, there are some other problems of urban planning in medium sized cities (previously detailed in the preliminary findings) that can be identified in the GUP of Târgu Mureș. An insufficient correlation of relevant regulation and strategic documents - ex. with the GUP of neighboring administrative units of Corunca and of Sâncraiu de Mureș- could be one them.

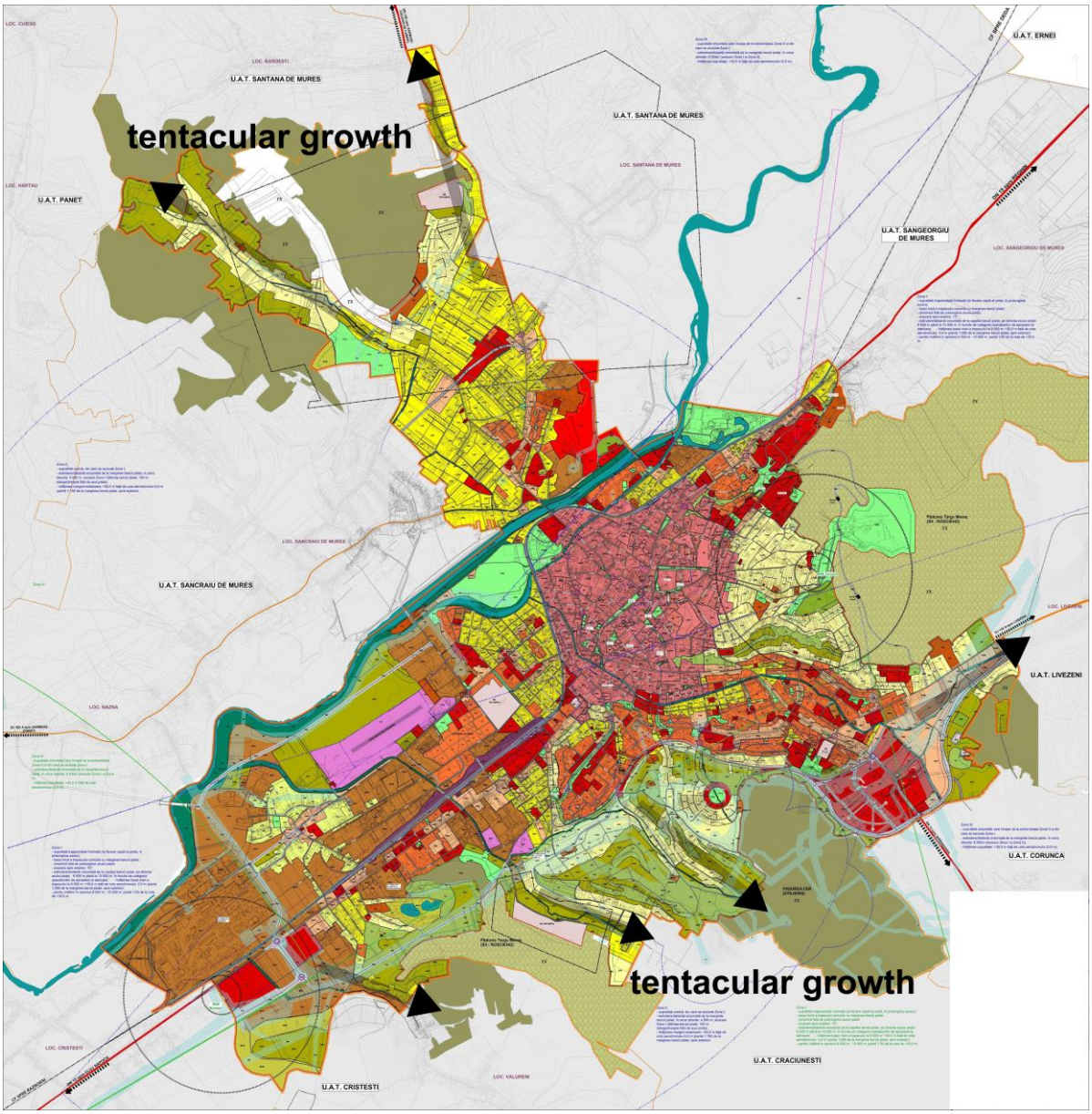

Fig. 3. GUP of Târgu Mureș: tentacular urban development

The often occuring/recurring problem - an incomplete differentiation of regulations for the three basic types of urban development interventions and corresponding urban territorial regulation units (maintaining / protection, urbanization and restructuring areas) - is also present. In many existing or proposed urbanized areas, only few of the compulsory public services and new blue and green infrastructure elements (ex. urban parks, public gardens, sport and leisure facilities etc.) are to be found. Similarly, there are no regulations regarding recommended local species of vegetation.

A separate general conceptual plan (tranpositioned in the urban regulation plan) would be welcome to illustrate the desired integration of the built urban body into a wider territorial ecosystem through a continuous and consistent network of natural areas and other blue and green infrastructure components (Ványolós and Lihăt, 2021).

Several encouraging design and detailed regulation elements can be found in the GUP, despite the shortcomings,: as part of the

ecosystem services, specific provisions for enhancing open space in highrise residential areas erected in the socialist/ postsocialist period (greener car parking, protection, increase and development of vegetation surfaces, green roofing for better energy efficiency and also promoting urban gardening as a feature of the provisioning, regulating and supporting ecosystem services etc.), a generic regulation concerning urban rainwater management, distinct, special regulations for the ecological reconstruction of the landscape of some damaged, polluted former industrial sites (ex. former brick manufacturing platform) or that of designed to compensate the transfer of agricultural land to the buildable area while maintaining a land reserve for provisional ecosystem services (ex. agricultural prodution for local consumption needs).

case study #4:

The GUP of Miercurea Ciuc (**Fig. 4.**, Planwerk, 2012) is one of the few urban planning documentations in Romania for the last 30 years that proposed a reduction of the buildable area (with 9%, to approx. 1790 ha). As the title is stating it, the GUP considered an ecological planning approach combined with elements of design integration and overall landscape structure tehories, resulting in a strategic and regulation framework for a sustainable urban development based on the concept of the green city well anchored in a local, regional context. The ”Green city in the heart of Seklerland” formula is a synthesis of a multitude of qualities and potentials of the city, meant to support and guide its development. Miercurea Ciuc is not only an administrative centre, but also of a specific geographical, cultural-spiritual landscape.

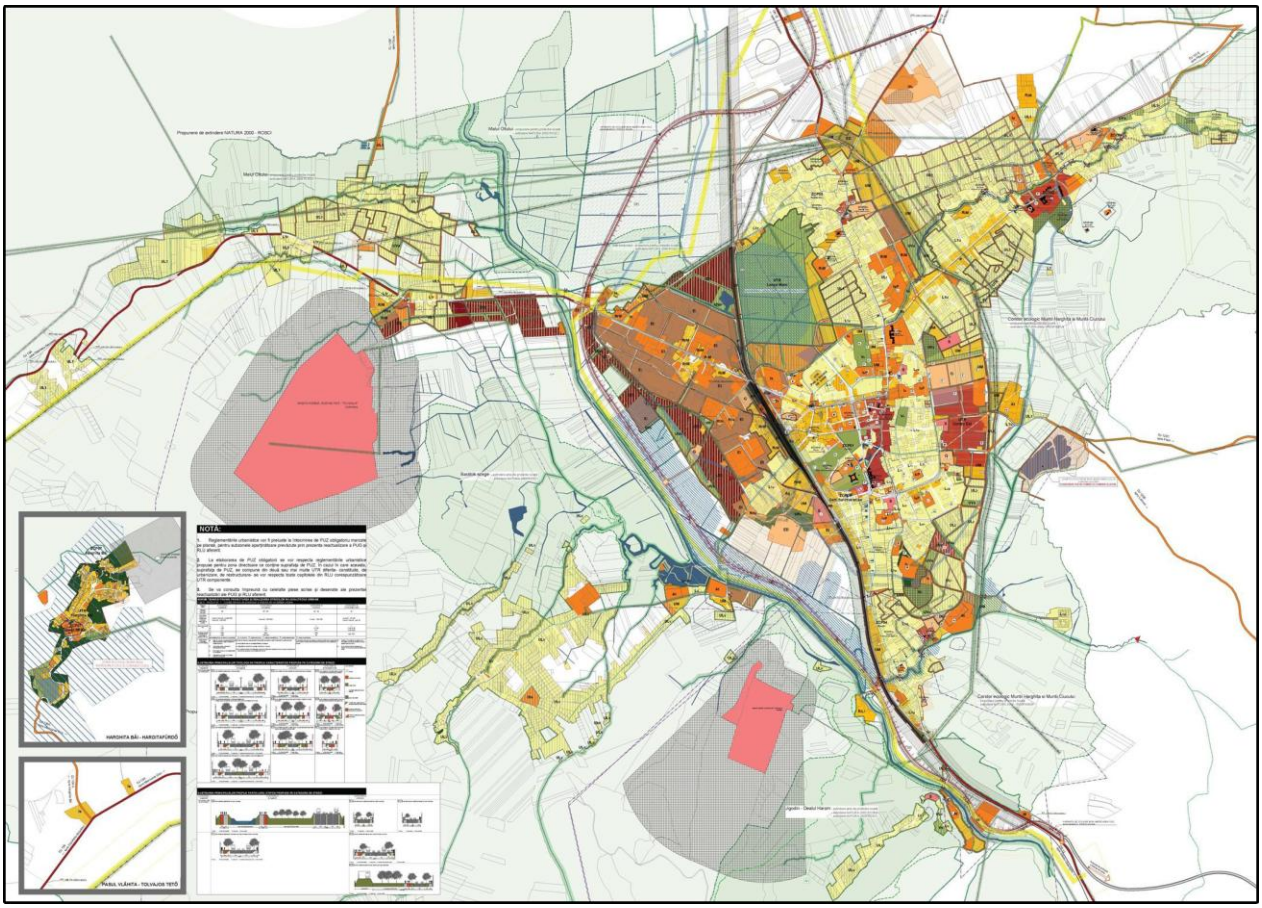

Fig. 4. GUP of Miercurea Ciuc: a framework for a sustainable urban development

Extending the urban green infrastructure with of new elements is among the main objectives of the GUP.

The spatial-functional reconfiguration promoted a more efficient landuse, limiting possible overlapping of contradicting activities and thus having an enhanced functioning and diversification of connections between different zones, with emphasis on new pedestrian and cycle routes.

The green character remains indispensible for both major functional components of the city profile, living an tourism. An urban development, building from the center to the peripheries represents the main principle for an efficient and rational, a sustainable spatial management, keeping the land resource available for future generations, too. Three main components make up the core idea of a sustainable land management: (I) brownfield type development in inner urban zones that need functional restructuring and/or ecological reconstruction comes first, before the greenfield type development at the outskirts, (II) any increase of the buildable area will be considered on the basis of data containing an evaluation of land needed for proposed development, (III) any extension of the buildable area will be phased, when densification criteria of inner urban zones is met. The optimization of the spatial scheme is the result of the synthesis of complex data and conclusions from various preliminary studies (demographic, social, economic, environment etc.).

Miercurea Ciuc is a green city given the omnipresence of neighboring natural landscape within the city, the two main mountains (Ciucului and Harghitei) are dominant elements of urban perspectives, working as backgrounds for all defining views. Natural landscape has a pivotal role in the identification of the local urban brand.

Fig. 5. GUP of Miercurea Ciuc: concept of optimized landuse and reduction of the buildable area

As illustrated in a schematic concept plan (**Fig. 5.**), the GUP counters the risk of urban sprawl, of disolution of the built city body in the surrounding natural landscape, instead integrating it organically through a complex network of blue and green infrastructure elements (park, gardens, sport-leisure surfaces, ecological corridors, protected areas and protection zones etc.), that delivers three of the ecosystem services (provisioning, regulating and supporting) to provide conditions of wellbeing in the city.

This concept plan has three main components: (1) the newly proposed Lunca Mare natural park and the ecological corridor, a green walkway along the Șumuleu stream between the Șumuleu hillside natural area and the Olt riverbank, (2) the middle green belt linking the Olt riverbank to the Șuta lakeside, and (3) the green corridor between the Șuta lakeside and the protected natural area of Jigodin-Csihányos, through the central urban zones and the Olt riverbank.

Although in the implementation phase local administration could not stick to the strict idea of buildable area reduction and the corresponding more efficient landuse, most strategic and regulation proposals of the GUP regarding ecological perspective are still valid: new elements of the blue and green infrastructure providing socio-cultural, regulating and supporting ecosystem services, the detailed regulations for protected natural areas within the existing legal framework, a list of recommended local vegetation species.

Conclusions

As main findings of the current research regarding the ecological perspective in nowadays' urban (landscape) planning in Transylvania, the following can be concluded:

- few clear, recognizible contemporary design and planning theory elements that strategic and regulation proposals are based on
- insufficient correlation between relevant strategic and regulation planning documents involved on the horizontal (neighboring settlements) and on the vertical (county, regional and national) administrative level
- difficulties, inconsistencies in the implementation phase due to sometimes unclear, not precise enough monitoring provisions in the legal framework
- in most GUPs detailed regulations for protected natural areas, habitats, in line with the existing legal framework
- unsustainable land management: in most cases a significant increase of the buildable, in contrast with the demographic decrease and the lack of a significant building pressure (the GUP of Miercurea Ciuc remains an exception)
- in some cases, new socio-cultural (recreational) and regulating services provided through proposed regulation in the newly urbanized areas, while in very few cases other ecosystem services (provisioning and supporting)
- in few cases separate, detailed and localized urban biodiversity provisions

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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A MINI REVIEW ON BIOTECHNOLOGICAL POTENTIALS OF BIOACTIVE COMPOUNDS AND BIOPRODUCTS ISOLATED FROM CYANOBACTERIA

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Abstract: Cyanobacteria are well-distributed, because of their ability to acclimate to various environments. Recently, cyanobacteria have received more research attention due to increasing pollution problems and global warming. They have many potential applications in the biotechnology sectors such as pharmaceuticals, bioplastics production, and cosmetics. Cyanobacteria produce many biologically active compounds that are utilized as anti-inflammatory, antiviral, antibacterial, and antifungal agents. The bioactive metabolites extracted from cyanobacteria include alkaloids, fatty acids, lipopeptides, and amides. In this minireview, the potential of some biotechnical applications are summarized to provide an account of the recent advancements in cyanobacteria research.

Keywords: Cyanobacteria, biotechnology, bioactive compounds, antimicrobial compounds, bioplastics, cosmetics

1. Introduction

Cyanobacteria is one of the most common microorganisms that live in different ecosystems on this planet. These microorganisms have been able to survive in environments such as exposed rocks, highly saline waters, polar regions, hot springs, arid deserts, and other extreme environments, and can form symbiotic relations with various organisms (Hu et al., 2012; Kumar et al., 2019; de la Cruz et al., 2020). Cyanobacteria are among the most important organisms that produce biomass, as they play a main function in the biogeochemical recycling of elements in the environment (Kumar et al., 2015; Van Goethem and Cowan, 2019), such as the nitrogen and carbon cycles, and many applications of biotechnology such as a biofuel, biofertilizer, bioplastics production, bioremediation, secondary metabolites production, pigments, and nitrogen fixation

(Shih et al., 2013; Garlapati et al., 2019; Yong et al., 2021). Cyanobacteria are considered as a significant source of metabolites that are mainly used as biopesticides, toxins, pharmaceutical compounds, cosmetic compounds, and growth factors (Al-Haj et al., 2016; Hassan et al., 2022), as shown on **Fig. 1**.

In recent decades, one of the major challenges that the healthcare system may face is the emergence of multi-drug resistant (MDR) bacteria, the cause of which is attributed to the excessive use of antibiotics by humans. As a result of the increase in resistance, there has become a problem for effective treatment using antibiotics, and therefore there has been an urgent need for research and exploration of new sources of antimicrobials (Laxminarayan et al., 2013; Strieth et al., 2022). Cyanobacteria are a significant unexplored source of several new bioactive compounds (Encarnação et al., 2015; Nuryadi et al., 2020).

Cyanobacteria are among the most powerful and unconventional sources of drugs against many diseases (Swain et al., 2015). Many bioactive metabolites have been isolated from cyanobacteria, which have demonstrated the potential for further more drug exploration (Mazard et al., 2016; Lange et al., 2018; Shishido et al., 2019; Schwarzenberger et al., 2020; Hassan et al., 2022; Lamare and Chaurasia, 2022; Yadav et al., 2023). Many secondary metabolites are produced by large multienzyme complexes, usually either nonribosomal peptide synthetases (NRPSs), polyketide synthases (PKSs), or PKS-NRPS hybrids, where large multienzyme complexes modify and assemble individual peptides into a single active molecule (Welker et al., 2012). Cyanobacteria produce a wide range of bioactive compounds such as polyketides, polysaccharides, alkaloids, lipids, carotenes, fatty acids, vitamins, phycocyanin, and proteins, which possess many characteristics such as antiviral, antifungal, antibacterial, algicidal, anti-inflammatory, anti-aging, and anticancer activity (Mimouni et al., 2012; Demay et al., 2019; Verma et al., 2022; Yadav et al., 2023). Cyanobacteria include many different orders, with filamentous and colonial cyanobacteria being among the most productive sources of natural products (Mazard et al., 2016). Jones et al. (2011) found that the production of filamentous cyanobacteria of the total known secondary metabolites is about 26% of the production, which belongs to the genera *Lyngbya*, *Nostoc*, *Microcystis*, *Oscillatoria*, and *Anabaena* (van der Merwe, 2015). Niveshika et al. (2016) recorded the appearance of the compound EMTAHDCA extracted from the cyanobacterium *Nostoc* sp. MGL001, which showed antibacterial activity at a concentration of 150 µg/mL.

Cyanobacteria blooms increasingly worldwide, posing a major threat to aquatic ecosystems and humans (Zhang et al., 2022). The cyanobacteria bloom causes hypoxia in aquatic environments, where the cyanobacteria accumulate, die, and decompose, resulting in the emergence of toxic compounds such as hydrogen sulfide (H_2S), and others (Huang and Zimba, 2019), which causes changes to the structure of the microorganism community and the resulting impact on animal and plant organisms (Liu et al., 2009). Many human health risks are associated with direct or indirect exposure to toxic compounds resulting from the reproduction and blooming of cyanobacteria. The health issues include mouth ulcers, acute inflammation of the stomach and intestines, skin rashes, shortness of breath (Gallitelli et al., 2005), vomiting, diarrhea (Codd et al., 2020), headaches, nausea (Thawabteh et al., 2023), eye and ear infections (Lévesque et al., 2016), and may cause cancer (Žegura et al., 2011; Zhao et al., 2013; Hernandez et al., 2021). Dermal exposure to toxic cyanobacterial compounds causes many symptoms, including skin irritation, which

ranges from mild to moderate, in addition to skin allergy in some individuals (Nielsen and Jiang, 2020). Pilotto et al. (2004) found that there was a small percentage, about 20% of healthy people, who developed skin reactions caused by cyanobacteria as a result of ordinary water recreation, and this reaction did not require any treatment because it was mild. Many skin-related problems have been reported with occupational or recreational exposure, including skin rashes, irritation, sores, peeling, swelling, and allergies resulting from contact with water containing toxic compounds of cyanobacteria (Stewart et al., 2006). The present article provides an overview of the biotechnological applications of cyanobacteria and their diverse uses in pharmaceuticals, cosmetics, and bioplastics.

