POSTER PRESENTATION ABSTRACTS

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Exploring the biotechnological capabilities of *Leucoagaricus gongylophorus* for CAZymes and FOLymes Production

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Leucoagaricus gongylophorus, a fungus from the Basidiomycota division, forms a mutualistic relationship with leaf-cutting ants Atta and Acromyrmex. While it's known to produce carbohydrate-active enzymes (CAZymes) and lignin-oxidizing enzymes (FOLymes) within ant nests, details about its independent growth and enzyme production are scarce. This study focuses on *L. gongylophorus* strain LEU18496, symbiont of Atta mexicana, to evaluate its potential in enzyme production. Cultures were grown with various carbon sources—glucose, carboxymethylcellulose (CMC), xylan, and pectin for 35 days. Growth occurred on all sources, with glucose yielding the highest biomass (8.6 g/L). Secretome analysis showed varied protein concentrations, with pectin cultures reaching 242.4 µg/mL. Enzyme activity for endoglucanase, xylanase, and pectinase was measured at intervals. Endoglucanase peaked at day 14 in CMC cultures (45.8 mU). Xylanase peaked at day 21, while pectinase activity followed similar trends. Future plans involve proteomic analysis using mass spectrometry to identify CAZymes and FOLymes, correlating this data with enzyme production potential. This research sheds light on *L. gongylophorus*'s biotechnological significance in enzyme production, crucial for industries like biorefineries.

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The Relevance of Cannabinoid Receptor Ligands in Alzheimer's Disease Management

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Despite more than a century since its discovery, Alzheimer's disease (AD) remains a major healthcare challenge without effective treatments. The potential of Cannabis sativa in neurodegenerative disorders is promising, with the endocannabinoid system (ECS) being a crucial target for neuroprotection. This research evaluated the effects of chronic, intermittent exposure of two cannabinoid receptor ligands (JWH-133 and Cannabix-ir® Medium Flos—an EU GMP-certified batch of Cannabis sativa L.) on recognition memory, brain metabolism, and ECS modulation in APP/PS1 mice. The animals were treated with cannabinoid ligands, alone or combined with donepezil, over 90 days. Cognitive performance, brain metabolism, and ECS modulation were assessed. Post-testing, animals underwent immunohistochemistry analysis. The study demonstrated that chronic therapy partially reversed recognition memory impairment and decreased anxiety-like behavior. Additionally, there was a reduction in Aβ plaque deposits, decreased cerebral glucose metabolism and lower CB2 receptor expression. Enlarged astrocytes and enhanced M1mAChR expression were also observed. The findings underscore the crucial role of the extended ECS in cognitive decline and AD pathology, providing essential preclinical evidence for further exploration of cannabinoid receptor ligands in AD treatment.

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Antibacterial effect of recombinantly expressed peptide Salivaricin B

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The search for novel antibacterial peptides has become increasingly important in the face of rising antibiotic resistance, which poses a significant threat to public health worldwide. In this study, we focused on Salivaricin B, a lantibiotic peptide with promising antibacterial properties. We successfully produced

Salivaricin B through genetic modification techniques that allowed its recombinant expression.

To evaluate the efficiency of recombinant peptide production, we compared the growth and cultivation conditions of genetically modified *Escherichia coli* and *Streptococcus salivarius* K12, the host organisms used in the production process. Our results revealed significant differences in the growth patterns of these bacterial strains, with *E. coli* exhibiting more robust growth under the experimental conditions.

In addition, the antibacterial activity of Salivaricin B was tested against various bacteria to assess its antagonistic effects. The investigations confirmed a significant lantibiotic activity of Salivaricin B, which inhibited the growth of several microorganisms.

These results highlight the potential of Salivaricin B as a novel antibacterial agent and provide important insights for the development of future antimicrobial therapies.

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Effect of Modification of Temporin a with Dab and Dap on Photo- and Cytotoxicity and Antiproliferative Activity

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Cancer is one of the leading causes of deaths worldwide and existing chemotherapeutics exhibit low selectivity, resulting in both cancerous and healthy cells being affected. Therefore, the need for antitumor agents with no adverse effect and better efficacy is increasing. A promising class of biologically active substances are antimicrobial peptides (AMP), which exhibit diverse functions including antibacterial and antitumor, combined with low to no toxicity to normal eukaryotic cells. In this study we examined the impact of the modification of Temporin A, a naturally occurring AMP, with two different unnatural amino acids Dab (FLPLIGRVL-Dab-GIL-NH2) and Dap (FLPLIGRVL- Dap-GIL-NH2) in position 7 on the cytotoxicity, phototoxicity and antiproliferative activity in comparison to the parent peptide. The novel peptides were synthesized following the Fmoc/OBut strategy for SPPS and analysed for structure and purity using HPLC-MS technique. Cytotoxicity and phototoxicity were evaluated with Neutral Red Uptake in vitro test on BALB/c 3T3 cell lines, The antiproliferative activity was assessed on cell cultures using the MTT dye reduction assay and the results were discussed.

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Study of antitumor activity of temporine derivatives, containing citrulline and ornithine

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The antitumor effects of temporins can be attributed to their amphipathic α-helical structure. This structural feature allows temporins to target and interact with cancer cell membranes selectively. As a result, these interactions lead to cell death via apoptosis. In this research we used the Fmoc/OBut strategy to synthesize new temporin analogs introducing in position 7 the unnatural amino acids citrulline (Cit) and ornithine (Orn). The antitumor potential of analogs was examined on cell cultures using the MTT dye reduction assay. This method is based on the metabolism of MTT to insoluble formazan by mitochondrial reductases. Antiproliferative activities were expressed as IC50 values (concentrations required for 50% inhibition of cell growth). The obtained structure-activity relationships will be discussed. Briefly, temporins and their synthetic analogs show great potential as novel anticancer agents due to their selective cytotoxicity towards cancer cells and ability to induce apoptosis. Further research is needed to optimize their therapeutic potential and advance them towards clinical applications.

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New Temporin A and F Analogues Modified With Unnatural Amino Acids Dap And Dab And Antibacterial Activity Against Bacillus subtilis

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Temporins are a family, composed of short antimicrobial peptides, isolated from the skin of the European red frog Rana temporaria. In recent years they have attracted the attention of the scientific community due to the wide range of antimicrobial properties which they possess and could be used in the fight against the growing antimicrobial resistance. The purpose of this investigation was to synthesize new Temporin A and F (Temporin Dab and Dap) analogues and examine the effect of minor modifications in the side chain on the growth of Bacillus subtilis NBIMCC3562. Temporin Dap and Dab are analogues of Temporin A where natural Arg residue is replaced by diaminopropionic acid and diaminobutyric acid, respectively.

Their antibacterial activity was assessed by determining the minimal inhibition (MIC) and minimal bactericidal concentrations (MBC). The MIC of the newly synthesized analogues was assessed by a broth microdilution method, whereas their MBC was determined by a plate count method. The obtained anti-Bacillus activity of the analogues will be further discussed. The best antibacterial activity was observed for Temporin F and Dab.

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Synthesis and biological activity of Monofluorenated Analogue of Aurein 1.2

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Aurein 1.2 (Gly-Leu-Phe-Asp-Ile-Ile-Lys-Lys-Ile-Ala-Glu-Ser-Phe-NH2), is a short multi-functional antimicrobial peptide isolated from Litoria aurea and L. raniformis skin secretions. In recent years the multidrug-resistant bacterial pathogens have become a global problem. Antimicrobial peptides (AMPs) are increasingly sought-after and researched antimicrobial agents due to its desired pharmacological properties. Many antimicrobial peptides also act as anticancer peptides (ACPs). They have the ability to pass through cell membranes and destroy bacteria or cancer cells. Herein, Aurein 1.2 was chosen as a template for rational modification to achieve a more potent biologically active peptide. We synthesized new analogues by replacing the Phe in the 3rd and 13th position sequentially and simultaneously with 4-fluoro-phenylalanine in the primary structure of Aurein 1.2. The introduction of fluorine into the aromatic ring aims to improve the pharmacodynamics of the new peptides. H-Gly1-Leu2-Xxx3-Asp4-Ile5-Ile6-Lys7-Lys8-Ile9-Ala10-Glu11-Ser12-Xxx13-NH2 The obtained new analogues were tested in vitro for cytotoxicity, phototoxicity and antiproliferative activity on a panel of tumor and normal cell lines. The relationship structure biological activity will be discussed.

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Heterologous expression of a LanM homologous protein, from Mesorhizobium qingshengii J19, involved in yttrium immobilization

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The biological importance of Rare Earth Elements (REEs) has been explored, with recent studies focusing on uncovering the bacterial mechanisms associated with the selective recognition and binding of LnIIIs. A recent breakthrough in this field was the discovery of a Lanmodulin (LanM), a selective LnIII-binding protein. Lanmodulin possesses four EF-hands typically associated with Ca binding but demonstrates a natural affinity and selectivity for trivalent LnIIIs over Ca. This study explores the role of an unidentified EF-hand domain-containing protein, homologous to LanM, found in the strain Mesorhizobium qingshengii J19. This protein was studied for its ability to bind metals. The EF-hand gene from M. qingshengii J19 was overexpressed in Escherichia coli BL21 cells to validate its functionality. Engineered cells exhibited increased Y accumulation, reaching up to 3-fold higher levels than control cells. Simultaneously adding Nd and Y at equal concentrations led to higher levels of both elements accumulated in the engineered cells. Metal-protein binding assays with the purified protein confirmed its selectivity for Y, along with its capacity to bind Nd. These results highlight the potential functionality of this protein from strain M. qingshengii J19, suggesting its exploration for future applications in the recovery and recycling of Y from the environment.