2. Bioactive compounds produced by cyanobacteria

Cyanobacteria is one of the most important living organisms as a source of natural products, as it is capable of producing a number of bioactive compounds, as it is considered a modern and rich source of these compounds (Demay et al., 2019; Kini et al., 2020; Nowruzi 2022a).

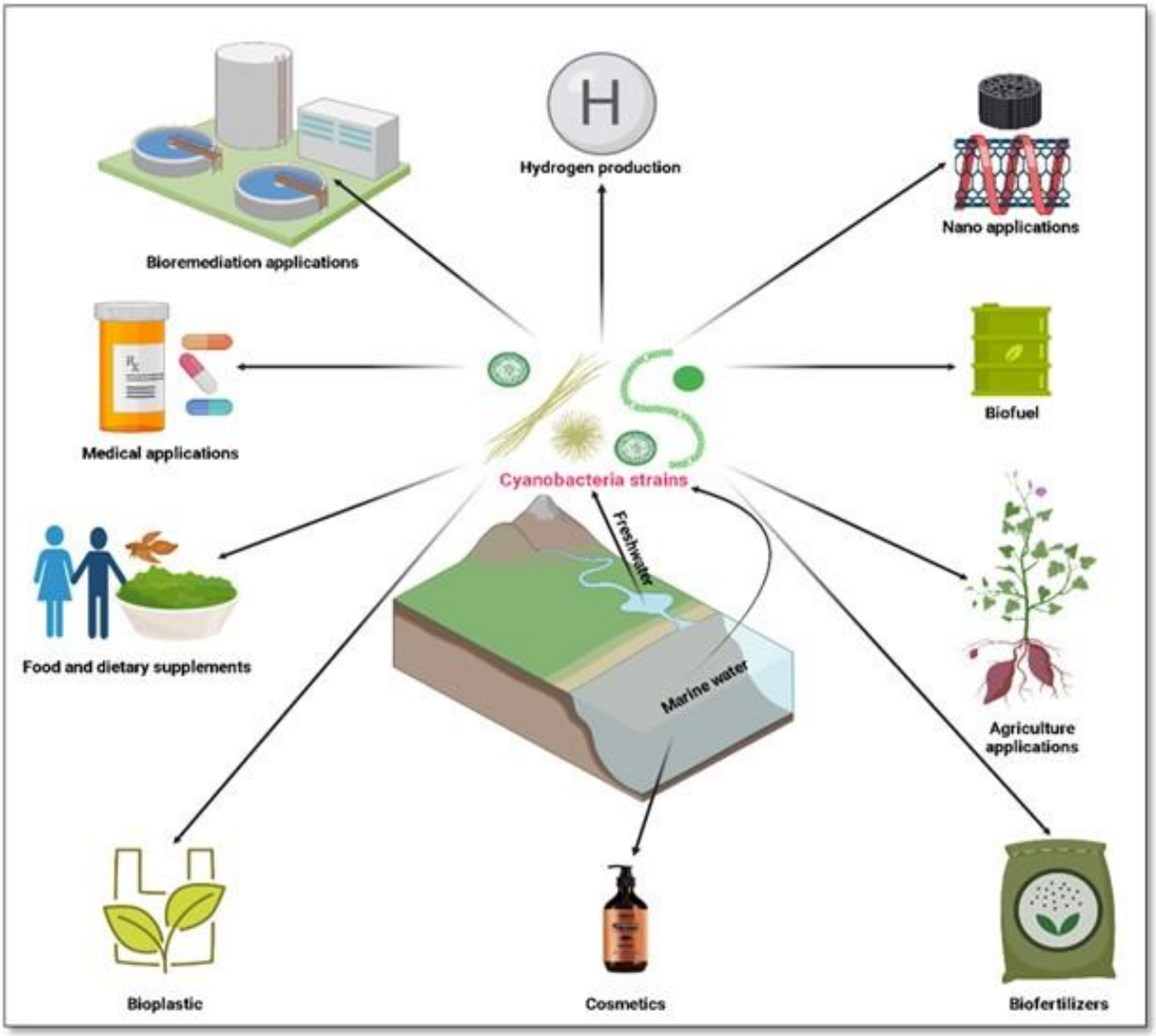

Fig. 1. Biotechnological applications of cyanobacteria

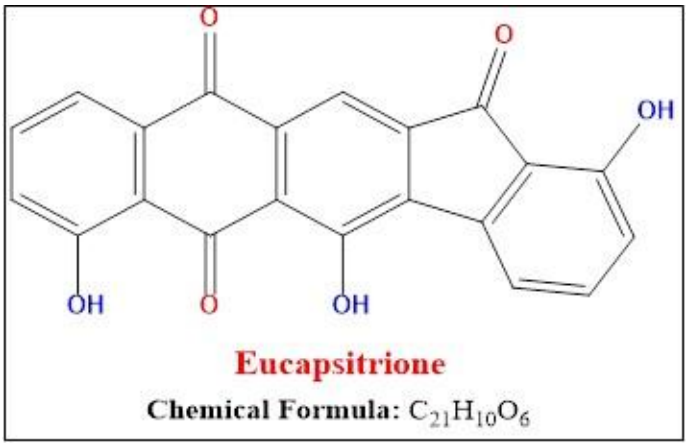

Fig. 2. Structure of Eucapsitrione compound derivative produced by the cyanobacterium *Eucapsis* sp.

Several studies and literature reviews have shown that there are about 19 strains of cyanobacteria that can produce more than 20 bioactive compounds, with most of these compounds tending to be lipopeptides (Abed et al., 2009).

Bioactive compounds are usually effectual against tissues, cells, and organisms at low concentrations, either beneficially or detrimentally to these organisms, and may cause harmful or beneficial effects on humans and other organisms.

Cyanobacteria produce some antimicrobial compounds and these compounds are used in the food industry and food conservation (Sung et al., 2013; Sun et al., 2016). Cyanobacteria can produce about 85 groups of secondary metabolites, which exhibit strong antimicrobial activity (Singh et al., 2016). Eucapsitrione (**Fig. 2**) is an anthraquinone-derived molecule which isolated from the *Eucapsis* sp. (Sturdy et al., 2010). A brief details on the bioactivity of cyanobacteria is given below.

2.1. Antibacterial activity

During recent decades, an alarming rise in antibiotic-resistant bacterial strains has been reported (Falaise et al., 2016; Hamdani et al., 2020). For this reason, alternative sources of antimicrobial compounds must be found (Stincone and Brandelli, 2020). Cyanobacterial extracts are rich sources of different classes of compounds such as peptides, siderophores, polyketones, lipopeptides, heterocyclic compounds, and alkaloids (Vijayakumar and Menakha, 2015; Řezanka et al., 2018; Saurav et al., 2019). Cyanobacteria produce secondary metabolites that have antibacterial activity against Gram-positive and Gram-negative bacteria (Swain et al., 2017; Demay et al., 2019; Cepas et al., 2021; Chauhan et al., 2022; Lykov et al., 2023). There are many secondary metabolites produced by cyanobacteria, including peptides, which contain many compounds such as tenuecyclamide A and D, lyngbyazothrin A, kawaguchipeptin A and B, scytonemin A, borophycin, scyptolin A, and muscoride A, which have documented activities against some types of pathogenic bacteria. Asthana et al. (2009) recorded antibacterial activity of hapalindole isolated from *Nostoc* CCC537 and *Fischerella* sp., against *Enterobacter aerogenes* MTCC2822, *Staphylococcus aureus* ATCC25923, *Pseudomonas aeruginosa* ATCC27853, *Salmonella typhi* MTCC3216, and multi-drug resistant strains of *Escherichia coli* GS 2003/01, 02, 03 and *Escherichia coli* ATCC25992.

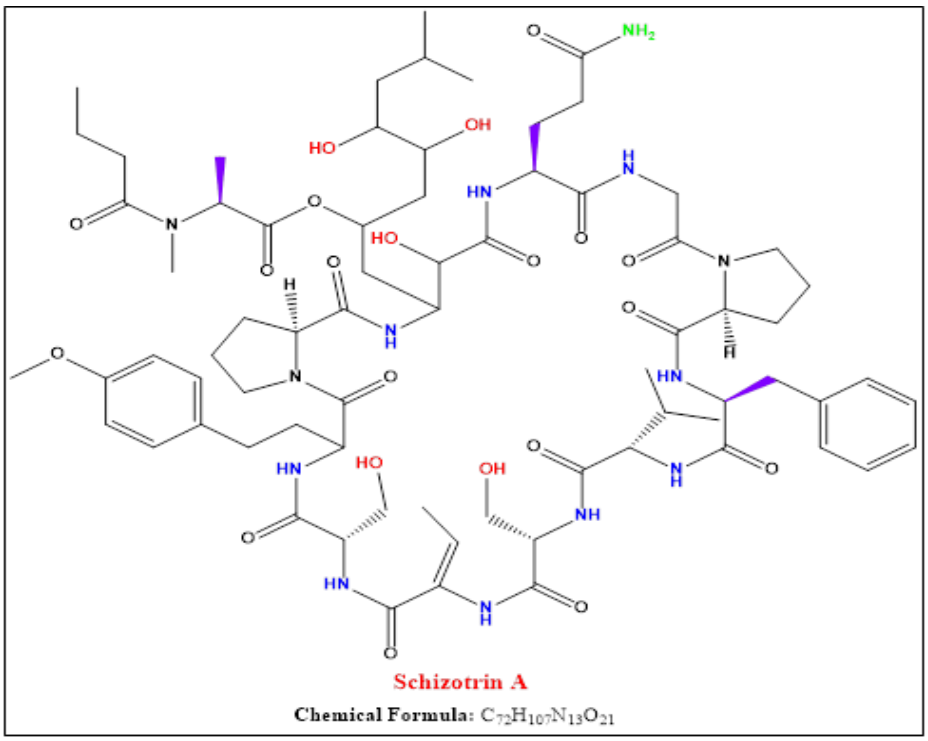

Fig. 3. Structure of antimicrobial compound schizotrin A

2.2. Antialgal activity

In testing the antialgal effect of compounds produced by cyanobacteria, it was found that approximately 10 families of metabolites have an antialgal effect on microalgae. Studies on cyanobacteria that were isolated from two strains, *Nostoc linckia* CALU 892 and *Scytonema hofmanni* UTEX 2349, showed powerful antimicrobial activity against various strains of microalgae and cyanobacteria (Mason et al., 1982; Gromov et al., 1991). There are two compounds, schizotrin A and ambigols, that exhibit antimicrobial activity against fungi, bacteria, and protozoa (**Fig. 3**). Also, these compounds are shown to inhibit the process of photosynthesis (anti-algal effect), which provides promising solutions in the fight against algae and are alternatives to chemical pesticide compounds based on PSII inhibition (Demay et al., 2019).

2.3 Antifungal activity

Cyanobacteria produce many antifungal compounds including nostodione A, fischerellin A, tolytoxin, nostocyclamide, hapalindole, tjipanazole, carazostatin, toyocamycin, and scytophycin which are commonly produced by some genera and species of the cyanobacterial orders such as Oscillatoriales, Nostocaltoriales, and Stigonematales (Abed et al., 2009), as shown **Fig. 4**. Cyanobacteria produce many peptides such as tolybyssidin A and B, fischerellin A and B, lobocyclamide B, scytonemin A, cryptophycin 1 and 52, AK-3, nostocyclamide, hormothamnin A, hassallidin A and B, laxaphycin A and B, calophycin, majusculamide C, hectochlorin, and lyngbyabellin A and B, which have been reported to have antifungal activities (Swain et al., 2017). Vestola et al. (2014) recorded hassallidin A and B (glycosylated lipopeptides) from *Hassallia* sp., with antifungal activity against *Candida* sp., which recorded the lowest inhibitory concentration value of about 4.8 mg/mL.

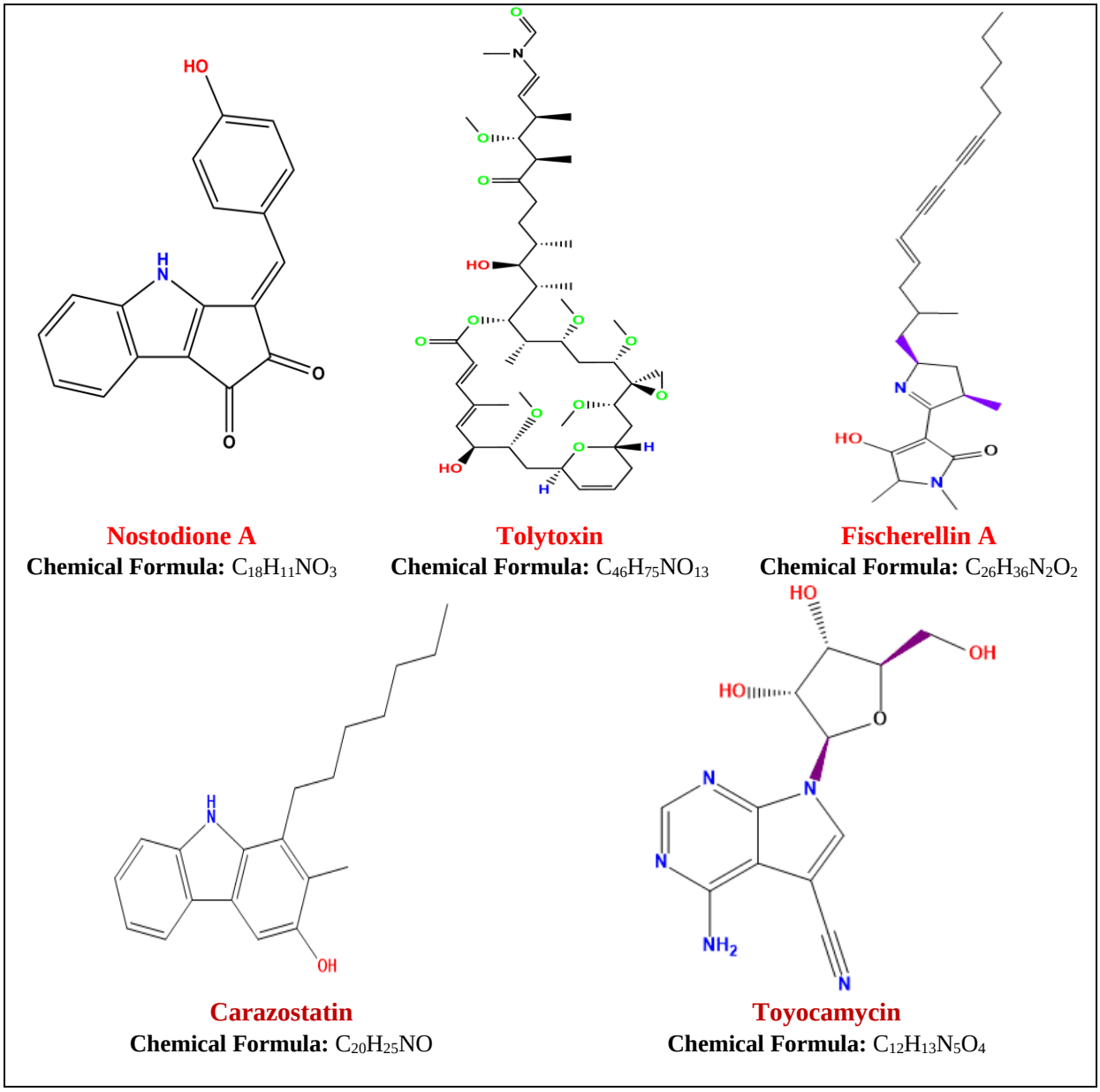

Fig. 4. Structure of some antifungal compounds produced by cyanobacteria

2.4. Antivirals activity

Through studies, it was shown that cyanobacteria can produce antivirals, and the activity of these antivirals has been determined against both human immunodeficiency virus (HIV-1 or HIV-2) and Herpes simplex virus (HSV-1 or HSV-2). Aplysiatoxins (**Fig. 5**) have also been shown to inhibit the activity of Chikungunya virus (CHIKV) but are also considered toxins produced by cyanobacteria (Chlipala et al., 2010; Gupta et al., 2014). The cyanovirin-N analogs isolated from *Cyanothece* sp., and *Nostoc ellipsosporum* showed antiviral activity against large groups of viruses (Boyd et al., 1997; Matei et al., 2016). Cyanovirin-N analogs showed activity against the measles virus, feline immunodeficiency virus, HIV-1, HIV-2, HHV-6, and SIV virus (Boyd et al., 1997; Dey et al., 2000). Therefore, it is clear that cyanobacteria produce many bioactive compounds that act against viruses, bacteria, algae, and fungi. **Table 1** shows some bioactive compounds that are isolated from cyanobacteria.

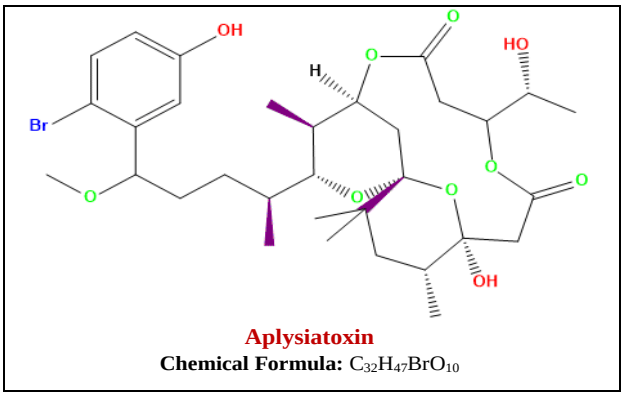

Fig. 5. Aplysiatoxins (cyanotoxin) produced by cyanobacteria

3. Bioplastics from cyanobacteria

Plastics are one of the important materials that are used in many important industries such as the automotive industry, medical equipment, household electrical appliances, computers, etc. Plastic materials are derived from petrochemical materials that are not usually biodegradable and are not renewable, which leads to many problems in the environment, humans and living creatures. In recent years, researchers' interest has increased in the importance of studying bioplastics and their use as an alternative to plastics derived from petrochemicals, as they are made from sustainable resources such as cornstarch, oils, living organisms, and fats (Chua et al., 1999; Chen and Patel, 2012; Aslam et al., 2023). Cyanobacteria produces polyesters from polyhydroxyalkanoates (PHAs) (Gomes et al., 2020; Koller, 2020), a type of thermoplastic that has properties similar to synthetic polypropylene. Among the most common PHAs, polyhydroxybutyrate (PHB) is produced by several genera of cyanobacteria, and this species also exhibits thermoplastic processability, hydrophobicity, biocompatibility, and biodegradability (Hai et al., 2001; Das and Maiti, 2021). Among the cyanobacteria that produce PHB are species such as *Scytonema geitleri* (Singh, et al. 2019), *Arthrospira platensis* (Duangsri et al., 2020b), *Spirulina* sp. (Kordi et al., 2020), *Synechocystis* sp. PCC 6803 (Koch et al., 2020), *Anabaena* sp. (Simonazzi et al., 2021), *Synechocystis* sp. (Rodríguez Lorenzo et al., 2022), and *Synechococcus leopoliensis* (Mariotto et al., 2023).