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The role of genetic for disease control in aquaculture: preliminary data

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Over the past few years, veterinary research has aimed to identify methodologies for preventing and contrasting diseases with a non- chemotherapeutic approach that avoids the exclusive use of drugs. Among these, the possibility of exploiting genetic resources of resistance/resilience is of great interest, enhancing the immunogenetic and biological resources that allow animal welfare and the hygienic quality of food products. According to a 2021 FAO report, aquaculture is of fundamental importance to meet an always growing food demand. In aquaculture, infectious and parasitic diseases generate huge economic losses. One of the possible strategy to prevent the onset of the most common pathologies is represented by genetic improvement

for disease resistance. In this study, the main genes that encode for proteins of the acute phase of infections and antimicrobial peptides such as lysozyme, cytokines associated with the innate response (IL-6) and cytokines associated with the response adaptive (IFN-) have been investigated. RT- PCR assays were developed or optimized and the discovery, in the target genes, of novel polymorphisms (SNPs) associated with a resistant/ susceptible phenotype is ongoing.
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Experimental validation and characterization of in silico predicted FDA-approved drugs as antibacterial agents

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Antibiotic resistance has become a global problem, therefore, there is an urgent need to develop new-generation antibiotics. In this project, systematic approaches combining computational and experimental studies were developed for the inhibition of the bacterial ribosome, one of the main targets of antibiotics, to reduce the cost and period for drug design. Virtual libraries were prepared with FDA-approved drugs, and the affinities of the compounds to different functional regions of the bacterial ribosome were predicted by molecular docking. Following in silico-prediction, confirmation and characterization of the drug candidates with high docking scores were aimed within the scope of this study. Firstly, the antibacterial activity of these candidates was investigated against *E. coli*. Then, the toxicity of the selected drugs on human cells was evaluated. Next, IC50 values were determined by in vitro translation inhibition analysis based on luciferase enzyme synthesis with *E. coli* S30 extract. The findings from this project and the developed systematic approach will provide very useful information in solving the problem of antibiotic resistance.
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Keywords: Bacterial ribosome; antibiotics; protein synthesis; *E.coli*

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Influenza a vaccine candidate based on virus-like particles formed by the coat protein of phage Beihai32

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Effective control of influenza A infection could potentially be achieved by developing broad-spectrum vaccines based on conserved antigens. We developed such a candidate vaccine against influenza A virus based on the extracellular domain of the transmembrane protein M2 (M2e) linked to the coat protein (CP) of the single-stranded RNA phage Beihai32, which is capable of forming virus-like particles (VLPs). Four copies of the M2e peptide were linked to the C-terminus of CP. The fusion protein was efficiently expressed in Escherichia coli and formed spherical VLPs of about 30 nm in size. Subcutaneous immunization of mice with VLPs induced high levels of M2e-specific IgG antibodies in serum and provided mice with protection against lethal influenza A virus challenge. In order to further evaluate the capacity of phage Beihai32 CP as a carrier of large peptides, we fused the 238 a.a long green fluorescent protein at the C-terminus of CP. The fusion protein was expressed in E. coli and formed VLPs. Therefore, the CP of phage Beihai32 may become a promising carrier for presentation of even large antigens in vaccine development.
This research was funded by the Ministry of Science and Higher Education of Russia (project 122112300052-5).

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Cuantification of galantamine from natural extracts and testing it as preventive antidote in organophosphates exposure

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Objectives: Obtaining natural extracts from Narcissus poeticus; Identification, and cuantification, of galantamine from natural extracts; Testing its cytotoxicity and antidote effect in organophosphates exposure.
Materials and methods: The extracts were obtained by maceration and ultrasound-assisted methods. Quantification of galantamine was carried out using a system equipped with a diode-array detector (HPLC-DAD). The intrinsic toxicity and antidote effect were measured on human fibroblasts cell line (NHDF-Lonza) using the MTT cell growth assay kit.
Results: The extract obtained by maceration showed a higher concentration of galantamine (7,92 mg ± 0.01 /100 mg dry extract) than the one obtained by ultrasound method (4.53 mg ± 0.05 mg/100 mg dry extract). MTT test results: galantamine from natural extract shows lower cytotoxicity (5,8±0,002%) than the one obtained by chemical synthesis (10,4±0,005%); the antidote effect of galantamine from natural extract is comparable, (cell viability 90.63±0,003%) with that obtained by chemical synthesis (cell viability 84.62±0,07%).
Conclusions: The extract obtained by maceration method

from *N.poeticus* showed the highest concentration of galantamine. The intrinsic cytotoxicity and the protective effect of galantamine from the natural extract are comparable to that obtained by chemical synthesis. Obtaining galantamine from natural extracts is less polluting than chemical synthesis.

Keywords: galantamine, natural extracts, diazynon

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Changes of the Morpho-Productive Performances of Silkworm Bombyx Mori Romanian Breed by Dietary Addition of Yeast

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The aim of this work is to evaluate the effects of the addition of yeast in nourishing of silkworms B. mori Galben Centurat Baneasa breed, 5th instar, on morpho-productive performance. 300 larvae were randomly distributed on 3 groups, 2 replicates per group: 1) control (C group) fed classical mulberry leaves; 2) experimental 1 (E1 group) fed C diet and yeast 1 x 107 concentration; 3) experimental 2 (E2 group) fed C diet and yeast 1 x 109 concentration. The larvae were measured on the first, fifth, seventh, and ninth days (D) of the trial. The LSD test was utilized for statistical evaluation. A concentration of 1 x 109 (E2-group) yeast improve significantly larva parameters as follow: i) weight > 4.8% vs. C group, respective 1.69% vs. E1 (P = 0.04); ii) length > 1.9% compared to C group, and 1.04% compared to E1 group (P=0.03). In D9 the larva weight was 4.85 higher than D1, while larva length was 2.12 times higher than D1. Whichever the yeast concentration, the cocoons characteristics were not affected (P>0.05). No mortality or cases of disease were recorded. In conclusion, the yeast enhances mulberry leaves, improving larval performance without changing the parameters of the cocoons.

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Capsid protein of Physalis Mottle Virus as a carrier of the M2e peptide of influenza A virus

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Vaccination is the most effective way to protect against influenza. The use of conserved viral antigens, such as the M2e peptide, opens up the prospect of developing a "universal" vaccine with broad strain specificity. However, M2e is poorly immunogenic and requires linking to a highly immunogenic adjuvant or carrier. Virus-like particles (VLPs) formed by capsid proteins of plant viruses are such promising carriers. They are inherently safe and have adjuvant properties. The capsid protein of Physalis Mottle Virus (PhMV) can self-assemble into spherical VLPs. In our work, we attached 4 tandem copies of the consensus sequence of the M2e peptide of human influenza A viruses to the N- terminus of the PhMV capsid protein. The recombinant fusion 4M2eh-PhMV protein was successfully expressed in *Escherichia coli* and formed VLPs structurally similar to VLPs formed by unmodified PhMV capsid proteins. Immunization of mice with 4M2eh-PhMV particles induced high titers of anti-M2e antibodies in serum and protected animals from lethal challenge with influenza A virus.

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The impact of animal welfare on sheep reproductive function based on blood analysis

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The study aimed to observe and correlate blood biochemical and hematological parameters and general welfare of sheep, emphasizing the impact on their reproductive function in a flock of 100 sheep. The flock was divided into three samples evaluated according to physiological and reproductive status, and the results of blood tests were correlated with welfare and reproductive performance parameters. Careful management and optimization of these parameters have led to significant improvements in the reproductive function and productivity of sheep.The study emphasizes the importance of routine monitoring of blood tests for the effective management of welfare and reproductive health in meat sheep.In carrying out the statistical study, computerized calculation formulas were used, followed by manual computation of reference values according to the degree of freedom (df).A 95% confidence level was set for the relevance of the results. This percentage gives us a high degree of confidence that the entire on-farm sheep population has parameters between the upper and lower limit of the reference values. These findings suggest that, by maintaining optimal physiological conditions, the reproductive success and overall welfare of sheep can be significantly improved, leading to increased productivity and sustainability in the sheep sector.

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Synthesis and antiproliferative activity of ferrocene peptidomimetics

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Modified synthetic peptides, also known as peptidomimetics, are characterized by structural features aimed at overcoming the therapeutic limitations of natural peptides. Recent work on ferrocene-containing peptidomimetics highlights the turn-inducing potential of ferrocene-1,1'-diamine. Due to the distance between the ferrocene rings of 3.3 Å, amino acids and short peptides attached to the ferrocene scaffold can form intramolecular hydrogen bonds in the resulting beta-sheet-like structures. The aim of this work was to synthesize and test the antiproliferative activity of six peptidomimetics Ac–L/D–Ala–NH–Fn–NH–L/D– AA–Boc [Fn = ferrocenyl, AA = Val (1,4), Leu (2,5), Phe (3,6)], derived from ferrocene-1,1'-diamine as a turn-inducing scaffold. The bioconjugates 1-6 were tested for their antiproliferative activity against healthy (HFF-1) and tumor (MCF-7 and HepG2) cell lines. The results of the MTT assay showed the highest antiproliferative activity of the ferrocene conjugates with Phe (3,6), which caused visible disruption of morphology and death of the HepG2 tumor cell line. The stronger inhibitory effect of conjugate 6 on the HepG2 tumor cell line compared to the healthy HFF-1 cell line also indicates its therapeutic potential.

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Investigations on the bioactive properties of the anthocyanins from black chokeberry (aronia melanocarpa)

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Black chokeberry (Aronia melanocarpa) contains a large variety of valuable biologically active compounds. The aim of the study was to assess the properties of bioactive compounds from the black chokeberry pomace. The ethanolic extract prepared from black chokeberry is rich in anthocyanins (0.28±0.01 g cyanidin-3-glucoside/g dw) with high antioxidant activity. The molecular modelling technique was used to test the potential inhibitory properties exerted by the main anthocyanins found in the black chokeberry on the activity of the enzymes associated to the metabolic syndrome The three-dimensional models of the α-amylase, α-glucosidase and lipase were used as receptors for the cyanidin-3-O-galactoside and cyanidin-3-O-arabinoside in the docking procedure. Careful analysis of the resulting docking complexes indicated that the tested anthocyanins are able to attach in the vicinity of the active site of the enzymes, therefore interfering with their activity. Moreover, the anthocyanin extract exhibited antimicrobial activity against Staphylococcus aureus and Listeria monocytogenes. In conclusion, the alcoholic extracts of black chokeberry are rich in bioactive compounds with high antioxidant capacity, inhibitory activity against the metabolic syndrome associated enzymes and promising antimicrobial activity.