There are some promising bioplastics including PHA, polyesters, starch, polysaccharides, and cellulose (Storz and Vorlop, 2013). PHA is a fatty substance that is stocked in the cells of cyanobacteria and other living organisms where it is used as an exporter of carbon and energy. It is also produced through the microbial fermentation processes of alkanoic acids, sugars, alkenes, alkanes, and lipids and is then accumulated as granules in the cytoplasm (Reddy and Mohan, 2015). PHB was reported for the first time in 1966 in cells of the cyanobacteria *Chlorogloeopsis fritschii*. It was observed that the largest production of biopolymer was in a type of filamentous cyanobacteria of the type *Nostoc muscorum* Agardh, where the accumulation rate was about 78% dry cell weight (dcw) in heterotrophy with the restriction of nitrogen, glucose, and supplementation of acetate and valerate (Steinbüchel and Valentin, 1995). However, another cyanobacterial species, *Anabaena fertilissima* under mixotrophic cultivation with the addition of acetate, citrate, and deprivation of nitrogen and phosphorus, showed the highest cumulative percentage so far estimated at 85% dcw (Samantaray and Mallick, 2012)

Table. 1. Some bioactive compounds isolated from cyanobacterial strains

Cyanobacterial strains	Bioactive compounds	References
Antibacterial activity		
Lyngbya majuscula	Malyngolide	Dobretsov et al. (2010)
Lyngbya sp.	Lyngbyazothrin	Swain et al. (2017)
Microcystis aeruginosa	Kawaguchipeptin B	Dahms et al. (2006)
Microcoleus lacustris	Abietane	Swain et al. (2017)
Nostoc commune	Comnostins	
	Noscomin	Jaki et al. (2000)
Nostoc insulare	Norharmane	Volk and Furkert (2006)
Nostoc muscorum	Muscoride A	Nagatsu et al. (1995)
Nostoc spongiaeforme	Tenuecyclamides	Banker and Carmeli (1998)
Nostoc sp.	Nostocarboline	Swain et al. (2017)
Oscillatoria redekei	Coriolic acid	
Schizothrix sp.	Schizotrin A	Pergament and Carmeli (1994)
Scytonema sp.	Scytonemin	Swain et al. (2017)
Scytonema ocellatum, Tolypothrix conglutinate	Tolytoxin	
Antivirals activity		
Lyngbya lagerheimii	Sulpholipid	Jha and Zi-Rong (2004)
Lyngbya majuscula	Cyclic polypeptide	
Microcystis ichthyoblabe	Ichthyopeptins A and B	Pandey (2015)
Nostoc ellipsosporum	Cyanovirin-N	Burja et al. (2001)
Nostoc flagelliforme	Nostoflan	Hayashi et al. (2008)
Nostoc sphaericum	Indolocarbazoles	Cohen (2002)
Oscillatoria raoi	Acetylated sulfoglyco-lipids	Reshef et al. (1997)
Phormidium spp.	Caylobolide B	Andrianasolo et al. (2005)
Phormidium tenue	Galactosyldiacylglycerols	Jha and Zi-Rong (2004)
Scytonema	Scytovirin	Bokesch et al. (2003)
Spirulina platensis	Spirulan	Hayashi et al. (1996)
Antialgal activity		
Fischerella muscicola	Fisherellin	Dahms et al. (2006)
Gomphosphaeria aponina	Aponin	Bhadury and Wright (2004)
Nostoc linckia	Cyanobacterin LU-1	Gromov et al. (1991)
Nostoc spongiaeforme	Nostocine A	Hirata et al. (1996)

Phormidium tenue	Galactosyldiacylglycerols	Murakami et al. (1991)
Scytonema hofmanni	Cyanobactericin	Abarzua et al. (1999)
Calothrix fusca	Calophycin	Swain et al. (2017)
Hapalosiphon fontinalis	Hapalindole	Burja et al. (2001)
Antifungal activity		
Hapalosiphon fontinalis	Fontonamide	Burja et al. (2001)
Hyella caespitosa	Carazostatin	
Lyngbya majuscula	Majusculamide C	Pandey (2015)
Nostoc commune	Nostodione	Bhadury and Wright (2004)
Nostoc sp.	Cryptophycin	Singh et al. (2005)
Nostoc sp. UHCC 0450	Swinholides	Humisto et al. (2018)
Plectonema radiosum and *Tolypothrix tenuis*	Tubercidin and toyocamycin	Pandey (2015)
Schizothrix sp.	Schizotrin A	Pergament and Carmeli (1994)
Scytonema hofmanni	Cyanobacterin	Swain et al. (2017)
Scytonema ocellatum	Tolytoxin	Patterson and Carmeli (1992)
Scytonema sp.	Scytonemin	Swain et al. (2017)
Scytonema pseudohofmanni	Scytophycins	Burja et al. (2001)
Scytonema and *Tolypothrix*		Ishibashi et al. (1986)
Tolypothrix tenuis	Toyocamycin	Banker and Carmeli (1998)
	Tubercidin	Swain et al. (2017)
	Toyocamycin	
Tolypothrix tjipanasensis	Tjipanazoles	Bonjouklian et al. (1991)

Cyanobacteria are a promising source for bioplastic production on a commercial scale, but they do not cover the need for various uses of plastic. Thus, genetically modified strains that have a greater ability to accumulate PHA must be produced in order to produce bioplastics on a commercial level. **Table 2** shows some types of bioplastics that are synthesized or produced by cyanobacteria.

Table. 2. Some types of bioplastics produced or synthesized by cyanobacteria

Bioplastic compounds	**Cyanobacteria species**	**Reference**
Poly-3-hydroxybutyrate (PHB)	*Anabaena cylindrica* 10 C	Lama et al. (1996)
	Anabaena sp. VIT-BMN 1	Gopi et al. (2014)
	Arthrospira platensis	Duangsri et al. (2020a)
	Aulosira fertilissima	Samantaray and Mallick (2012)
	Calothrix elenkinii TISTR 8285	Tarawat et al. (2020)
	Calothrix scytonemicola TISTR 8095	Kaewbai-Ngam et al. (2016)
	Calothrix sp. TISTR 8110	Tarawat et al. (2020)
	Chelatococcus daeguensis TAD1	Xu et al. (2014)
	Chlorogloeopsis fritschii TISTR 8547	Tarawat et al. (2020)
	Chroococcus hansgirgi TISTR 8561	Itthirit et al. (2021)
	Hapalosiphon intricatus TISTR 8227	Tarawat et al. (2020)
	Leptolyngbya sp. NIVA-CYA 255	Kettner et al. (2022)
	Myxosarcina sp. TISTR 8678	Tarawat et al. (2020)
	Nostoc hatei TISTR 8405	
	Nostoc microscopicum TISTR 8664	
	Nostoc muscorum TISTR 8871	
	Nostoc muscorum	Panda et al. (2005)
	Nostoc piscinale TISTR 8180	Tarawat et al. (2020)
	Oscillatoria jasorvensis TISTR 8980	
	Oscillatoria sp. TISTR 8623	
	Oscillatoria willei VIT-BMN 9	Gopi et al. (2014)
	Phormidium sp. TISTR 8462	Tarawat et al. (2020)
	Phormidium sp. TISTR 8640	
	Phormidium sp. VIT-BMN 3	Gopi et al. (2014)
	Scytonema geitleri	Singh et al. (2019)
	Spirulina platensis	Campbell 3rd et al. (1982)
	Spirulina sp. LEB 18	da Silva et al. (2018)
	Synechococcus MA19	Nishioka et al. (2001)
	Synechococcus sp. PCC 7002	Zhang et al. (2015)
	Synechococcus sp. TISTR 8503	Tarawat et al. (2020)
	Synechococcus sp. VIT-BMN 2	Gopi et al. (2014)
	Synechocystis salina	Meixner et al. (2018)
	Synechocystis sp. CCALA192	Troschl et al. (2018)
	Synechocystis sp. PCC 6714	Lackner et al. (2019)
	Synechocystis sp. PCC6803	Khetkorn et al. (2016)
	Synechocystis sp. PCC 6803	Zhang et al. (2017)
	Synechocystis sp. PCC 6803	Tarawat et al. (2020)
	Synechocystis sp. VIT-BMN 4	Gopi et al. (2014)
Poly-3-hydroxybutyrate-co hydroxyvalerate [P(HB-co-HV)]	*Nostoc muscorum*	Shetye and Mendhulkar (2022)
Polylactic acid (PLA)	*Arthrospira platensis*	Park and Lee (2022)
Polyhydroxyalkanoates (PHA)	*Arthrospira maxima*	De Philippis et al. (1992)

	Arthrospira platensis	Morais et al. (2015)
	Calothrix scytonemicola TISTR 8095	Kaewbai-Ngam et al. (2016)
	Chlorogloeopsis fritschii PCC 6912	Hai et al. (2001)
	Mastigocladopsis sp.	Kaewbai-Ngam et al. (2016)
	*Nostoc muscorum*a gardh	Bhati and Mallick (2016)
	Spirulina subsalsa	Shrivastav et al. (2010)
	Synechococcus sp. strain MA19	Hai et al. (2001)
	Synechocystis sp.	Lau et al. (2014)
Poly(-3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)	*Anabaena ambigua* TISTR 8001	Tarawat et al. (2020)
	Anabaena Spiroides TISTR 8075	
	Calothrix elenkinii TISTR 8285	
	Chlorogloeopsis fritschii TISTR 8547	
	Hapalosiphon intricatus TISTR 8227	
	Nostoc hatei TISTR 8405	
	Nostoc microscopicum TISTR 8664	
	Nostoc muscorum TISTR 8164	
	Nostoc muscorum TISTR 8871	
	Nostoc piscinale TISTR 8180	
	Nostoc sp. TISTR 9131	
	Oscillatoria sp. TISTR 8623	
	Phormidium sp. TISTR 8462	
	Phormidium sp. TISTR 8640	
	Tolypothrix distorta TISTR 8985	
Polyhydroxyvalerate (PHV)	*Anabaena cylindrica* 10 C	Lama et al. (1996)

4. Potential applications of cyanobacteria in cosmetics and skin care products

Despite the different technological applications of cyanobacteria, including the different pharmacological applications resulting from different species of cyanobacteria, there are many molecules that work on the skin as well, due to the fact that these species have the ability to renew their cells and protect themselves from external influences (environmental conditions), (Mourelle et al., 2015). Cyanobacteria produce by-products that may be used in the manufacture of personal care and cosmetic products, and they need further study on their mechanisms of action (Borowitzka 1995; Mourelle et al., 2017). Cosmetics aim to improve the morphology, structure and appearance of the skin by using active ingredients that have the ability to adapt to different skin types, and protect the skin from physical and chemical factors such as ultraviolet (UV) radiation, xenobiotics and, desiccation, which are among the major factors for flogging deterioration and aging (Mourelle et al., 2017; Morone et al., 2022b). Although the aging process is a natural physiological phenomenon, it may occur in an accelerated manner due to many mechanisms such as oxidative stress, which occurs because of free radicals, which causes of chemical havoc resulting from its high reactivity.

The production of phycobiliproteins (PBP), carotenoids, scytonemin (SCY), and phenolic compounds that are essential to the skin and makes these organisms important in the field of skin care. The aforementioned molecules play a significant role in anti-aging, because of their ability to protect against sunlight and their ability to act as antioxidants, in addition to their capability to produce enzymes that inhibit the degradation of the extracellular matrix (Morone et al., 2019; Favas et al., 2021).

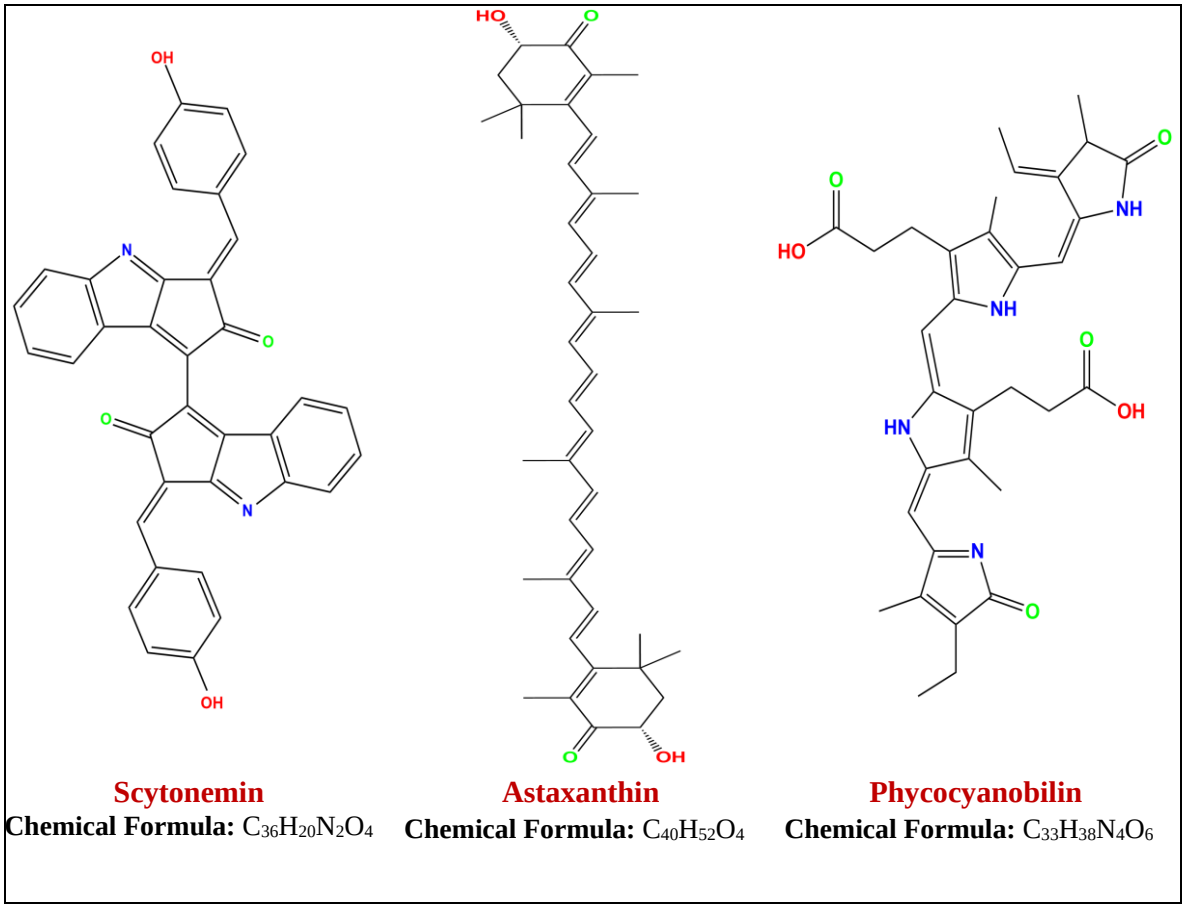

Fig. 6. Structure some carotenoids compounds produced by cyanobacteria

Cosmetics cover a large of these products such as anti-aging products (Morone et al., 2022a), UV protection (Martins et al., 2022), and skin moisturizing creams (Nowruzi, 2022b). Many current studies have focused on the active molecules in cyanobacteria and their potential in cosmetics, including the production of carotenoids, which act as antioxidants. Carotenoids are produced by some genera of cyanobacteria such as *Wollea*, *Synechocystis* and *Leptolyngbya* (Morone et al., 2019; Nowruzi et al., 2020b), and in the treatment of psoriasis by some genera such as *Leptolyngbya* and *Alkalinema aff. pantanalense*, *Nodosilinea antarctica*, *Cuspidothrix issatschenkoi* and *Cyanobium gracile* (Lopes et al., 2020). Cyanobacteria also produce active compounds belonging to the PBP family, which is a group of fluorescent proteins of different colors that produce various compounds including phycoerythrin (PE) and are produced from genera and species such as *Spirulina platensis* (Kamble et al., 2018), *Nostoc* sp. (Nowruzi et al., 2020a), *Halomicronema* (Patel et al., 2022), *Phormidium* sp. (Sonani et al., 2018) and *Microcystis aeruginosa* (Tanabe and Yamaguchi, 2018).

The PBP family also produces a pigment called phycocyanin (PC), which is produced by some genera and species cyanobacteria such as *Synechococcus* sp. (Lin et al., 2022), *Plectonema* sp. (Husain et al., 2021), *Spirulina platensis* (Gabr et al., 2020), *Arthrospira* sp. (Chentir et al., 2019), *Plectonema boryanum* (Mahfooz et al., 2017), *Geitlerinema* sp. H8DM (Patel et al., 2018), *Euhalothece* sp. (Mogany et al., 2018), *Cyanobacterium aponinum* PCC 10605 (Lin and Ng, 2021), *Leptolyngbya valderiana* (Maity and Mallick, 2023), *Pseudanabaena limnetica* (Tribhuvan et

al., 2023), and *Desertifilum tharense* UAM-C/S02 strain (Hernández–Martínez et al., 2023). A bioactive compound called allophycocyanin is also produced by cyanobacteria that include *Anabaena* sp. PCC (Ducret et al., 1998), *Phormidium* sp. A09DM (Sonani et al., 2015), and *Lyngbya* sp. A09DM (Rastogi et al., 2015). Furthermore, there is a phycoerythrocyanin (PEC) compound produced by cyanobacteria such as *Mastigocladus laminosus* (Duerring et al., 1990), *Westiellopsis prolifica* (Sai et al., 1993), *Anabaena variabilis* (Zhang et al., 1997), and *Leptolyngbya* sp. PCC 6406 (Hirose et al., 2019).

Cyanobacteria posses the ability to produce potentially active antioxidizing colorings compounds, which are utilized in cosmetic manufacture as natural pigments and cosmetic antioxidants. The pigments represent carotenoids (**Fig. 6**) such as astaxanthin from the genus *Synechocystis* sp. PCC 6803 (Shimada et al., 2020), and blue pigments phycocyanobilin from the genus *Spirulina*, which can be used in the manufacture of cosmetics such as lipsticks and eyeliners (Hamed, 2016). Many studies have documented the ability of scytonemin as an antioxidant compound, which is a carotenoid compound produced by cyanobacteria such as *Nostoc commune* (Venckus et al., 2018), *Scytonema* sp. R77DM (Rastogi et al., 2014), *Rivularia* sp. HKAR-4 (Rastogi et al., 2013), *Lyngbya* sp., (Fuentes-Tristan et al., 2019), *Leptolyngbya mycodia* (Naeimpoor and Sheibani Madrahi, 2022), and it can be used as a UV protection (cosmetic sunscreen).

Conclusions

Cyanobacteria are found in many aquatic ecosystems and adapt to live in various conditions, as they are distributed everywhere in the world, in addition to being plain to grow and maintain in the laboratory and under minimal conditions of nutritional requirements. As a result of the many characteristics of cyanobacteria, they are considered a promising candidate for the production of a large assortment of bioactive compounds. Cyanobacteria are used in many biotechnological applications due to their being a very attractive choice in the production of secondary metabolites. Many bioactive compounds have been isolated and extracted and have been identified such as antioxidants, antimicrobials, anticancer, antivirals, anti-UV, anti-aging, and anti-toxins. In recent years, cyanobacteria have been utilized in many applications, as they have been used in agricultural applications for the production of biofertilizers, in the treatment and reduction of pollutants due to their being environmentally friendly, in the exploration of many medicines and cosmetics, in the production of biofuels, and in the manufacture of nutritional supplements, vitamins and fodder. Therefore, efforts and research must be intensified to achieve high-quality products from cyanobacteria through biotechnological methods.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SHORT OVERVIEW OF OXIDATIVE STRESS IN MENTAL DISORDERS

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Abstract: This short overview explores the relationship between oxidative stress and mental disorders, focusing on the association with psychiatric pathologies such as Alzheimer's disease, schizophrenia, autism, depression, and the impact of sleep deprivation. The mechanisms of mitochondrial disfunction and oxidative stress in these pathologies are described, including the physiological function of limited free radicals in signal transduction, gene transcription, neuronal plasticity and memory. Key free radicals, including hydroxyl and superoxide are highlighted, along with compounds generating free radicals. Moreover, the potential therapeutic implications of dietary supplements (zinc, selenium, magnesium, vitamin C, E, CoQ_{10}) and lifestyle interventions with antioxidant properties are presented, laying the groundwork for future research in the field of mental health.