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Determination of polyphenolic content and antimicrobial activity of grape seed extracts

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Grape seeds are waste from wine production industry, that can be valorized in valuable bio-products. The aim of this study was to determine the polyphenolic content of eight grape seed extracts from Bulgarian grape cultivars. The total phenolic content, total flavonoids and procyanidins in grape seed extracts were determined. It was found that, the seed extracts of Pino Noir, Syrah and Cabernet Franc grapes have higher polyphenolic content than other extracts. The antimicrobial activity of the seed extracts from Pino Noir, Syrah and Cabernet Franc grapes against Staphylococcus aureus, Bacillus cereus and Escherichia coli were studied by agar diffusion method. The minimum inhibitory concentrations of this grape seed extracts against studied microorganisms were determined and compared with results obtained from other authors. Staphylococcus aureus was more sensitive to the studied grape extracts than other microorganisms. It was concluded that the studied grape seeds have a high potential as source of natural antimicrobial agents and as alternative to reuse of bio-products from winemaking

Keywords: grape seeds, polyphenolic content, antimicrobial activity, minimum inhibitory concentrations

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Optimizing variant detection in solanum melongena using nanopore sequencing and bioinformatics tools

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Variant detection is pivotal for advancing plant breeding programs, especially in Solanum melongena, which lacks the extensive molecular markers available in other Solanaceae species. Nanopore sequencing, a cutting-edge technology by Oxford Nanopore Technologies, offers real-time sequencing of long amplicons (>10 kb) with a portable device. This study leverages

nanopore sequencing to identify single nucleotide polymorphisms (SNPs) across various eggplant cultivars. We conducted a comprehensive evaluation of mapping software (BWAmem, Minimap2, and Bowtie2) and variant identification tools (Samtools, Freebayes, and Clair3) to determine the most effective combinations for analyzing genes of interest. Our findings indicate that Minimap2 and BWA-mem are superior for long-read mapping, while Clair3 and Samtools exhibit the highest accuracy in SNP detection. Additionally, optimizing protocols for amplifying large gene amplicons proved essential. These results underscore the potential of nanopore sequencing in resource-limited laboratories, providing a robust method for variant detection in Solanum melongena and enhancing molecular breeding efforts.

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Evaluation of Spinal Muscular Atrophy (SMA) carrier screening program awareness in Çanakkale population

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Introduction and Aim: Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder causing progressive muscle weakness. With a carrier frequency of 1/40-1/60, it is a common genetic disorder. Following the ACMG's 2008 recommendation for widespread carrier screening, Turkey initiated a national SMA screening program in December 2021 targeting individuals planning pregnancy, premarital couples, and pregnant women in the first trimester. This study at Çanakkale Onsekiz Mart University Medical Genetics Clinic assesses awareness of this screening among individuals of reproductive age.

Methods: We surveyed 63 reproductive-age participants with various clinical symptoms at the Medical Genetics outpatient clinic, using an 11-item questionnaire.

Results: Of the participants, 76.2% were female with an average age of 33. While 73% were aware of SMA, 58.7% did not know about Turkey's SMA screening program. Among the informed, 39.7% learned about it via social networks, 33.3% from the Medical Genetics Clinic, and 22.2% from primary care providers. Additionally, 66.7% of participants or their partners had not undergone SMA screening.

Conclusion: The study reveals a critical need for improved awareness efforts, particularly among reproductive-age individuals. Many in Çanakkale remain unaware of the SMA screening program, indicating a need for more effective outreach to enhance participation

Keywords: SMA, Screening, Genetic

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Can bacterial metallophores be exploited in platinum group metals extraction?

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Platinum-group metals (PGMs) are critical raw materials, primarily sourced from South Africa and Russia, making their supply vulnerable to global instability. Increasing the recycling efficiency of PGMs, while minimizing energy and water consumption, is crucial for sustainability, contributing to decrease the reliance on mining activities. The current work describes the production and use of an immobilized bacterial metallophore for the recovery of platinum from aqueous solution, intended for ion exchange chromatography. Several carbon and nitrogen sources were tested to optimize metallophore production by strain FBOM7R9A2. The metallophore was purified by FPLC, analysed by HPLC and immobilized by cross-linkage on a biopolymer. Pt binding kinetics and efficiency were assessed using ICP-MS. Genomic analysis identified genes involved in metallophore synthesis (antiSMASH) and their regulatory domains, which possessed palindromic recognition/binding sites for the IdeR repressor protein. Optimal metallophore production occurred at 72 hours with glucose or glycerol, and glutamate as a nitrogen source. Glycerol produced the purest extract, as judged by HPLC. The immobilized metallophore showed a higher affinity for Pt over Fe, with an 85.5-93.7% binding efficiency within 1h. This work highlights the potential of bacterial-derived materials to answer the challenges of economic and environmental sustainability.