Keywords: oxidative stress, mental disorder, dietary supplements, antioxidants

1. Introduction

Limited amount of free radicals have beneficial effects for the body, being involved in signal transduction, gene transcription, inflammatory response, neuronal plasticity, and memory (Uttara et al., 2009).

The most important free radicals are hydroxyl (OH·), superoxide ($O2^{-}$·) and nitric monoxide (NO·) as well as substances capable of producing free radicals such as hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^{-}$). Reactive oxygen species (ROS) are formed especially because of metabolic processes that require oxygen. The body's antioxidant defense includes glutathione (GSH), arginine, tocopherol, ascorbic acid, retinol and polyphenols derived from tea, the activity of these compounds being complemented by enzymes with an antioxidant effect e.g. superoxide dismutase (SOD), catalase (CAT), GSH reductase and GSH peroxidase (Sharma et al., 2022). SOD catalyzes the transformation of superoxide to hydrogen peroxide and oxygen, and hydrogen peroxide is converted to water and oxygen by catalase (Rodriguez-Rocha et al., 2013).

Oxidative stress (OS) is induced by a modified equilibrium between the generation of free radicals and the antioxidants effect, and it can lead to impairment of the cellular functions or mitochondrial dysfunction. The brain is highly vulnerable to the impact of free radicals due to the intense oxidative metabolism, the amount low in antioxidants that cross the protective barrier separating the bloodstream from the brain and the increased content of polyunsaturated fatty acids. Although the average weight of the human brain is only 1400 g, it consumes in aerobic energy metabolism more than 20% of the overall oxygen in the organism in order to provide energy to the 86 billion neurons (Cobley et al., 2018). The quantity of oxygen available to the brain is extremely carefully controlled at the level of the *prima fascia* precisely because of the possibility of ROS generation (Fukuto et al., 2012).

OS enhances the process of oxidative degradation of lipids, especially of membrane fats with the formation of compounds such as malondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE), acrolein which can bind to proteins or DNA leading to a change in their function (Reed, 2011). In the case of carbohydrates, due to their reducing character, they react with ROS, the compounds resulting from nonenzymatic combination with proteins are called advanced glycation end-products (AGEs), implicated in the etiology and progression of certain diseases, such as diabetes mellitus, cardiac impairment, and neurodegenerative conditions (Ahmed, 2005). In the case of proteins, the products generated through the influence of ROS and reactive nitrogen species (RNS) are protein carbonyls and nitrated proteins.

This short overview emphasizes the link between OS and cognitive dysfunction, highlighting a few dietary supplements with antioxidant properties. These supplements have the potential to contribute to the prevention and treatment of imbalances associated with OS in the context of mental disorders.

1. General aspects regarding mitochondrial dysfunction in neurological and psychiatric diseases

Mitochondria are cellular organelles with an extremely dynamic structure, continuously subjected to fission and fusion processes in order to sustain a healthy mitochondrial function. The disruption of these repair mechanisms leads to mitochondrial disfunction (Ježek et al., 2018; Ren et al., 2020).

Mitochondria play a crucial role in ensuring proper development of numerous processes at the neuronal level, such as: the main source of energy (ATP) through the process of oxidative phosphorylation, obtaining precursors and initiating the synthesis of heme - glycine and succinyl-CoA, a buffer role in regulating calcium concentration during signal transduction, therefore metabolic changes at the mitochondrial level have profound repercussions on the good functioning of neurons and can be responsible for the occurrence of numerous neurodegenerative diseases (Wang et al., 2020).

Among the mechanisms incriminated in mitochondrial disorders is OS, mitochondria being responsible for the production of ROS. ROS can modify the concentration gradient of Ca^{2+} on either side of the cell membrane through direct damage of Ca^{2+}-regulating proteins, with the mitochondrial increase of [Ca2+]. Intramitochondrial, ROS produce changes in the activity of NADH dehydrogenase, cytochrome c oxidase and ATP synthase, with direct damage to cellular energy metabolism (Sousa et al., 2023).

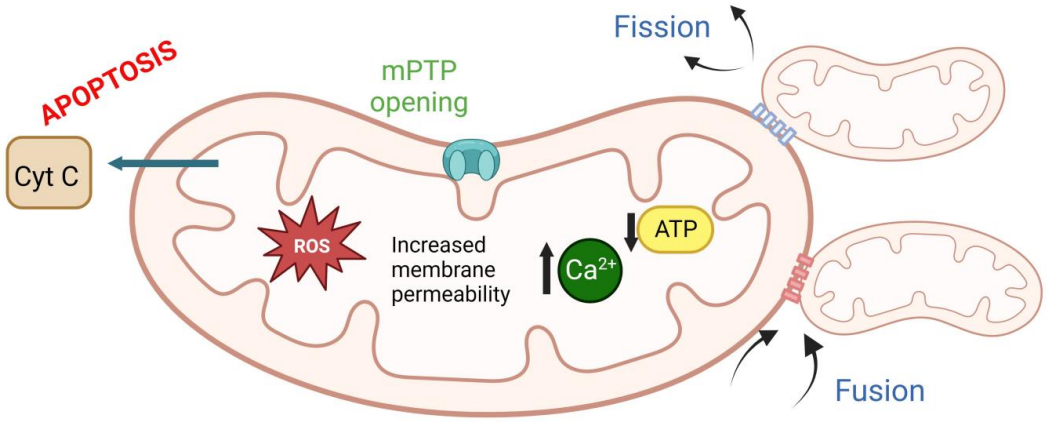

Fig. 1. Mitochondrial dysfunction (MD) pathways (1. Increase in the intramitochondrial concentration of Ca^{2+}, 2. Opening of the mPTP, 3. The liberation of cytochrome c into the cytoplasm, initiating cellular apoptotic processes, 4. Modification of mitochondrial fission and fusion processes, 5. Decrease of ATP concentration by modification of the electron transport chain) (after Norat et al., 2020)

Apart from OS, mitochondrial dysfunction can also arise due to alterations in mitochondrial DNA. Changes in the permeability of the mitochondrial membrane, triggered by the opening of the mitochondrial permeability transition pore (mPTP), result in the release of cytochrome c into the cytoplasm, leading to the initiation of cell apoptosis and mitophagy (see **Fig. 1**) (Liu et al., 2018; Ciocca and Pizzamiglio, 2023). Considering the increased energy requirement and the limited regeneration capacity of neurons, the proper functioning of mitochondria is essential for the survival of neurons (Johri and Beal, 2012). Mitochondrial disfunction is currently the most incriminated pathological process in the ethology of neurological disorders such as: Huntington's disease, Parkinson's disease, schizophrenia, multiple sclerosis and Alzheimer's disease (Wu et al., 2019).

2.1. Oxidative Stress and Alzheimer`s disease

Alzheimer's disease is a gradually advancing neurodegenerative condition identified by cognitive decline. While the precise origin of Alzheimer's disease remains elusive, emerging evidence indicates that OS plays a pivotal role in its development. Multiple mechanisms have been shown to be involved, including mitochondrial dysfunction, accumulation of transition metals, genetic factors, and amyloid beta-mediated processes.

Multiple studies have reported elevated markers of OS in Alzheimer’s disease, especially lipid peroxidation is greatly enhanced in neurons (Misrani et al., 2021). This highlights the complex interplay between OS and the development of hallmark characteristics associated with Alzheimer's disease.

Studies indicate that OS is implicated in the creation of significant pathological characteristics, including the aggregation of amyloid beta into plaques in Alzheimer's disease and hyperphosphorylated tau into neurofibrillary tangles (Ionescu-Tucker and Cotman, 2021). Furthermore, a significant decrease in glucose metabolism was described, which is thought to be at least partially caused by oxidative inactivation of enzymes implicated in glycolysis, the Krebs cycle, and

ATP biosynthesis (Butterfield and Halliwell, 2019). This defect in glucose metabolism further exacerbates the energy deficit in affected brain regions.

Overall, OS is not only a consequence of this pathology but also a contributing factor to its progression.

2.2. Oxidative Stress and depression

OS has been associated with the onset and progression of depression. Research shows a complex interplay between excessive exposure to free radicals and the development of depression. Various factors including smoking, alcohol dependence, obesity, and intense physical activity are associated with depression, all of which contribute to elevated levels of ROS. Chronic stress triggers the release of cortisol, leading to dysfunction in mood regulation, psychomotor drive, and impaired neurogenesis. Additionally, the uncontrolled release of glutamate into synapses, known as glutamatergic hyperactivity, is followed by stressful stimuli. This consequently leads to neurotoxicity, and neuronal death. These OS-induced alterations in the neuronal system, shown by a decreased hippocampal volume, are a dominant factor in the development of depression (Cecerska-Heryć et al., 2022).

The impact of OS extends to DNA damage, suggesting a plausible connection between OS and accelerated aging processes (Cecerska-Heryć et al., 2022). Furthermore, elevated levels of malondialdehyde (MDA) are noted in depressed patients. Supporting these findings, additional studies show reduced levels of crucial antioxidants such as tocopherol, zinc, and coenzyme Q10, contributing to an impaired defence against free radicals. Furthermore, some evidence propose that OS and inflammation may interact in a bidirectional manner, with OS promoting inflammation and vice versa. This bidirectional relationship could further contribute to the onset and advancement of depression (Bhatt et al., 2020). An excess production of inflammatory markers is linked to cognitive alterations and the manifestation of depressive symptoms.

2.3. Oxidative Stress and Autism

OS contributes significantly to the pathophysiology of Autism Spectrum Disorders (ASD). Multiple studies have shown increased markers of OS and decreased levels of antioxidants in individuals with ASD. These markers include abnormal lipid peroxidation, decreased levels of GSH and SOD, and reduced actions of antioxidant enzymes. Neurons, which are unable to produce GSH, are especially vulnerable to the harmful effects of OS. This may contribute to the observed neurological abnormalities in ASD (Pangrazzi et al., 2020).

Research indicates that two factors, mitochondrial dysfunction and the accumulation of transition metals, contribute to the heightened production of ROS in individuals with ASD. This in turn worsens OS, resulting in oxidative deterioration of lipids, proteins, and DNA, inflammation, and other harmful processes that may result in the clinical symptoms of ASD (Liu et al., 2022).

Selenium and selenoproteins play a vital role in individuals with ASD, influencing various processes such as antioxidants, inflammation, and brain cholesterol metabolism. Abnormalities in red blood cell membranes are observed in children with ASD, such as reduced phosphatidylethanolamine levels and elevated phosphatidylserine levels.

It is important to note that both genetic and environmental factors are responsible for increasing OS in individuals with ASD. Genetic factors include polymorphisms in genes associated with GSH metabolism, OS, and detoxification pathways, as well as copy-

number variations, which play a role in ASD pathogenesis (Gonzales et al., 2023). Environmental factors include exposure to heavy metals, infections, drugs, and environmental toxins are also implicated in increasing OS in ASD.

2.4. Oxidative stress and sleep loss/ deprivation

Sleep deprivation refers to the condition of insufficient or inadequate sleep, which can have adverse effects on different facets of health, affecting mainly the brain, liver, kidney, stomach, testes, and heart (Neculicioiu et al., 2023).

One of the potential mechanisms linking sleep deprivation to health problems is OS. Sleep holds significance in maintaining cognitive function and overall well-being. Research has found that chronic sleep deprivation can lead to cellular damage and cognitive impairments because of intense OS. After a period of sleep deprivation, the antioxidant defence mechanisms start to decrease, contributing to impairment of both short- and long-term memory (Atrooz and Salim, 2020).

Furthermore, studies have found a correlation between sleep loss and triggering OS in the gut through ROS accumulation. The reason for the accumulation of ROS during sleep loss is not well known. Levels of ROS may arise due to heightened production, diminished elimination, or a combination of both factors. Some studies have found that NADPH oxidase potentially led to gut dysbiosis induced by the hyperproduction of ROS. The accumulation of intestinal ROS may have systemic effects on gut microbial profiles and immunity, due to their high potential of cellular damage (Vaccaro et al., 2020).

Long periods of wakefulness have additionally shown a more active metabolism, and neuron activity, as well as increased glucose consumption, compared to periods of sleep. These findings correspond to an elevated oxygen-dependent ATP synthesis within the mitochondria, herewith, increasing the production of ROS. Moreover, sleep deprivation can produce a stress response. The activation of the hypothalamic-pituitary-adrenal axis holds a pivotal position in mediating the interaction between stress, sleep deprivation, metabolism, and its potential to induce OS. These results emphasize the crucial significance of prioritizing quality sleep as part of overall health maintenance.

2.5. Oxidative Stress and schizophrenia

OS can potentially contribute to the onset of schizophrenia by disrupting the balance of thiol status. Thiols, including GSH, are essential for maintaining antioxidants and ROS in equilibrium within the body. This disruption in thiol status may have implications for the pathophysiology of schizophrenia. Studies have shown that individuals with schizophrenia often exhibit reduced levels of antioxidants, such as GSH, and elevated levels of markers of oxidative damage compared to healthy individuals (Cuenod et al., 2022).

Moreover, OS can be a consequence of obstetric complications that have been linked to schizophrenia. In addition to its direct impact on the redox control system, OS can also affect DNA metabolism and epigenetic marking, potentially contributing to the vulnerability to schizophrenia (Fraguas et al., 2019).

This growing evidence indicates that OS could represent a shared mechanism by which different genetic and environmental factors impact neurodevelopmental processes underlying schizophrenia. The vulnerability-stress-inflammation model of schizophrenia integrates OS, highlighting the potential for stress to contribute to a persistent pro-inflammatory state. This increased inflammation could be seen within the cerebral

and circulatory systems of these patients (Ermakov et al., 2021).

Additionally, OS in schizophrenia is not only a consequence of genetic factors but can also be influenced by environmental factors such as childhood trauma, initiating the increase of pro-inflammatory cytokines and promote ROS generation. One area of interest represents the impact of OS on PV (parvalbumin) neurons, a specific type of inhibitory interneurons in the brain. Dysfunction or impairment of PV neurons has been associated with several psychiatric disorders, including schizophrenia. In conclusion, OS is considered a potential pathogenic mechanism in schizophrenia and individuals with schizophrenia are believed to be in a state of OS (Cuenod et al., 2022).

3. Diet, dietary supplements and phytochemicals with antioxidant activity

3.1. Diet, macro, and micronutrients

A well-balanced intake of both macro and micronutrients supports mental equilibrium (Quan et al., 2023). Even though glucose is the primary energy substrate for neurons, several studies have shown that the outcome of a high-carbohydrate diet increases the risk of depression by influencing the neuronal metabolism of serotonin leading to the stimulation of inflammatory processes and a reduction in the expression of brain-derived neurotrophic factor (BDNF) (Pinna et al., 2022; Colucci et al., 2020).

Regarding the consumption of fats, both high-fat diets and obesity are major factors that exacerbate depressive states. There are studies suggesting that the expression of the long isoform of the leptin receptor (LepRb) and the cannabinoid receptor type 1 (CNR1) is influenced, selective deletion of these receptors leading to behaviors related to depression (Gallego-Landin et al., 2021; Li et al., 2022). Alternatively, a high-protein diet is linked with a reduced risk of depression, probably attributed to its rich concentration of essential amino acids such as tryptophan, a precursor to serotonin (Reuter et al., 2021).

- *Ketogenic diet*. Ketone bodies become the primary source of energy for cells, including the neurons, during carbohydrate deprivation, and this condition is beneficial for patients with epilepsy as it helps reduce the frequency of epileptic seizures (Dowis and Banga, 2021). Building on this fact, recent studies are focusing on the benefits of the ketogenic diet in other conditions such as Alzheimer's, Parkinson's, migraines. The mechanisms by which this diet offers beneficial effects in neurological disorders are intricate, but there are some evident clues: it modulates the levels of BDNF, enhances mitochondrial function (Dyńka et al., 2022). The neuroprotective impact of the diet is also associated with gut microbiome's composition, as ketone bodies have an impact on the diversity and abundance of the microbiome (Tao et al., 2022). Even though the ketogenic diet holds therapeutic potential in various neurological conditions, its benefits should continue to be assessed through clinical studies in future research.
- *Micronutrients*
  - ✓ **Zinc.** Zinc acts as a cofactor for numerous enzymes participating in carbohydrates, lipid, and protein metabolism, influencing immunity. Several studies highlight the beneficial effect of supplementing zinc when combined with antidepressants at concentrations ranging from 25 to 220 mg for 8 to

12 weeks for the treatment of depression (Quan et al., 2023). The effect is both anti-inflammatory and an elevation of the concentration of BDNF was observed (Wu et al., 2021).

- ✓ **Magnesium.** Among its numerous biological roles, magnesium activates many enzymes involved in metabolism. Numerous studies have shown an inverse relationship between dietary magnesium intake and the risk of experiencing depression, modulating N-methyl-D-aspartate (NMDA) nerve signaling (Del Chierico et al., 2021). Supplementation of 248 to 500 mg/day for 6 to 8 weeks helps maintain mental equilibrium (Quan et al., 2023).
- ✓ **Selenium.** Selenium is involved in several physiological functions, having anti-oxidative and anti-inflammatory effect. However, supplementing with selenium should be preceded by measuring its levels in the blood, as there are controversies surrounding both under and over-dosage. Both scenarios pose a risk factor in promoting depression (Maruki et al., 2022). However, a recent study by *Pereira ME et al*. supports the beneficial antioxidant and anti-inflammatory action of selenium in patients with Alzheimer's disease. Selenium is a trace element that is a crucial component of selenoproteins, such as selenoprotein P, which holds a crucial function in the central nervous system by maintaining an antioxidative status and, as a result, mental health equilibrium. The recommended daily intake of selenium is generally in the range of 55 to 70 micrograms per day for adults (Pereira et al., 2022). Regarding the antioxidant effect, *Cardoso et al.* observed that there was an increase in the activity of GSH peroxidase following selenium supplementation in patients with Alzheimer disease (Cardoso et al., 2019).

3.2. Vitamin C

Vitamin C (ascorbic acid) is a cofactor in a variety of biological processes, being renowned for its antioxidant properties. In recent years, research has revealed that vitamin C plays a role in maintaining mental health by regulating the metabolism of neurotransmitters and, consequently, neuronal activity (Figueroa-Méndez and Rivas-Arancibia, 2015). The recommended dietary allowance (RDA) for vitamin C is established at 75-90 mg on a daily basis. While there are suggestions to intake 3 grams of vitamin C daily, it's important to note that this may lead to side effects such as nausea, vomiting, and diarrhea. (Kaźmierczak-Barańska et al., 2020).

Sim M et al. performed a study emphasizing the importance of vitamin C supplementation in the vitality of healthy young adults (20-39 years), concluding that vitamin C at doses of 500 mg twice daily for one month enhanced motivation for work and improved ability to stay focused positively influencing performance on cognitive tasks that demand prolonged attention (Sim et al., 2022). The presumed mechanism of action appears to involve vitamin C's role in dopaminergic transmission (it acts as a cofactor for dopamine-β-hydroxylase), in the serotoninergic, glutaminergic, cholinergic neurotransmissions by modulating hydroxylation reactions (Moritz et al., 2020).

Even though there are several studies demonstrating the anxiolytic and antidepressant impacts of vitamin C, advanced research is needed for this molecule to become a candidate in psychiatric therapy.

3.3. Vitamin E

Vitamin E (α-tocopherol) is a lipid-soluble vitamin, enhancing immunity and reducing OS (Wang et al., 2023). *Atiq A et al.* published an article regarding the impact of vitamin E using an experimental model of Parkinson's disease, reducing α-synuclein expression, increasing the expression of dopamine transporter in the *substantia nigra* and activating the nuclear factor erythroid-2-related factor 2 (Nrf2) pathways (Atiq et al., 2023).

In the same context, another study conducted on an experimental model of acute and chronic stress demonstrated that the preventive administration of vitamin E reduced OS markers (Al-Sowayan, 2020).

3.4. CoQ_{10}

CoQ_{10} is a part of the electron transport chain. It has anti-inflammatory and antioxidant effects that have been studied in the context of various neurological diseases (Sanoobar et al., 2013; Pandya et al., 2013).

In a randomized, double-blinded study, the impact of supplemental CoQ_{10} on patients with bipolar disorder yielded to changes of total antioxidant capacity and total thiol groups in the serum (Dai et al., 2022).

With these effects, CoQ_{10} is a new potential candidate in addition to those previously described in the prevention and treatment of psychiatric syndromes.

3.5. Phytochemicals (flavonoids, polyphenolic compounds)

Recent studies concluded that flavonoids, especially those derived from berries, have the potential to alleviate depression by exerting antioxidant properties, functioning as neuromodulators, and fostering cognitive well-being (Ali et al., 2021). Also, blackcurrant extracts increased expression of BDNF in the hippocampus of mouse models and reduced OS and inflammation (Currie et al., 2023).

In a recent review emphasizing the significance of nuclear factor erythroid-2-related factor 2 and natural flavonoid activators, it was reported that flavonoids like curcumin, quercetin, and resveratrol were shown to lower OS, increasing GSH concentration *in vitro*, reducing depressive-like behaviors in an experimental model, and decreasing malondialdehyde (MDA) levels (Zuo et al., 2022).

Quercetin is a flavonoid with proven antioxidant and anti-inflammatory properties. Quercetin`s anti-stress effects are achieved through a combination of mechanisms that involve regulating neurotransmitters like serotonin, suppressing the Hypothalamic-Pituitary-Adrenal axis, promoting neurotrophic factors that support brain health and resilience to stress, inhibiting the responses of microglial and astrocyte cells to stress (Colunga Biancatelli et al., 2020; Wang et al., 2020; Zhang et al., 2020).

Resveratrol is another natural compound, having a polyphenolic structure. Resveratrol has shown the potential to protect dopaminergic neurons from methamphetamine-induced neuronal cytotoxicity (Zeng et al., 2021).

The consumption of natural products or extracts containing flavonoids or other natural compounds with antioxidant properties has certain limitations, one of which is the variable content of the active substance in these fruits or extracts.

Conclusions

In conclusion, this short overview highlights the pivotal role of OS in the development and progression of various mental disorders, suggesting a multifaceted relationship between OS and mental disorders, involving disruptions in neurotransmitter balance, Hipothalamic-Pituitary-Axis hyperactivity, DNA damage, and antioxidant deficiencies. Furthermore, it explores the potential therapeutic implications of dietary supplements and lifestyle interventions with antioxidant properties, providing a foundation for future research in the mental health therapeutic field.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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THE ROLE OF A LANDSCAPE REHABILITATION STUDENT PROJECT IN CURRENT LANDSCAPE EDUCATION - HEALING URBANIZATION'S FOOTPRINT

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Abstract: The Giurgeului Depression has been significantly impacted by human intervention, resulting in numerous scars on the landscape. A comprehensive rehabilitation plan is imperative for the Suseni quarry area to restore its natural features. This initiative, spearheaded by the local council and municipality, collaborates with the Sapientia Hungarian University of Transylvania's Department of Horticulture and the Babeș-Bolyai University's Faculty of Biology and Geology. They've launched a research scholarship program for students focused on a research and landscape rehabilitation plan tender within Suseni's administrative region. Large scars created by human activities, adjacent to a valuable natural environment, significantly alter the natural landscape features and are detrimental to the visual and ecological relationship systems. Those need urgent rehabilitation. Student projects serves as a potential model for transforming natural landscapes affected by human actions. It aims to teach landscape design through experimental approaches, aligning with modern research-driven design methods for strategic planning. Moreover, it seeks to enhance student projects for practical implementation by local authorities, contributing positively to the area's ecosystem and landscape. Much has already been taken from this landscape, and it is now time to give something back to the area, the local ecosystem, and the landscape.

Keywords: students project, quarry, reclamation, rehabilitation concept, footprints, renaturalization

Introduction

This paper explores the process of translating landscape design by using the example of student work for an originally natural landscape marked by human interventions. It aims to address the question of how students can be taught by an experimental approach to landscape design, particularly in light of new strategic planning tasks that demand research-oriented design methods and

how can a student project be enhanced and used by local authorities.

According to Scully and Kerr (2014), the concept of student workload holds significant importance and complexity. In these students work publication, we present a collection of inspired designs, innovative concepts, and visionary projects crafted by the next generation of landscape architects. Each project showcases the dedication and passion of these talented students as they push the boundaries of design, seeking to create harmonious relationships between people and their surroundings.

The recognition of creative landscape analysis as an experimental process of translation yields numerous productive implications for teaching landscape design, particularly in relation to formulating a design problem (Tietjen, 2013). This publication not only celebrates the artistic prowess of these students but also highlights their deep understanding of ecological systems, cultural contexts, and the social impact of their designs. Their projects aim to foster connections, promote well-being, and inspire a sense of wonder in the natural world.

The Landscape Planning module at the Technological and Higher Education Institute of Sapientia is offered during the third year of their four-year Bachelor's program in Landscape Architecture. This 14-week module primarily focuses on introducing research concepts and methods that are relevant to landscape architectural inquiries, with a specific emphasis on their potential application in landscape planning and design.

Project-Based Learning (PBL) has been fully integrated. PBL entails the use of authentic problems that are closely aligned with real-world situations (Al-Balushi and Al-Aamri, 2014) and empowers students to conduct investigations at their own pace and in their own unique ways, resulting in a highly effective approach for enhancing student engagement and fostering critical thinking skills (Wurdinger et al., 2007).

In addition, the incorporation of group work in this module promotes collaborative learning, which has been found to be more effective and leads to improved critical thinking performance and deeper learning. This emphasis on collaborative learning is essential to the overall goals of the module (Gokhale, 1995; Newman, et al., 1995).

1. Call for tenders, terms and conditions

Regarding the rehabilitation concept plan of the Suseni quarry (**Fig. 1.**), the initiative was instigated by the local council and municipality. The university's lecturers and students received their cordial support during the preparation of the proposal. The Harghita County Association and the Harghita County Council, in collaboration with the Suseni Mayor's Office, the Sapientia Hungarian University of Transylvania, Department of Horticulture, and the Faculty of Biology and Geology of the Babeș-Bolyai University, launched a research scholarship program for students pursuing their bachelor's, master's, and doctoral degrees, specialized in this field.

The tender process consisted of two stages. The first stage was an open design competition, and the winning design would be further developed in the second stage with the involvement of associated higher education institutions.

I. In the first stage, a research plan was formulated and a recycling proposal was visualized. This phase included important studies related to landscape rehabilitation, relevant conclusions, and proposals presented in written and drawing formats.

Fig. 1. Upper view of Suseni quarry (from the tender)

II. In the second stage, the winning research plan and recycling proposal were further developed in detail so that the commissioning institution could utilize it effectively in the subsequent planning process.

The tender call emphasized the importance of multidisciplinary thinking, specifically the interdisciplinary approach in landscape planning, which is necessary for a task of this magnitude. It requires the collaboration of various disciplines, and therefore the professional diversity of the teams was a fundamental requirement for the application process and for achieving a high-quality outcome.

2. Methodology

The research methods employed in this context are diverse and vary based on the specific goals of inquiry. These methods encompass various approaches, ranging from the accumulation of existing design knowledge through logical argumentation to case study research methods. Comparative analysis based on levels of intervention and design means is utilized to facilitate this type of research.

Given that the majority of the students share a similar educational background with limited or no prior experience in rigorous research, their baseline cognition level in research is generally low. To address this, action research has been undertaken during module deliveries to evaluate the effectiveness of Project-Based Learning (PBL) approaches in fostering research-oriented learning among our undergraduate landscape students.

3. Location

Human intervention has notably affected the Giurgeului Depression, leaving multiple scars on its landscape. The Suseni quarry area necessitates a comprehensive concept plan for rehabilitation to reinstate its natural landscape features. The creation of such a plan marks the first crucial stage in the successful healing of the landscape, a pivotal factor for attaining positive results.

The large scars created as a result of human activities significantly alter the natural landscape features. These wounds, including mine areas, are detrimental to the visual and ecological relationship systems. They represent a significant change in the ecological, growing area and landscape features, referred to as "errors in the landscape." Landscape planning tools can aid in correcting these errors through the process of landscape rehabilitation, which can accelerate the healing of landscape wounds and injuries.

The research and landscape rehabilitation plan tender is situated within Suseni's administrative region (**Fig. 2.**), specifically along county road number 138, positioned between the settlements of Liban and the settlement center, bordering a significant natural environment. The quarry predominantly consists of andesite, a resilient dark gray volcanic rock well-suited for road construction and paving purposes. Andesite mining started in the late 1930s and is still ongoing in the Suseni mine, owned by the Lafarge mining company with French capital. It is one of the largest and most modern quarries in Romania, with six extraction levels covering an area of almost 100 ha, and an annual extraction volume of approximately 1 million tons of stone. The extracted stone, totaling over one hundred million tons, was utilized in the construction of railway networks and airports throughout Romania. The mining period in the mine lasts for approximately two to three years, after which it may be closed.

The quarry's location is highly advantageous, given its spectacular setting and accessibility by railway from the direction of Voşlobeni. Moreover, the county road passes over the mine, which makes it a suitable tourist destination after its closure. Giurgeului Depression tourism represents almost a quarter of the county's tourism. The quarry currently represents a landscape wound that requires healing and utilization of its touristic potential to host cultural-community events, festivals, landscape awareness and nature conservation programs, and educational trails. Recently, in cooperation with World Wildlife Fund (WWF) Romania and the Natura 2000 authority, a preliminary mine rehabilitation schedule was prepared, which included building a lookout and strengthening the copper interfaces.

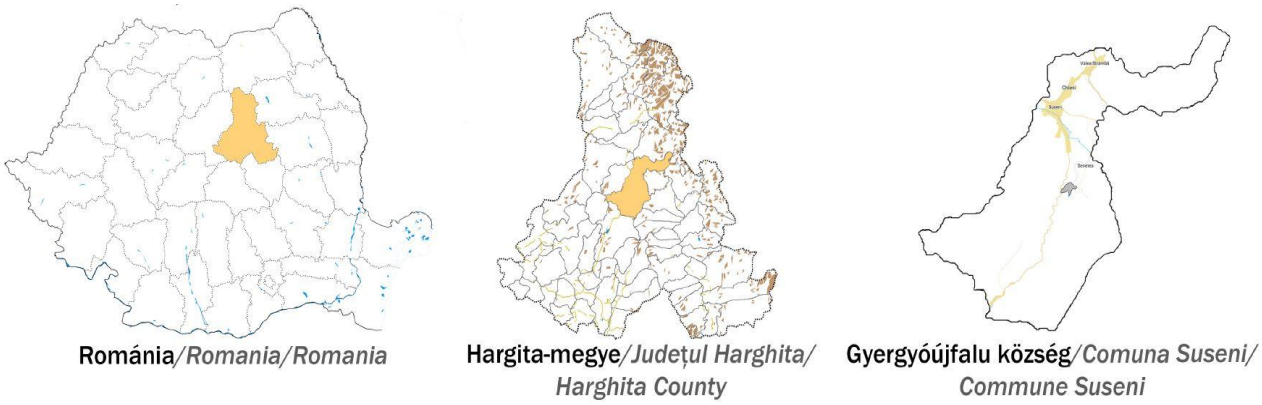


Fig. 2. Location (from students' work, Team 4)

4. Work process

4.1. Tune in

At the onset of the work process, a number of preliminary studies were conducted in collaboration with students, including pre-documentation works, landscape philosophy, historical land use studies, and the process of development and transformation of the various landscape elements. A broader examination revealed that the Suseni quarry is not a unique mining site within the Giurgeului Depression, but rather a landscape wound. This was further evidenced by the proximity of the Voșlobeni quarry.

4.2. Site visit or fieldwork

At the heart of fieldwork lies the essence of learning in real time and real places, with the outdoors serving as the primary laboratory for landscape architecture education. Developing the ability to perceive and comprehend the landscape is considered the initial step in cultivating a keen visual sense. This process involves observing the forms and functions of the landscape, which cannot be accomplished at a professional level without engaging in activities such as drawing, sketching, measuring, and creating cross-sections. By physically experiencing the landscape, designers gain a deeper understanding of its form, emphasizing the significance of bodily engagement in comprehending and manipulating the landscape as a designer. (Fekete and Toorn, 2021).

During the site inspection, the area was evaluated for its characteristics, including its accessibility, walkability possibilities, and visual connections. From the lookout point along County Road No. 138, the extensive size of the mine area and the resulting landscape wound were evident. The impact of the quarry could be felt not only at the settlement level but also at the landscape scale level, making it a defining visual element. The Voșlobeni quarry, located on the opposite hillside, was also visible from the lookout point. Thus, the presence of quarries in the Giurgeului Depression can be considered a characteristic landscape feature. Observing the huge soil profiles, layers, stratifications, level differences, and the perception of the "lunar landscape" and destruction amidst nature near the mine are all defining experiences (**Fig. 3.**).

Mining activities are still being carried out in the area, and their negative impacts such as noise and dust can be felt in the surrounding environment. As landscape and garden architect Attila Csemez points out, "something must be sacrificed for something". While mining operations are still ongoing and expected to continue for a few more years, it is important to plan for the eventual return of the area to nature and human use, especially considering the high-quality raw materials that were extracted from this quarry and used for urban development over the years.

In certain locations, signs of nature reclaiming the area are visible as pioneer vegetation emerges in small patches. However, to accelerate this process, human intervention is required. This can be achieved through the implementation of engineering and biological methods, as well as landscape planning interventions that promote nature-based solutions in harmony with the ecosystem. By strengthening the natural connection systems and supporting local ecosystem systems, the area can be reintroduced into the cycle of use.

Fig. 3. View above the mine in the direction of Voșlobeni (personal photo)

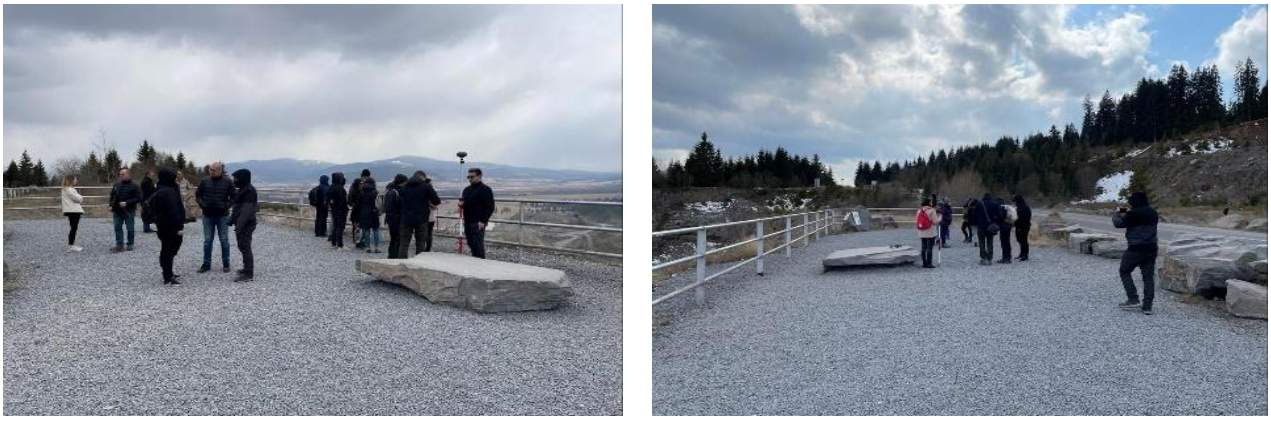


Fig. 4. Site survey (personal photos)

4.3. Analyzes

The analyses encompass a broad spectrum of scales, ranging from landscape-level analyses, such as topographical and hydrographic conditions, as well as ecosystem networks, to area studies and on-site analyses (**Fig. 4.**) that delve into more specific aspects, such as accessibility, terrain dynamics, visual relationships, (**Fig. 5.**) surrounding plant associations (**Fig. 6.**), and geological conditions (**Fig. 7.**).

4.4. Concepts and post-utilization suggestions

The post-utilization of mining areas requires careful consideration and planning. The concepts (**Fig. 8.**, **Fig. 9.**, **Fig. 10.**) and after-use suggestions should take into account the extent and location of the mining areas, as well as the economic purpose and extraction technology used.