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Sustainable biotools for efficient metal and COD removal from ındustrial wastewater

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The treatment of industrial wastewater to remove heavy metals and organic pollutants, and restoring acceptable water quality is a major challenge in wastewater management. Biological treatments, including bioaugmentation with selected bacteria for pollutant degradation and the use of bacterial biopolymers with metal-binding properties, are gaining interest in addressing this challenge. In this study, two approaches were explored to reduce iron and COD levels in the wastewater to meet water reuse standards of the wastewater in a bicycle component manufacturing industry. One approach involves using a biopolymer from Ochrobactrum tritici 5bvl-1 as a biosorbent for iron removal. The biosorption efficiency of this biopolymer in

iron solutions ranged from 55.0% to 100%, with a maximum biosorption capacity observed at 3 mM of iron, reaching 46.3 mg/g. Initial tests of the biopolymer on industrial wastewater showed 60% iron removal. The second approach involved the selection of bacteria capable of degrading surfactants (Tween 40 and 80) and forming biofilm to potentially bioagument biological reactors to enhance COD removal. Four bacterial strains from the genera Pseudomonas, Acinetobacter and Diaphorobacter were selected. These strains were able to remove about 50-60% of COD from the industry wastewater, highlighting their potential for improving treatment efficiency.

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Evaluation of using ACF supplement in the production of foot-and-mouth disease vaccine

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Serum plays a vital role in in vaccine production. Nowadays, biomanufacturers have focused on working with serum-free (SFM), protein-free media (PFM), or animal-component-free (ACF) media additives in vaccine production due to some disadvantages of animal-derived products, which can potentially contain adventitious contaminating agents. Additionally, the substantial costs and required special risk assessments by both the supplier and the user have driven this shift. The use of SFM, PFM and ACF supplements in vaccine production minimizes animal-based risks while also contributing to antigen purity and quality. This study aimed to evaluate the use of ACF supplements and observe their effects on the viral titer and plaque formation of foot and mouth disease virus (FMDV). For this purpose, different concentrations of ACF supplement were used directly adding to the FMDV culture medium. Overall, the results suggest that the soybean peptone-based ACF supplement is a simple and feasible option for supplementing the vaccine antigen production process when it is optimized in FMD virus culture media.

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The impact of smoking on the oral microbiome and proteomic-metabolomic salivary profile in the Slovak population under 25 years

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The human oral cavity is a complex environment hosting over 700 species of microorganisms, predominantly bacteria from genera such as Prevotella, Fusobacteria, and Proteobacteria, which are essential for maintaining oral and overall health. Human saliva contains various compounds, including metabolites produced by oral bacteria, proteins, hormones, lipids, and other small molecules, all of which participate in metabolic pathways influencing health. Smoking increases anaerobic pathogens, such as Porphyromonas gingivalis and Streptococcus salivarius, which are linked to oral diseases. Elevated levels of salivary proteins, such as CRP and CXCL10, and metabolites like spermidine and neopterin in smokers, indicate heightened immune responses and inflammation, contributing to various diseases. Therefore, salivary metabolites and proteins hold potential as biomarkers for diagnosing periodontitis, oral cancer, and other disease. This study investigates the differences in the oral microbiome between smokers and non-smokers under the age of 25 in Slovakia. It explores the impact of tobacco on health through proteomic and metabolomic analyses of saliva using mass spectrometry. By analysing the diversity of oral bacteria and salivary profiles, this research aims to enhance understanding of the oral microbiome's role in overall human health.
This study is supported by project FPPV-55-2024.

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Unravelling the calpain protease family in the unicellular flagellate *Euglena gracilis*

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Euglena gracilis, a versatile photosynthetic organism, holds immense potential for biotechnological applications. Its unique metabolic pathways and ability to thrive in diverse environments make it a valuable model for studying biological processes and evolution. Calpains are calcium-dependent cysteine proteases that are well conserved across a wide range of organisms. They are of interest of many studies due to their involvement in various pathological processes. Recent research has confirmed a great expanse of calpains in unicellular eukaryotes. Employing a Hidden Markov Model to target the calpains' specific CysPC domain, we identified a diverse repertoire of 27 calpain-like proteins in *Euglena gracilis*. These proteins exhibited distinct structural features, suggesting their involvement in a wide range of cellular processes. Notably, we discovered two transmembrane calpains and one predicted to localize in mitochondria, suggesting a key role in metabolism of *Euglena gracilis*. Phylogenetic analysis uncovered the evolutionary relationships of these proteins with calpain homologs from other organisms, providing insights into the evolution of the calpain family. Our findings contribute to a deeper understanding of calpain structure and distribution in *Euglena gracilis* and offer

new avenues for biotechnological exploitation.
This research was funded by VEGA:1/0694/21 and FPPV-57-2024 grant.

Keywords: *Euglena gracilis*, calpains, evolution, bioinformatics

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Spontaneous lung hernia and rib fractures requiring chest wall reconstruction

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Lung hernia is a rare pathology that is known and described for at least 500 years. Recent advances in the materials and methods used for chest wall reconstruction can improve the surgical possibilities when facing difficult cases and high-risk patients. We present the case of an obese woman that developed an important spontaneous lung hernia and multiple spontaneous rib fractures, following a violent coughing episode, that resulted in acute respiratory distress. Surgical repair was sucsesfully realised by rib osteosynthesis with titanium rib clips and the chest wall was reconstructed with a Gore-Tex prosthesis.