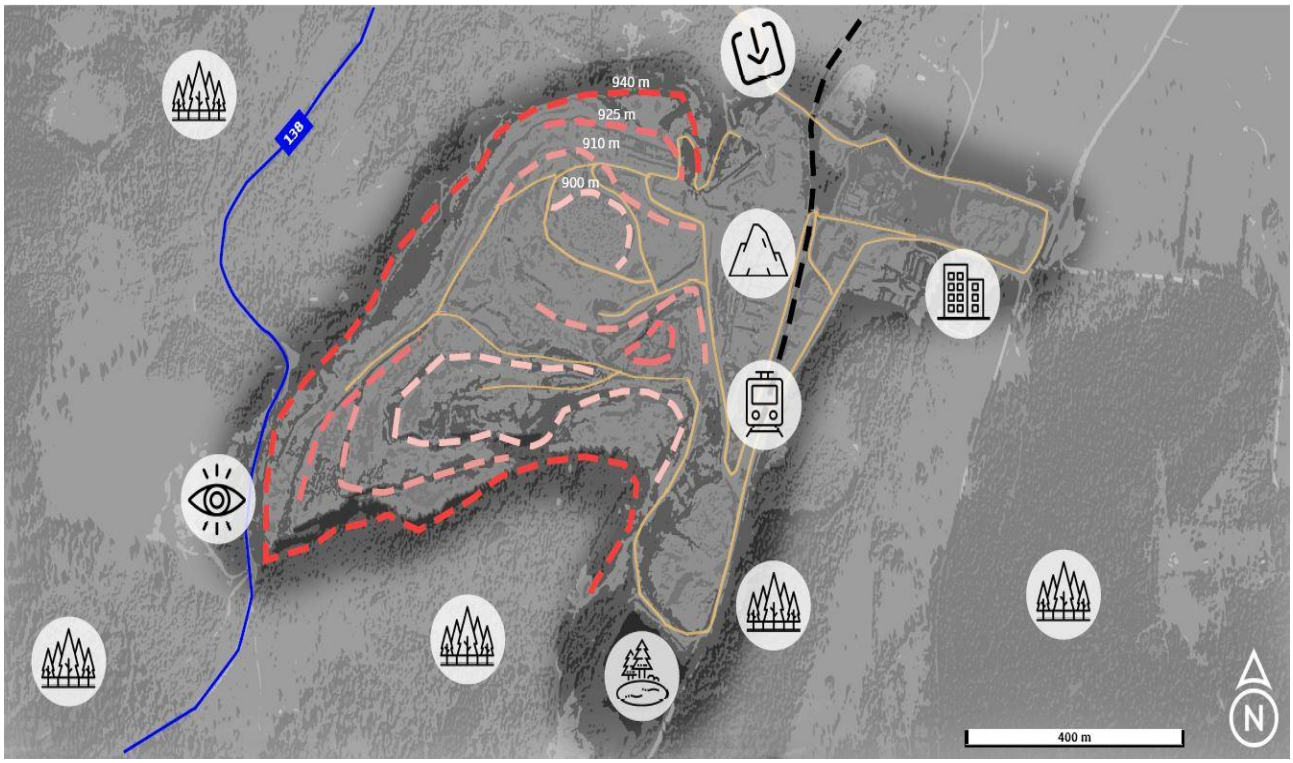
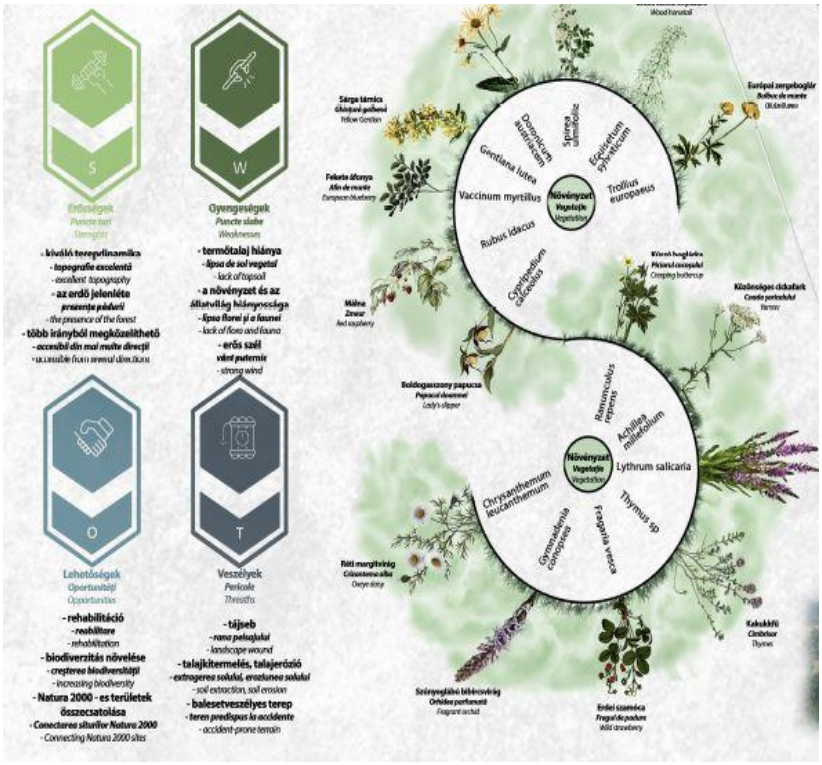


Fig. 5. Examination of the design area (from students' work, Team 2)

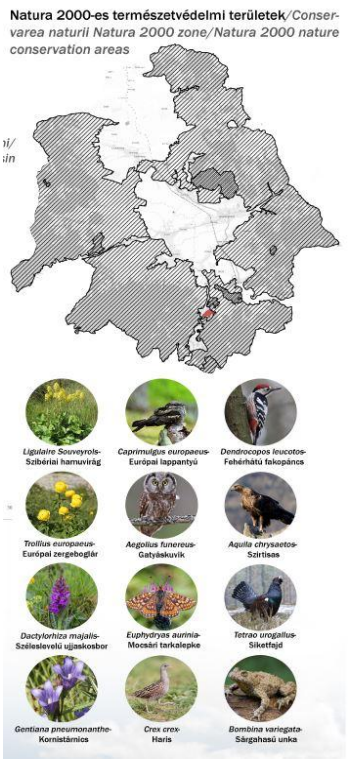
The proposed plans should be in harmony with nature and aim to manage the area in cooperation with it, serving both human and natural needs, including the protection of natural values and passing them on to future generations. The plans should include sustainable solutions that preserve and enhance the surrounding flora and fauna, and ensure a balanced and resilient ecosystem.

The function scheme (**Fig. 11.**) is a visual representation that illustrates the relationships and interdependencies between the independent spatial units within a given external space and landscape. It provides a framework for the activities carried out within these spatial units, defining their respective functions. Through the function scheme, it is possible to strategically optimize the placement of existing and proposed functions within the area. This enables efficient utilization of the available resources, while ensuring that the functions are in line with the goals of the landscape plan.

When devising post-use proposals, it was crucial to manage the area comprehensively, considering its functions to ensure optimal utilization and prolong the area's lifespan. After consulting with the municipality and assessing the local characteristics, it was deemed appropriate to incorporate some form of tourism. Accordingly, post-use proposals (**Fig. 13.**) were formulated, including a tent camp serving as a stopover on a hiking route, a nature trail, a sculpture park, a cultural center, a community space, an adventure park, LandArt installations (**Fig. 12.**), and more.



(from students' work, Team 1)



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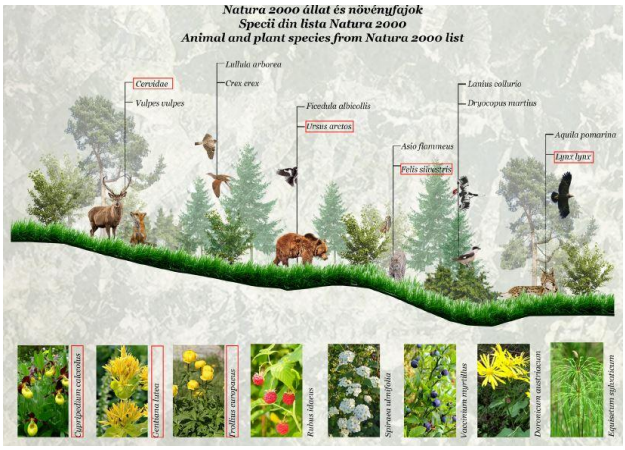
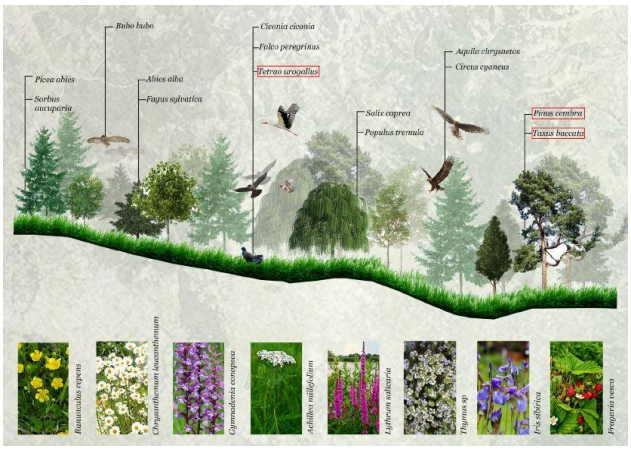


(from students' work, Team 5)



(from students' work, Team 5)

Fig. 6. Examination of surrounding Natura 2000 areas (from students' work)

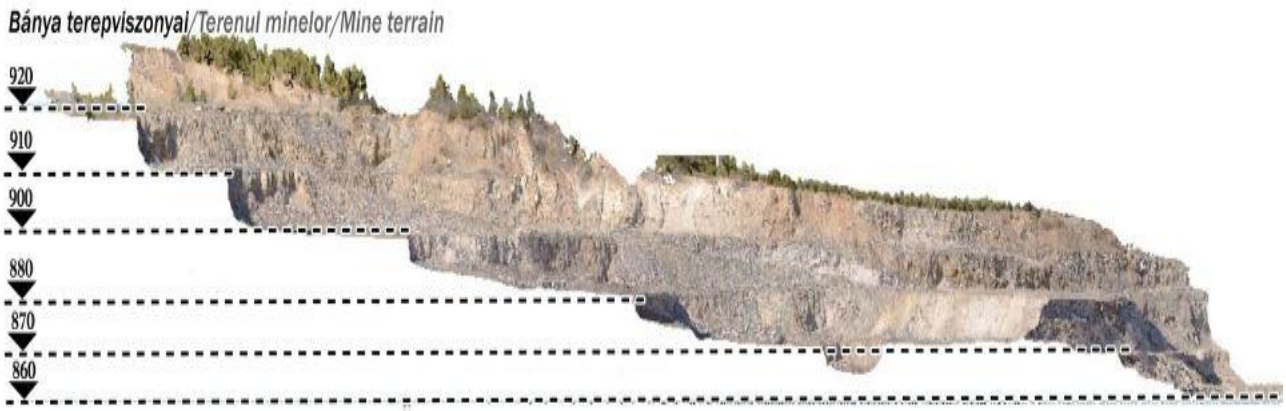


Fig. 7. Examination of the terrain conditions of the mine (from students' work, Team 4)

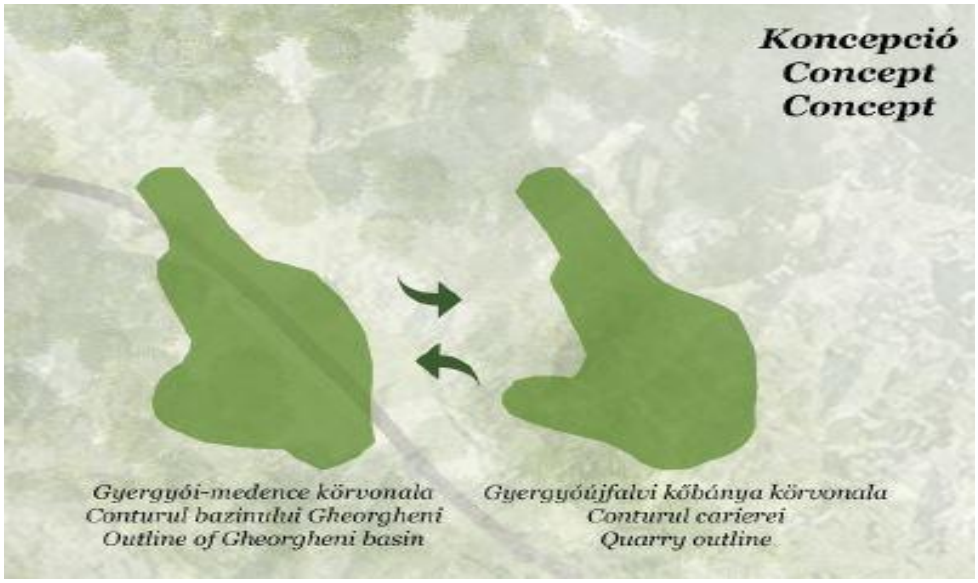


Fig. 8. Concept diagram based on analogy (from students' work, Team 5)

Fig. 9. Concept diagram, renaturalization and multi-use model (from student's work, Team 2)

Fig. 10. Concept figure, renaturalization process in several steps (from student work, Team 1)

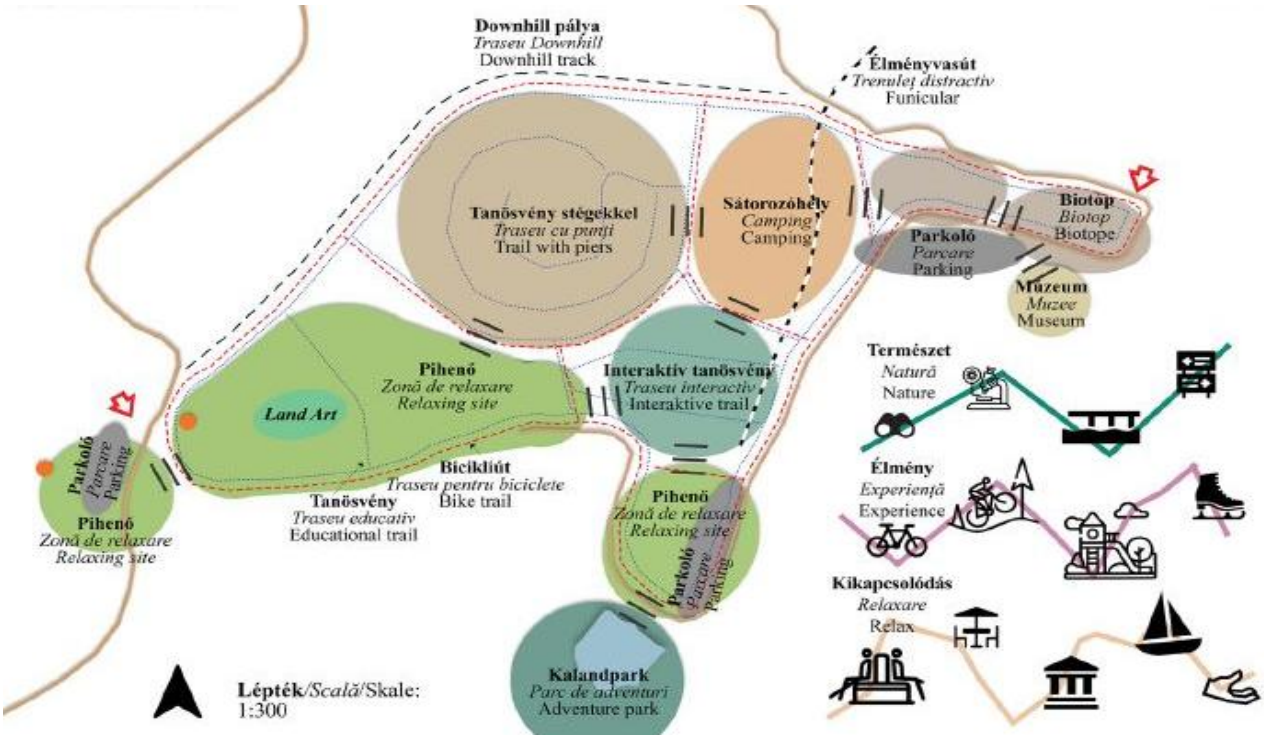


Fig. 11. Function scheme (from students' work, Team 3)

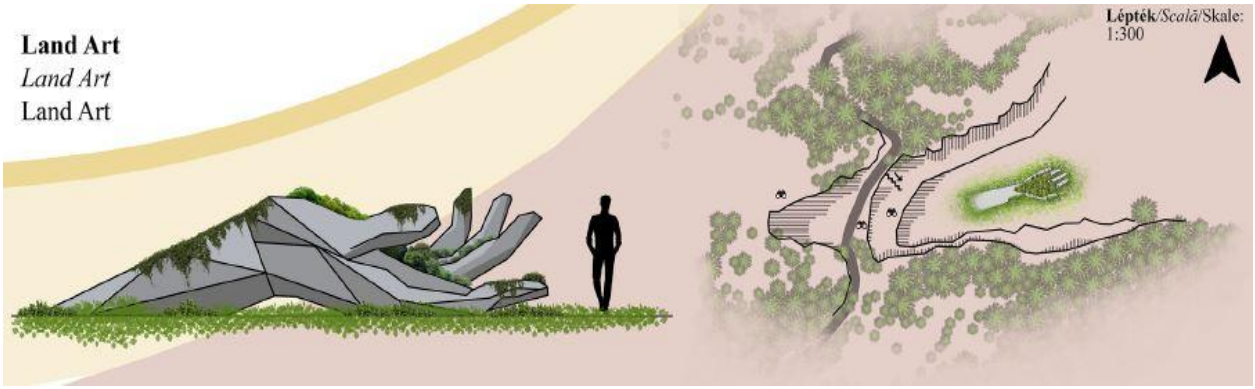


Fig. 12. Land Art, using the materials provided by the area (from students' work, Team 3)

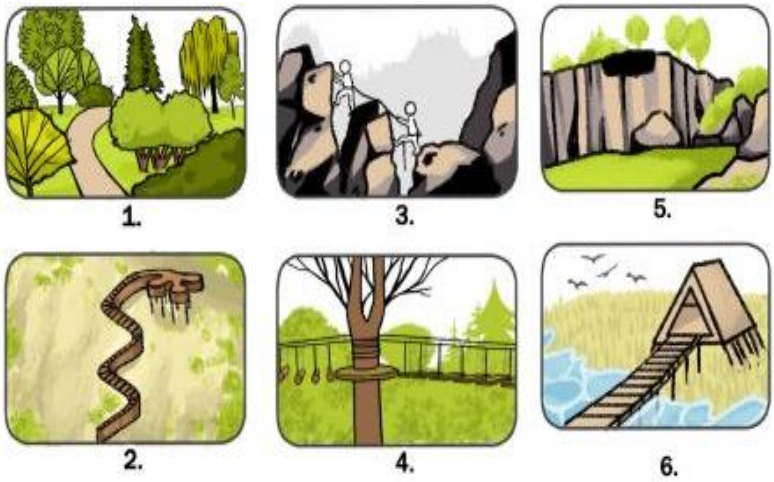


Fig. 13. Post-use proposal (from students' work, Team 4)

4.5. Results announcement, winning team

The project was developed in collaboration with the Harghita County Association, the Harghita County Council, the Mayor's Office of Suseni, the Department of Horticultural Engineering at the Faculty of Sapientia Transylvanian Hungarian University in Târgu Mureș, and the Faculty of Biology and Geology at Babeș-Bolyai University in Cluj Napoca (Maszol.ro, 2022). The award ceremony was attended at Sapientia by Csaba Borboly, the president of the Harghita County Council, who emphasized the importance of the project's focus on proximity to nature. The students examined various possibilities and created proposals for the proper utilization of several hectares of land (Borboly, 2022).

During the awards ceremony, the field work of the participating teams was briefly presented, following which the best proposals were awarded. The competition was highly competitive, with the scores of the awarded teams being very close. The team composed of Andrea András, Róbert Csutak, Erika-Andrea Kálmán, Ákos László, and Kriszta Anna Sándor won the prize for the best application (**Fig. 14.**). The motto of their concept and post-utilization proposal was "From dust to clean air", which effectively describes the current state of the area characterized by dust, and the desired state of clean air, which could be best provided by nature itself. Their proposal aims to connect the surrounding nature reserves by creating an arboretum-like bridge.

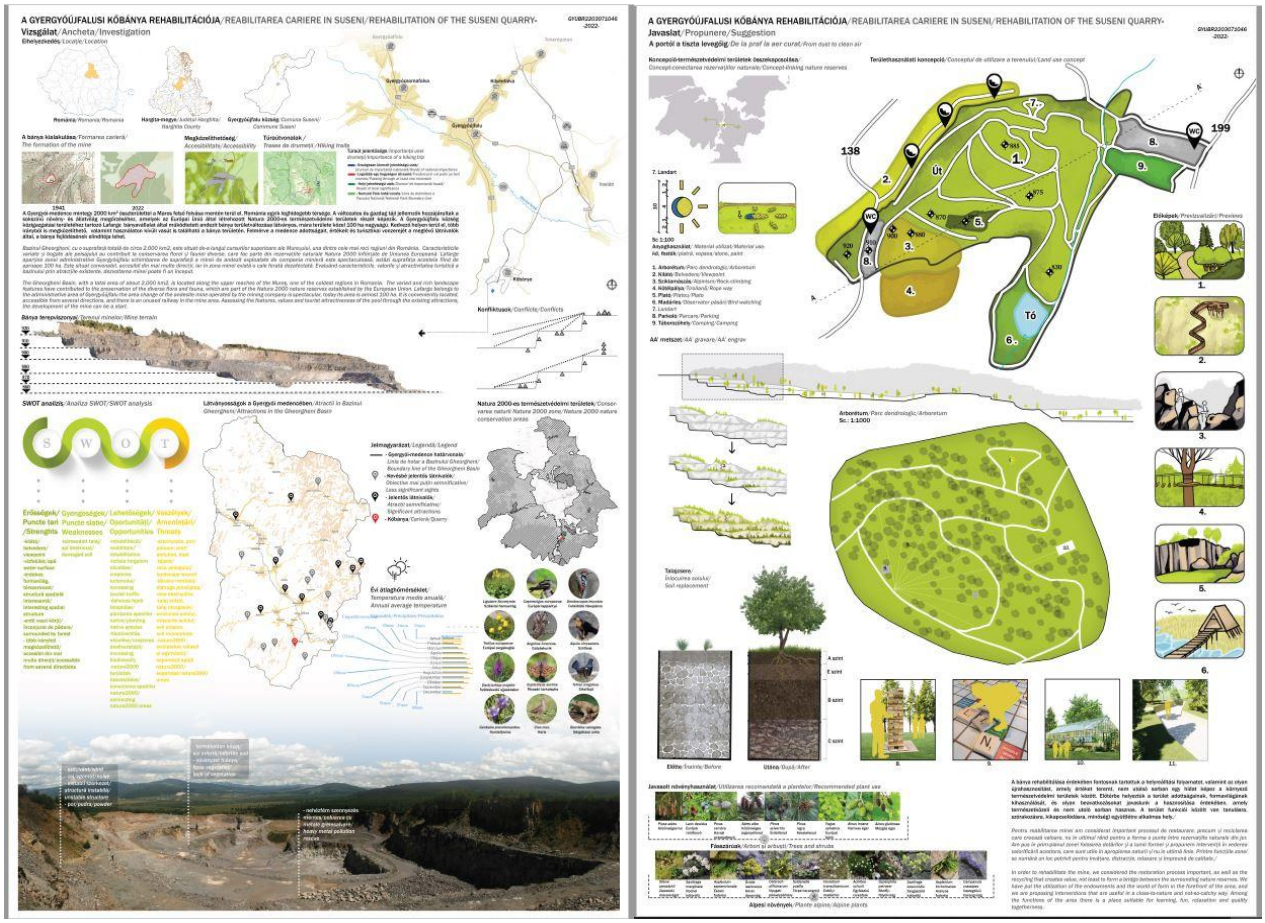


Fig. 14. Analysis and proposal poster of the winning team (from students' work, Team 4)

The second prize was awarded to the group of university students consisting of Anett-Rita Bálint, Zsuzsa Kencse, Laura-Kitty Kopacz, Ráhel Portik-Szabó, and Simó Csenge-Melánia, while the third prize went to the creative team composed of Áron Vitos, Ambrus Adrienn, Orsolya Bálint, Szabolcs Csiza, and Áron Géczi. In addition, a special prize was awarded to the application prepared by Erzsébet Hegedüs, Norbert Köllő, Siklody Szabolcs, Klementina Székely, and Helga Tóth-Pál, as well as the design application devised by Barbara Irisz Dobos, Andrea Izabella Orbán, Mónika Pál, Péter Adrienn, and Péter Bernadett (Maszol.ro).

Conclusions

It's great to see that the competition was successful and that multiple teams were awarded for their proposals. It shows that there were many innovative and thoughtful ideas that could contribute to the sustainable use and revitalization of the mining area. We hope that the winning proposals and ideas will be considered seriously and implemented in the future.

It is important to acknowledge that undergraduate students in landscape architecture and other design-related disciplines have varying learning experiences and thinking styles. Therefore, it is crucial to establish realistic targets that align with their individual capabilities and cognitive characteristics.

Based on our experience, it is of utmost importance to guide students through the entire process and enable them to establish a connection between research and design in their minds. By doing so, they will be better equipped to utilize research findings to support their design proposals and decision-making throughout their career development. This emphasis on bridging the gap between research and design empowers students to integrate these two elements effectively.

The current design competition serves as an exemplar to underscore the significance of establishing and preserving inter-disciplinary relationships as well as fostering positive communication with local authorities, all of which can be mutually advantageous. Such applications present students with opportunities to professionally test themselves, apply their knowledge and experience in real-world contexts, and cultivate their creativity.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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12. Team 2 - GYUBR2202281159 - Áron Vitos, Ambrus Adrienn, Orsolya Bálint, Szabolcs Csiza, and Áron Géczi
13. Team 3 - GYUBR2203070752 - Barbara Irisz Dobos, Andrea Izabella Orbán, Mónika Pál, Péter Adrienn, and Péter Bernadett
14. Team 4 - GYUBR2203071046 - Andrea András, Róbert Csutak, Erika-Andrea Kálmán, Ákos László, and Kriszta Anna Sándor
15. Team 5 - GYUBR2205231036 - Anett-Rita Bálint, Zsuzsa Kencse, Laura-Kitty Kopacz, Ráhel Portik-Szabó, and Simó Csenge-Melánia

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LIVERWORTS AND MOSSES FROM ROMANIA WITH MEDICINAL POTENTIAL

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Abstract: Liverworts and mosses are terrestrial plants that contain biologically active substances that give them important medicinal qualities. After reviewing the available literature on the pharmacological activity of the most used liverworts and mosses, we present 34 species found in Romania, used for the treatment of common diseases in folk medicine around the world. Their uses in traditional medicine are sometimes confirmed by pharmacological research, especially external ones (as antimicrobial or cytotoxic remedies). The species of liverworts and mosses are mentioned with their distribution in Romanian flora and the habitats where they live.

Keywords: liverworts, mosses, Romania, medicinal species

Introduction

Liverworts and mosses (bryophyte) are non-vascular plants, separated into a distinct phyla: Anthocerophyta (hornworts), with approximately 200 - 250 species (Villarreal et al., 2010), Marchantiophyta (hepatic), with approximately 7000 - 9000 species (Von Konrat et al., 2010) and Bryophyta (mosses), with approximately 11,000 -13,000 species (Magill, 2010). Some studies mention that out of the approximately 450,000 species of plants, bryophytes with 20,000 – 25,000 species are the second largest group, as species, after angiosperms (Mishra et al., 2014).

They are lesser-known, small-sized plants, with morphological identification characters that are more difficult for most people to notice, but which contribute to a greater or lesser extent to the composition of the terrestrial globe's vegetal carpet, dominated by flowering plants.

Bryophytes have been used as medicinal plants in China and North America as early as 400 years ago (Asakawa et al., 1980; Benek et al., 2022). Phytochemical studies on several bryophyte species have found that they contain biologically active compounds (lipids, proteins, polyphenols, terpenoids, organic acids, fatty acids, etc.) with various bioactivities, including the antibacterial, antitumor, antifungal and insecticidal ones (Glime, 2007). Some researchers have also described their myorelaxant effects on the smooth muscles of the organs in the abdominal cavity, on the bronchioles, as their potential use in the fight against obesity (Saxena and Yadav, 2018;

Bukvički et al., 2012; Purkon et al., 2022; Motti et al., 2023).

Since people still today, in the first phase, turn to medicinal plants for the relief or treatment of various diseases, it is understandable why the research of these species can be considered a priority, both from a medicinal point of view and from the perspective of protecting biodiversity.

In Romania ethnopharmacology the medicinal use of plants was mostly documented by Dihoru and Boruz (2016) without mentioning the bryophyte species, by Butură (1979) and Alexan et al. (1983) which presents data only on *Polytrichum commune*, *Funaria hygrometrica* and *Pogonatum urnigerum* as species of medicinal bryophytes.

In this paper we aim to complete the list of medicinal plants from Romania with species of mosses and liverworts used for medicinal purposes in countries with a tradition both in folk medicine and in pharmacological research of this fascinating group of plants. The selection of medicinal species from the Romanian bryoflora was made from an approximate number of 979 species, of which: 4 hornworts, 217 liverworts and 758 mosses (Ștefănuț and Goia, 2012).

Materials and Methods

Information about bryophytes and their medicinal properties was gathered by searching scientific databases such as: PubMed, Elsevier, Google Scholar, Springer, Scopus and in similar online and offline books. The following keywords were used in the investigations: "ethnobotany", "ethnomedicine", "ethnobryology", "medicinal bryophytes", "ethnopharmacology", phytotherapy", "medicinal", "ailments".

In a first stage, 179 articles were selected, based on their titles and summaries, identified by using the keywords mentioned above. Subsequently, the study focused only on the articles that contained information on medicinally important bryophyte species, including their content in biologically active substances. In the end, the review was narrowed down to 80 articles that corresponded to the purpose of the work, to identify the bryophyte species with medicinal potential that grow in Romania.

The classification of mosses species is according to Goffinet et al. (2009) and of liverworts is according to Crandall-Stotler et al. (2009) and the nomenclature follows the World Flora Online. Their chorology in Romania is according to Mihai et al. (1998).

Results and Discussions

Bryophytes are early and primitive plants, diversified in a hostile environment, during the Upper Ordovician - Silurian phase of the primary radiation of terrestrial biota (Bateman et al., 1998), possessing secondary metabolites with an important role in biology, ecology and evolution them (Peters et al., 2018).

Life on Earth developed through the interaction of plants, animals, and microorganisms, and it is only natural that the secondary metabolites of bryophytes have medicinal qualities, as do those of vascular plants.

To date, biologically active compounds (lipids, proteins, polyphenols, terpenoids, organic acids, fatty acids, diterpenoids, bibenzyl, bis-bibenzyl, polyketides, etc.) with antibacterial, antitumor, antifungal, insecticidal activity have been reported (Glime, 2007; Novaković et al., 2021).

By consulting the bryological literature, 34 species of medicinal bryophytes for human use were identified from 979 species of Romanian bryoflora. These species are separated into a distinct phyla: Marchantiophyta and Bryophyta (mosses) and grouped into two categories in the

Red List of treated species: Almost Threatened (NT) (*Ditrichum pallidum*) and Least Concern (LC) the other 23 species (Ștefănuț and Goia, 2012).

Marchantiophyta (liverworts)

1. *Conocephalum conicum* (L.) Underw., fam. Conocephalaceae, in traditional medicine, is used to cure cuts, burns, scalds, and fractures, swollen tissue, snake bites, gallstones, jaundice, as antimicrobial, antifungal and antipyretic (Asakawa, 1998, 2007, 2015; Asakawa et al., 2013; Haris, 2008; Alam, 2012). According to Asakawa (1998) the species contains guianolides that showed antitumor activity against P-388 lymphocytic leukemia. The research conducted by Negi et al. (2020) showed good antifungal activity for the acetone extract of *Conocephalum conicum* (collected from Kumaon region of Western Himalaya: altitude 1400 m and 2100 m) against *Aspergillus flavus* and *Aspergillus parasiticus* species (aflatoxigenic species). The authors identified 30 main compounds in the acetone extract (riccardin C, citronellol, geranylgeraniol, phytol, spathulenol, globulol, steroids, fatty acids, etc.).

Methanolic extracts mainly contain monoterpene esters, sesquiterpene lactones and phenethyl glycosides, but do not contain macrocyclic bis-bibenzyls (Ivković et al., 2021). The species is used ethnomedicinally in China, India, Italy (Motti et al., 2023).

In Romania, it is found in the regions of Banat, Bucovina, Maramures, Moldova, Muntenia, Transylvania, Oltenia, distributed in moist places on the ground, on the humus on stones and rocks, on the side of the roads. The thallus emits an aromatic odor when broken. It is included on the Red List of treated species as Least Concern (LC) taxon.

2. *Frullania tamarisci* (L.) Dumort., fam. Frullaniaceae, is used ethnomedicinally in China as an antiseptic (Asakawa, 1998, 2007; Haris, 2008). The dietyl eter extract contains monoterpenes, sesquiterpenes hydrocarbons and lactones and oxygenated sesquiterpenes (Ludwiczuk and Asakawa, 2021).

In Romania it is found in Banat, Bucovina, Moldova, Muntenia, Oltenia, Transylvania.

It grows in the form of cortico-saxicolous mats. It is included on the Red List of treated species as Least Concern (LC) taxon.

3. *Marchantia polymorpha* L., fam. Marchantiaceae, is used ethnomedicinally for liver diseases, as well as for pulmonary tuberculosis, cardiovascular diseases, bladder stones, skin inflammations, insect bites, boils, abscesses and pimple eruptions, fractures, poisonous snake bites, burns, scalds and open wounds (Asakawa, 1998; Glime, 2007; Haris, 2008; Asakawa et al., 2013; Wang et al., 2016). It is used ethnomedicinally in Brazil, China, India, Europe (Estonia) (Glime, 2007; Motti et al., 2023).

Contains the sesquiterpenoids costunolide and tulipinoid (Kanasaki and Ohta, 1976; Glime, 2007); flavonoids (Wang et al., 2016); bis-bibenzyls (marchantin A, B, C, D, E, F, G, J, L, neomarchantin A, riccardin D, etc.) (Asakawa, 2017); and fatty acids (Lu et al., 2019). The following groups of compounds were identified in the methanolic extracts: terpenes, oils, sugars and bis-bibenzyls (marchantin A as one of the most dominant). These extracts showed antimicrobial activity against Gram positive bacteria (Ivković et al., 2021).

Various biological activities are reported for this species in the available literature: antipyretic, antidotal, diuretic (Askawa, 1998; Askawa et al., 2013); antioxidant activity (Wang et al., 2016); antifungal (*Candida*), antiviral, cytotoxic and apoptotic, cardiotonic, muscle relaxant, antioxidant, calcium inhibitor, inhibition of nitric oxide production and antitrypanosomal activity (Asakawa, 2017).

In Romania it is widespread in Banat, Bucovina, Dobrogea, Maramures, Moldova, Muntenia, Transylvania, Oltenia, Dobrogea, distributed on clayey, moist and shady soils, sometimes in swamps. It is included on the Red List of treated species as Least Concern (LC) taxon.

4. *Reboulia hemisphaerica* (L.) Raddi, fam. Aytoniaceae, is used ethnomedicinally to stop bleeding, treat wounds and bruises in China (Asakawa, 1998; 2007; Abay, 2011). The ethanol extract contains as the main biologically active substances terpenic and bis-bibenzyl phenolic compounds (riccardin C, marchantin C, M, N, O, marchantiaquinone) with antimicrobial, anticancer, antifungal, antiviral, cytotoxic, antioxidant, anti-inflammatory, analgesic effects, myorelaxant antiobesity and wound healing activities (Tosun et al., 2016; Asakawa, 2017).

In Romania it is spread in Banat, Dobrogea, Maramures, Muntenia, Transylvania, Oltenia, distributed on calcareous rocks. It is included on the Red List of treated species as Least Concern (LC) taxon.

5. *Riccardia multifida* (L.) Gray, fam. Aneuraceae. The methanol extract contains macrocyclic bis-bibenzyl derivatives, riccardin A and B, which inhibited KB cells at a concentration of 10 and 12 μg/ml, respectively (Alam, 2012). It is medicinally active for antileukemic and stomach pain and swelling in cattle (Azuelo et al., 2011; Alam, 2012).

In Romania it is found in Bucovina, Moldova, Muntenia, Transylvania, distributed in open spaces, on rotten logs, wet rocks, wet soil, swamps and peat bogs. It is included on the Red List of treated species as Least Concern (LC) taxon.

6. *Riccia fluitans* L., fam. Ricciaceae, is useful in healing wounds (Tosun et al., 2016) and infections (Lawarence et al., 2023). The methanol extract contains phytosterol mixtures and acetylene fatty acids (Asakawa, 2004) saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, acetylenic acids (Lu et al., 2019).

In Romania it is widespread in Banat, Dobrogea, Maramures, Moldova, Muntenia, Transylvania. It is a floating plant that grows in moist marshy places, preferring calm waters. It is included on the Red List of treated species as Least Concern (LC) taxon.

7. *Diplophyllum taxifolium* (Wahl.) Dum., fam. Scapaniaceae. The methanol extract contains essential oil with diplophyllin. Diplophyllin shows cytotoxic activity against human epidermoid carcinoma (Bandyopadhyay and Dey, 2022). In Romania it is widespread in Bucovina, Maramures, Muntenia, Transylvania, distributed on stone, siliceous rocks, in the mountain area. It is included on the Red List of treated species as Least Concern (LC) taxon.

Bryophyta (mosses)

1. *Atrichum undulatum* (Hedw.) P. Beauv, fam. Polytrichaceae. The chloroform/methanol extract contains: sterols (major: 24-methylcholesterol and 24-ethyl-22-dehydrocholesterol), carotenoids (-carotene, lutein, violaxanthin, and neoxanthin) (Dembitsky, 1993), fatty acids (major: linoleic acid, -linolenic acid, palmitic acid, oleic acid, and arachidonic acid) (Pejin et al., 2012), coumarin glycosides (Jung et al., 1994). It has an antimicrobial effect against the bacterial species *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Enterobacter cloacae, Listeria monocytogenes, Bacillus cereus, Micrococcus flavus* and *Staphylococcus aureus* (Sabovljevic et al., 2010). The anticancer effect is also mentioned in chinese ethnomedicine (Du, 1997).

In Romania it is found in Banat, Bucovina, Dobrogea, Maramures, Moldova, Muntenia, Transylvania, distributed on acidic clayey or

sandy soils, on calcareous soils in forests, from hilly to mountainous areas. It is included on the Red List of treated species as Least Concern (LC) taxon.

2. *Barbula unguiculata* Hedw., fam. Pottiaceae, has been used as an analgesic and decrease fever (Chandra et al., 2017). It is used in traditional medicine in India and the United States (Haris, 2008; Lubaina et al., 2014). There are no chemical or pharmacological studies on this species. (Vollár et al., 2018).

In Romania it is found in Banat, Moldova, Transylvania, Oltenia, Dobrogea, distributed in lowlands, hills, less in the mountains, on the ground, on fields, on roadsides, sometimes through forests, on walls, rarely on stones and rocks. It is included on the Red List of treated species as Least Concern (LC) taxon.

3. *Bartramia ithyphylla* Brid., fam. Bartramiaceae. The methanol and acetone extract contains macrocyclic biflavonoid (Marko et al., 2001) and is used in traditional Chinese medicine to suppress fear, calms nerves, irregular heartbeat, epilepsy, apoplexy (Du, 1997).

In Romania it is found in Bucovina, Maramures, Moldova, Muntenia, Oltenia, Transylvania, distributed on sandy soil with humus in forests, through the cracks of siliceous rocks and in the mountainous area. It is included on the Red List of treated species as Least Concern (LC) taxon.

4. *Bryum argenteum* Hedw. Fam. Bryaceae. The ethanolic extract contains flavonoids with antimicrobial activity against various bacterial (*Escherichia coli, Staphilococcus aureus, Bacillus subtilis, Micrococcus luteus*) and fungal strains (*Aspergillus niger, Penicillium ochrochloron, Candida albicans, Trichophyton mentagrophyes*) strains (McCleary et al., 1960; Karpiński and Adamczak, 2017; Markham and Given, 1988). *B. argenteum* showed the highest antimicrobial activity for *E. coli* and *S. aureus* (Vollár et al., 2018).

This species has also been used as an antidotal, antipyretic and antirhinitis treatment (Alam, 2012; Asakawa, 1998, 2015; Asakawa et al., 2013).