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Successful management of colorectal cancer with lung metastasis in a 74 year old patient a case report

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Introduction: Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer-related deaths worldwide. Its incidence continues to rise including advanced, metastatic stages, complicating treatment and prognosis. Still no consensus on treatment, advances in surgical techniques, particularly video-assisted thoracoscopic surgery (VATS), have significantly improved the management of pulmonary metastatic lesions.
Materials and Methods: 74-year-old patient diagnosed with CRC in 2016 (T2N1M0) followed by R0 surgical resection and adjuvant chemotherapy (FOLFOX 12 cures). After a disease-free interval (DFI) of 60 months, a lung nodule suspected to be a metastasis was found on follow-up PET- CT in the left upper lobe. A multidisciplinary team decided to perform VATS wedge resection to remove the nodule. Upon exploring the thoracic cavity, the nodule was identified and using two staplers, the lung parenchyma was resected, and the specimen was sent for pathological examination.
Results: Chest tube removal day 1 postop and the patient was discharged on day 2 with minimal pain management and no complications. Nodule was confirmed metastasis from CRC, with negative safety margins.
Conclusions: Surgery remains a leading option for CRC patients with lung metastasis. VATS compared to open thoracotomy helps decrease pain and hospitalization period while providing the same benefits.

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Next Generation Sequencing (NGS) panels in hereditary cancer: Efficacy and challenges

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Background/Objectives: Next Generation Sequencing (NGS) has transformed cancer susceptibility testing by allowing simultaneous analysis of multiple genes. Panel design varies among laboratories. This study evaluates the effectiveness and limitations of different NGS panel sizes.
Methods: We analyzed a 61-gene hereditary cancer panel for 701 patients (since February 2020) and a 171-gene expanded panel for 294 patients (since March 2023). Results were interpreted using Qiagen Clinical Insight Interpret software. Variant pathogenicity was assessed using QCI, Franklin, Clinvar, HGMD databases, and additional literature.
Results: The old panel detected pathogenic variants in 9.1% of patients and likely pathogenic variants in 5.7%. The new panel found pathogenic variants in 17.3% and likely pathogenic variants in 9.8% of patients. Of the pathogenic or likely pathogenic variants detected by the new panel, 30% were in genes not included in the old panel. The incidence of such variants increased from 14.8% (old panel) to 27.2% (new panel).
Conclusion: The new panel is superior in detecting pathogenic variants. However, it introduces challenges such as limited guidance, increased variants of uncertain significance, and incidental findings, complicating patient management and genetic counseling. Further research and guidance are essential for effective clinical use of large panels.

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Rare Case of Mucopolysaccharidosis Type III B - Sanfilippo Syndrome B

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Introduction: The mucopolysaccharidoses are inherited lysosomal storage disorders caused by the absence of functional enzymes that contribute to the degradation of glycosaminoglycans. The progressive systemic deposition of GAGs results in

multi-organ system dysfunction.
Case presentation: A 6 years old boy was the first child born of a consanguineous and healthy parents. On physical examination, he had coarse facial features, with thick hair, a low hairline, thick eyebrows, a wide and flat nasal bridge, protruding tongue, generalized hirsutism, short neck and hepatosplenomegaly. Furthermore, brain MRI were carried out at the age of two years and karyotype testing at the age of 6 years found to be normal. Genetic examination through whole exome sequencing revealed a homozygous mutation in the NAGLU gene.
Discussion: In genetic counseling, we knew that the family wants next pregnancy and explain them that everybody has two copies of each gene, one inherited from their mother, and one from their father. Couples can be screened for the genetic changes that cause Sanfilippo before they fall pregnant, and if there is a high risk, family planning options can be considered, such as IVF with genetic screening to select embryos that do not have the genetic change.

Keywords: Mucopolysaccharidose, glycosaminoglycan, Sanfilippo.

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Association of hereditary predisposition to thrombosis in childhood with hyperhomocysteinemia, folate cycle genes and leiden mutation

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Introduction: Thrombophilia is a complexly inherited pathology, in the etiopathogenesis of which both genetic and environmental factors play a role. Currently, a number of genetic variants of blood coagulation genes are known that are associated with a hereditary predisposition to thrombophilia, including in children. But studies have also established an association with thrombophilia of genes of other metabolic pathways.
Materials and methods: We analyzed 48 children aged 5 to 16 years with a history of episodes of thrombosis and hyperhomocysteinemia and 39 children in the control group.
Results: The study showed a significant association of the TT genotype of the variant MTHFR C677T (OR=3.8, CI:2.5-5.8, p<0.001) and the rare CC genotype of the MTHFR A1298C ((OR=3.1, CI:2.4-4.8, p<0.001) with thrombophilia in childhood. Heterozygous genotypes did not show a significant association with this pathology, but for the combined genotype AC+CT of the corresponding genetic variants, a significant association was established (OR = 2.7, CI: 2.2-4.5, p < 0.001). An association of heterozygous variant GA Leiden mutation (OR=4.9, CI:2.7-5.5, p<0.001).
Conclusions: Thus, we confirmed that not only genetic variants of blood coagulation genes are associated with thrombophilia in childhood, but also, independently of them, genetic variants of folate cycle genes.