It is used in traditional Chinese medicine (Haris E S, 2008). In Romania it is found in Banat, Bucovina, Dobrogea, Moldova, Muntenia, Oltenia, Transilvania from the lowland to the alpine area. It is distributed on cultivated and uncultivated land, on sandy soils, sea dunes, rocks covered with earth, in rock cracks and on roofs. It is included on the Red List of treated species as Least Concern (LC) taxon.

5. *Ptychostomum capillare* (Hedw.) D.T.Holyoak & N.Pedersen., fam. Bryaceae, is used in traditional medicine in the United States for activity against fire sickness, fever, and body aches (Motti et al., 2023). It has antimicrobial, antibiofilm, antioxidant, antigenotoxic and anticancer activities. The ethanolic extract has reduced amounts of ascorbic acid and α-tocopherol (Onbasli and Yuvali, 2021).

In Romania it is found in Banat, Bucovina, Dobrogea, Maramures, Moldova, Muntenia, Oltenia, Transylvania, distributed in forests, in rock cracks, rarely at the base of tree trunks, starting from low to subalpine regions. It is included on the Red List of treated species as Least Concern (LC) taxon.

6. *Climacium dendroides* (Hedw.) F. Weber & D. Mohr, fam. Climaciaceae, is used in traditional Chinese medicine to clear heat, eliminate dampness, relax muscles, rheumatism, and bone and muscle pain (Motti et al., 2023). *Climacium dendroides* contains: fatty acids, monoglycerols, terpenoids, alcohols, sterols, diterpenes, alkanes, wax esters, triterpenes, steroids, polyphenols, amino acids (Klavina et al., 2015). Ethanolic extracts demonstrated pronounced antibacterial activity

against *Bacillus cereus* and *Escherichia coli* species and antiproliferative activity on various animal and human cancer cell lines (Klavina et al., 2015). In Romania it is very widespread in Transylvania, Bucovina, Moldova, Muntenia and to a lesser extent in Banat, Oltenia and Maramureș. It is distributed on the ground in places with high humidity, often near lakes, swamps, in hygrophilous meadows and through forests, rarely at the base of wet trees. It is included on the Red List of treated species as Least Concern (LC) taxon.

7. *Cratoneuron filicinum* (Hedw.) Spruce, fam. Amblystegiaceae, has antibacterial activity evidenced by methanolic extracts obtained from biological material collected from Derventa (Serbia) (Bukvički et al., 2012). It has ethnomedicinal use in China for calming and soothing, heart problems - used for malum cordis (heart disease) in the Western Himalayas (Alam et al., 2015; Asakawa et al., 2013).

In Romania it is found in Banat, Bucovina, Maramures, Moldova, Muntenia, Transylvania, Oltenia. It grows in sparse mats on calcareous substrate, in very wet, floodable places, on the edge of water, in swamps, on stones and at the base of trees near water, in hilly and mountainous regions. It is included on the Red List of treated species as Least Concern (LC) taxon.

8. *Dicranum majus* Turner, fam. Dicranaceae, has ethnomedicinal use in China for clearing lungs and stops cough (Motti et al., 2023). Dry 70% ethanol extracts of *Dicranum majus* have anti-inflammatory effect (Marques et al., 2022).

In Romania it is widespread in Bucovina, Maramures, Transylvania, distributed on siliceous rocks, on moist soil, rotten trunks, in mountain forests and in the subalpine layer. It is included on the Red List of treated species as Least Concern (LC) taxon.

9. *Dicranum bonjeanii* De Not., fam. Dicraniaceae, is used ethnomedicinally in Canada and the United States as an absorbent (Motti et al., 2023). There are no chemical or pharmacological studies on this species.

In Romania it is widespread in Banat, Bucovina, Maramures, Muntenia, Transylvania. It prefers to grow in eutrophic swamps and calcareous habitats, while avoiding acidic substrates. It is included on the Red List of treated species as Least Concern (LC) taxon.

10. *Ditrichum pallidum* (Hedw.) Hampe, fam. Ditrichaceae, is used ethnomedicinally in China and India for convulsions, particularly in infants (Asakawa, 1998, 2007; Haris, 2008; Asakawa et al., 2013). There are no chemical or pharmacological studies on this species.

In Romania it is widespread in Banat, Moldova, Oltenia, Transylvania, distributed on the ground among calcareous rocks. It is included on the Red List of treated species as Almost Threatened (NT) taxon.

11. *Funaria hygrometrica* Hedw., fam. Funariaceae, is used ethnomedicinally in China and Germany for pulmonary tuberculosis, hemostasis, bruises, skin infections, athlete's foot dermatophytosis, blood vomiting, light sedative, nose inflammation and sinusitis, alopecia (Asakawa, 2007; Haris, 2008; Asakawa et al., 2013; Chandra et al., 2017). In Romania, it is used ethnomedicinally for its diuretic, sudorific, astringent and expectorant properties (Alexan et al., 1983).

The methanol, chloroform, and acetone extracts obtained from this species contain terpenoids and alkaloids with antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus* (Savaroglu et al., 2011).

In Romania it is widespread in Banat, Bucovina, Dobrogea, Maramures, Moldova, Muntenia, Transylvania, Oltenia, distributed on various substrates in mats, on the ground, fields, uncultivated places, in forests, through clearings, on dry sands, sometimes in marshes, on sea dunes, stones and rocks covered with

soil, rarely on rotten wood or at the base of trees. It is common in all areas. It is included on the Red List of treated species as Least Concern (LC) taxon.

12. ***Homalothecium sericeum*** (Hedw.) Schimp., fam. Brachytheciaceae, has medicinal activity as antimicrobial, antioxidant and insecticidal (Ozturk et al., 2018; Çolak et al., 2011).

The acetone extract of *Homalothecium sericeum* has highest antibacterial activity against *Pseudomonas aeruginosa* (Oztopcu-Vatan et al., 2011).

In Romania it is widespread in Banat, Bucovina, Dobrogea, Maramures, Moldova, Muntenia, Transylvania, Oltenia, distributed on sunny rocks, sparse forests on tree trunks. It is included on the Red List of treated species as Least Concern (LC) taxon.

13. ***Hypnum cupressiforme*** Hedw., fam. Hypnaceae. The ethanol, methanol, acetone and chloroform extracts contain biologically active compounds, such as flavonoids, phenolic acids and triterpenoids with complex antimicrobial, antioxidant, antifugic medicinal activity (Lunić et al., 2020; Çolak et al., 2011). Antimicrobial activity is against *Bacillus subtilis* and *Staphylococcus aureus* species (Savaroglu et al., 2011; Ertürk et al., 2015).

In Romania it is spread in Banat, Bucovina, Dobrogea, Maramures, Moldova, Muntenia, Transylvania, Oltenia, distributed on the ground, at the base of tree trunks, rotting wood, on stones and rocks covered with earth, in sess forests, hills, mountains and in the subalpine areas. It is included on the Red List of treated species as Least Concern (LC) taxon.

14. ***Hylocomium splendens*** (Hedw.) Schimp, fam. Hylocomiaceae, is used ethnomedicinally in Canada and Italy as a poultice for treating wounds (sores) (Motti et al., 2023).

The volatile oil extracted from *Hylocomium splendens* showed antimicrobial activity against *Escherichia coli, Yersinia pseudotuberculosis, Staphylococcus aureus, Enterococcus faecalis, Bacillus cereus, Mycobacterium smegmatis* and *Candida albicans* species (Cansu et al., 2013; Klavina et al., 2015) identified the following groups of substances from *Hylocomium splendens* extracts (collected from Latvia): fatty acids, monoglycerols, terpenoids, alcohols, sterols, diterpenes, alkanes, wax esters, triterpenes, steroids, polyphenols. amino acids. According to the cited authors, the ethanolic extracts demonstrated antibacterial activity against *Bacillus cereus* and *Pseudomonas aeruginosa* species.

In Romania it is widespread in Banat, Bucovina, Maramures, Moldova, Muntenia, Transylvania, Oltenia. It grows on acid soil, rocks, rotting trunks, in more or less lighted places, in mountain forests, in junipers and the alpine layer, rarely in the region of high hills. It is included on the Red List of treated species as Least Concern (LC) taxon..

15. ***Philonotis fontana*** (Hedwig) Brid., fam. Bartramiaceae, is used ethnomedicinally in China as antipyretic, drawing out toxins, sore throat, diuretic, urinary obstructions (Chandra et al., 2017). The ethanol extract obtained from *Philonotis sp*. contains flavonoids and carotenoids (Marko et al., 2001). Asakawa (1998) and Asakawa et al. (2013) mentions the species with antipyretic, antidotal activity, for andenopharyngitis.

In Romania it is widespread in Banat, Bucovina, Maramures, Moldova, Muntenia, Transylvania, Oltenia. It always grows in a wet place (in springs, spring streams), rocks with drainage in the mountain area. It is included on the Red List of treated species as Least Concern (LC) taxon.

16. ***Plagiomnium cuspidatum*** (Hedw.) T.J. Kop., fam. Mniaceae. The methanol extract contains sesquiterpenoids (Suire et al., 2000). This species demonstrated antimicrobial

activity (against *Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis*) (Yildirim Akatin et al., 2022) and antiproliferative activity (against cancer cell lines) (Vollár et al., 2018).

In Romania it is widespread in Banat, Bucovina, Dobrogea, Moldova, Muntenia, Transylvania, Oltenia, distributed on the ground, at the base of tree trunks, on exposed roots, in forests, sometimes in meadows, on humus on moist and shaded rocks, in hill and mountain regions. It is included on the Red List of treated species as Least Concern (LC) taxon.

17. ***Plagiopus oederianus*** (Sw.) H. A. Crum et L. E. Anderson, fam. Bartramiaceae, is used ethnomedicinally in China as a sedative, in epilepsy, apoplexy, cardiovascular diseases (Asakawa et al., 2013). It is not studied chemically and pharmacologically. In Romania it is widespread in Transylvania, Moldova and sporadically in Maramures, Muntenia, Oltenia. It grows on moist calcareous, sometimes siliceous rocks in mountain and alpine regions. It is included on the Red List of treated species as Least Concern (LC) taxon.

18. ***Pogonatum urnigerum*** (Hedw.) P. Beauv. Fam. Polytrichaceae, is cited as antifungal agent (Asakawa, 1998). In Romania, it is mentioned ethnomedicinally as a useful species against rheumatism (Alexan, 1983; Butură, 1979). It is little studied chemically and pharmacologically. Lu et al. (2023) studied the long-chain polyunsaturated fatty acid profile of *Pogonatum urnigerum* collected from Iceland.

In Romania it is spread in Banat, Bucovina, Maramures, Moldova, Muntenia, Oltenia, Transylvania. It grows on acid, dry or moist, light soils, in forests in hilly and mountainous areas. It is included on the Red List of treated species as Least Concern (LC) taxon.

19. ***Polytrichum commune*** Hedw., fam. Polytrichaceae, is used ethnomedicinally in Canada, China, Germany, Ecuador, India, Guatemala, United Kingdom as anti-inflammatory and antidotal, hemostatic, gallbladder and kidney, stones, to speed up the birth of a baby during childbirth, to strengthen hair (Glime, 2007, Asakawa, 2015; Bandyopadhyay and Dey, 2022). Alexan et al. (1983) mentions the use of this species in traditional Romanian medicine for its diuretic, sudorific, astringent and expectorant properties.

The methanol extract from *Polytrichum commune* contains luteolin, quercetin, astragalin, rutin (Nam et al., 2008) sterols, terpenoids, fatty acids, polyphenolics, carbohydrates, amino acids (Klavina et al., 2015). Ethanol extracts have high antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus* and antiproliferative activity on different animal and human cancer cell lines (Klavina et al., 2015).

In Romania it is widespread in Banat, Bucovina, Maramures, Moldova, Muntenia, Transylvania, Oltenia, distributed on acid soils, in peat bogs, in mountain forests and on the alpine area. It is included on the Red List of treated species as Least Concern (LC) taxon.

20. ***Polytrichum juniperinum*** Hedw., fam. Polytrichaceae, is used ethnomedicinally in Canada, China, India, United Kingdom for prostate diseases, urinary difficulties, sores, boils, and swelling (Glime, 2007; Motti et al., 2023).

The methanol extract obtained from this species contains anthraquinone derivatives, terpenoids, flavonoids, alkaloids and have demonstrated antimicrobial activity (against *Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus*) (Savaroglu et al., 2011).

Polytrichum juniperinum (collected from Latvia) contains fatty acids, monoglycerols, terpenoids, alcohols, sterols, diterpenes, alkanes, wax esters, triterpenes, steroids, polyphenols, aminoacids and the ethanolic extracts have demonstrated antiproliferative

activity on different animal and human cancer cell lines (Klavina et al., 2015).

In Romania it is widespread in Banat, Bucovina, Dobrogea, Maramures, Moldova, Muntenia, Transylvania, Oltenia, distributed in more or less lighted forests, in dry or wet resorts, sometimes on old decaying trunks, starting from the low regions to the alpine ones. It is included on the Red List of treated species as Least Concern (LC) taxon.

21. *Rhizomnium punctatum* (Hedw.) T.J. Kop., fam. Mniaceae, is used in the United States as a treatment for leg swelling (Abay, 2011; Motti et al., 2023). *R. punctatum* shows antimicrobial activity against the species *Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis* (Yildirim Akatin et al., 2022). Lu et al. (2023) studied the long-chain polyunsaturated fatty acids profile of *R. punctatum* collected from Iceland.

In Romania it is widespread in Banat, Bucovina, Maramures, Moldova, Muntenia, Transylvania, Oltenia, distributed on moist soil, often with gravel, near streams, springs in forests in the hills and mountains. It is included on the Red List of treated species as Least Concern (LC) taxon.

22. *Rhodobryum roseum* (Hedw.) Limpr., fam. Bryaceae. The ethanol extract contains piperine and methyl piperate that exert significant protective effects on cardiac myocytes (Hu et al., 2009). It is used ethnomedicinally as remedy for cardiovascular diseases, high cholesterol, being also cited for its sedative properties in China and India (Asakawa, 2007; Glime, 2007).

In Romania it is widespread in Bucovina, Moldova, Muntenia, Transylvania, Oltenia, distributed on the ground, sometimes on gravel or wet rocks covered with earth, in shady forests, in hilly and mountainous regions. It is included on the Red List of treated species as Least Concern (LC) taxon.

23. *Sphagnum girgensohnii* Russow, fam. Sphagnaceae is used ethnomedicinally in China as a surgical dressing (Haris, 2008; Motti et al., 2023). The ethanol extract obtained from *Sphagnum girgensohnii* contains p-coumaric acid and rutin (Zych et al., 2023).

In Romania it is widespread in Banat, Bucovina, Maramures, Moldova, Muntenia, Transylvania, Oltenia, distributed in very humid depressed places in mountain forests up to the alpine area. It is included on the Red List of treated species as Least Concern (LC) taxon.

24. *Sphagnum magellanicum* Brid., fam. Sphagnaceae, is used ethnomedicinally in China and Canada for surgical dressings, diapers (Motti et al., 2023). The ethanol extract contains sterols, triterpenoids- ursolic acid, fatty acids, fatty alcohols, n-alkanes, wax ester, phenolics (Baas et al., 2000; Alam, 2021). According to Klavina et al. (2015) *Sphagnum magellanicum* contains fatty acids, monoglycerols, terpenoids, alcohols, sterols, diterpenes, alkanes, wax esters, triterpenes, steriods, polyphenols, aminoacids and the ethanolic extracts have demonstrated antibacterial activity (against species *Bacillus cereus* and *Escherichia coli*) and antiproliferative activity (on different animal and human cancer cell lines). Zyck et al. (2023) identified in *Sphagnum magellanicum* extracts: p-coumaric acid, rutin and quercetin; the extracts show strong antioxidant activity.

In Romania it is widespread in Banat, Bucovina, Maramures, Moldova, Muntenia, Transylvania, Oltenia. It grows in peat bog. It is included on the Red List of treated species as Least Concern (LC) taxon.

25. *Sphagnum palustre* L., fam. Sphagnaceae, is used ethnomedicinally in China for surgical dressing, eye diseases (Motti et al., 2023) and in Korea for several diseases such as heart pain and stroke (Nam et al., 2011). The ethanol extract contains sterols, ursolic acid, fatty acids, fatty alcohols, n-

alkanes, wax ester (Baas et al., 2000). Coumarin, caffeic acid, quercetin, astragalin, chlorogenic acid, rutin were identified in the ethanolic extract (Nam et al., 2011; Zych et al., 2023). Eom et al. (2016) reported that ethanolic extracts of *S. palustre* (collected from Korea) showed inhibitory effect on aromatase activity.

In Romania it is spread in Banat, Bucovina, Maramures, Moldova, Muntenia, Transylvania, Oltenia. It grows in moist coniferous forests and marshy meadows. It is included on the Red List of treated species as Least Concern (LC) taxon.

26. *Sphagnum squarrosum* Crome, fam. Sphagnaceae, is used ethnomedicinally in China as a surgical dressing (Motti et al., 2023; Haris, 2008). The ethanol extract from *Sphagnum squarrosum* contain p-coumaric acid, rutin and apigenin (Zych et al., 2023) .

In Romania it is widespread in Banat, Bucovina, Maramures, Moldova, Muntenia, Transylvania, Oltenia. It grows in bogs, wet places, depressed places with increased humidity, in mountain forests, in the subalpine area. It is included on the Red List of treated species as Least Concern (LC) taxon.

27. *Weisia controversa* Hedw., fam. Pottiaceae, is used ethnomedicinally in China to clear heat and relieves toxicity, nose inflammation and sinuses (Motti et al., 2023). It has also been used to treat the liver disorder (Bandyopadhyay and Dey, 2022). It is not analyzed chemically and pharmacologically.

In Romania it is widespread in Banat, Bucovina, Moldova, Muntenia, Transylvania, Oltenia, distributed on the ground, in fields, uncultivated places, wet rocks, in lowland, hilly and mountainous regions. It is included on the Red List of treated species as Least Concern (LC) taxon.

We find that of the 34 bryophyte species existing in Romania and registered as medicinal plants globally, 22 are used in China, where traditional medicine is over 4000 years old (Tan and Vanitha, 2004). Chemical and pharmacological investigation of the gametophyte extract of the species mentioned in this article and the age of use of the 22 species from China constitute serious evidence that Romanian bryophytes represent an important natural source for obtaining new drugs for the treatment of human diseases.

Conclusions

The data in the article contains 34 species of bryophytes, with ethnomedicinal use in different parts of the world: China, India, Italy, Brazil, Estonia, United States of America, Canada, Romania, Germany, Ecuador, Guatemala, Great Britain, Korea.

2. Many species of bryophytes have antimicrobian action: *Rhizomnium punctatum, Polytrichum juniperinum, Polytrichum commune, Plagiomnium cuspidatum, Hylocomium splendens, Hypnum cupressiforme, Homalothecium sericeum, Funaria hygrometrica, Climacium dendroides, Ptychostomum capillare, Bryum argenteum, Marchantia polymorpha, Conocephalum conicum.*

3. Bryophytes contain secondary metabolites with therapeutic potential in the treatment of serious ailments, common today: *Diplophyllum taxifolium* has anticancer activity against human epidermoid carcinoma, *Polytrichum juniperinum* is used in prostate diseases, and *Riccardia multifida* has antileukemic activity.

4. The ethno-medicinal properties of *Sphagnum* species recommend their use as a dressing with good and fast absorption.

5. All species with medicinal potential have a degree of vulnerability, but *Ditrichum pallidum* requires more careful protection.

Chemical and pharmacological studies can focus on species with medicinal potential that

have been less studied and are mentioned in this paper.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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