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Study of genetic diversity of wheat collection samples for resistance to septoria

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Septoria tritici blotch poses significant biotic stress to wheat crops, making the development of resistant cultivars through molecular approaches both economically and environmentally sustainable. In Kazakhstan, wheat production is heavily affected by frequent disease outbreaks. This study focuses on 220 local and foreign varieties and lines of hexaploid wheat, aiming to assess their genetic diversity in resistance to Septoria. Molecular screening was performed to identify sources of resistance and 70 genotypes were found to possess Stb resistance genes: 18 carried the Stb2 gene (8.18%); 28 carried the Stb4 gene (12.72%); 7 carried the Stb7 gene (3.18%); and 17 carried the Stb8 gene (8.18%). The highest genetic diversity for Stb genes was observed in Kazakhstan wheat varieties (19.5%), followed by samples from CIMMYT (6.8%). Notably, four genotypes displayed the presence of two effective Stb genes: Omskaya-18 (Stb2, Stb8); Samgay (Stb7, Stb4); JAC161/TEMU51.80 (Stb2, Stb8); and KR12-5035 (Stb7, Stb2). The genetic diversity in Kazakhstani wheat samples was higher than in those from Russia, Brazil, and Canada, but lower compared to the germplasm diversity from CIMMYT and Mexico.
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Determination of potential biotechnological targets of brilliant blue by using combination of in silico target fishing and molecular docking techniques

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Brillant blue ($C_{47}H_{49}N_3O_7S_2$), also known as benzenemethanaminium or E133, is one of the most industrially used food colorant. Although use of brilliant blue is commonly accepted as safe in the food regulations, there is a growing concern in the nutrition and health experts due to its synthetic nature and presence of active groups in its molecular structure. In this regard, the present study conducted to investigate potential hu-

man targets of brilliant blue by using recent in silico tools. For this, 3D molecular structure of brilliant blue was obtained from PubChem Database (CID: 136664753). Target fishing studies was performed by using TargetNet, an open web server predicting the binding of multiple targets for the given molecules. Then, the results were further analyzed by molecular docking studies using Schrödinger Maestro Suite (ver. 13.9). According to the target fishing studies, 16 potential human targets including 5- hydroxytryptamine_receptor_1E, Bcl-2-related_protein_A1, Caspase-9, COUP_transcription_factor_2, Cytochrome_P450_2C9, DNA_(cytosine-5)-methyltransferase_1, DNA_dC dU-editing_enzyme_APOBEC-3A, Dual_specificity_protein_phosphatase_3, Galanin_receptor_type_3, G-protein_coupled_receptor_35, Hepatocyte_nuclear_factor_4-alpha, Nuclear_receptor_ROR-alpha, Perilipin-1, Perilipin-5, Toll-like_receptor_9, Tyrosine-protein_kinase_Fyn were determined. Molecular docking results revealed and validated the interactions, binding affinities between brilliant blue and the targets varied from -4.447 to -7.604 kcal/mol. Thus, a valuable information on the potential targets of brilliant blue was gained for the biotechnology research.

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Potential of grape seed extracts as dietary supplements

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Grape seed extracts (*Vitis Vinifera* L.) are a good sources of phytochemicals and are suitable raw materials for the production of antioxidative dietary supplements. The present study was carried out to assess the proximate composition, polyphenolic contents and antioxidant activity of grape seed extract of two red cultivars – Pinot Noir and Marselan. Proximate composition analysis of seed extracts - ash, crude protein, crude fat, total carbohydrate and total dietary fiber contents were determined using AOAC methods. The total phenolic compounds, total flavonoids and total procyanidins were also studied. The proximate composition of Pinot Noir grape seed extract and Marselan grape seed extract were similar. The total polyphenolic contains, total flavonoids and procyanidins of Pinot Noir seed extract were higher than results in Marselan seed extract. The contain of total flavonoids and procyanidins in Pinot Noir seed extract was higher than the ones in Merselan seed extract. The antioxidant activity of Pinot Noir seed extract was higher than activity of Marselan seed extract. The inhibitory potential of grape seed extract on fat-metabolizing pancreatic lipase was evaluated.

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A case report of cytogenetic analysis in couples with recurrent pregnancy loss

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Introduction: Spontaneous miscarriages continue to be a challenging problem for patients and clinicians. Approximately 15–20% of clinical pregnancies end in spontaneous abortions. Case report: 15 studies were conducted in couples with recurrent miscarriage and 7 in products of conception. The main results of the couples with recurrent miscarriage were: the frequency of chromosomal abnormalities which varied from 1.9% to 15% and in products of conception, the results observed were: the frequency of chromosomal abnormality was above 45% in approximately 65% of the studies; there was a predominance alteration of the numerical chromosomal.

Material Metod: For cytogenetic analysis, peripheral blood (2 ml) was collected in heparin vacutainers. For every case whole blood (0.5– 0.6 ml) cultures were set up in RPMI 1640 media containing 20% fetal bovine serum, antibiotic mixture, phytohaemagglutinin for 72h and Giemsa–trypsin using standard methodology. In each case, 15–20 metaphase plates were microscopically examined and scored and at least ten metaphases were analysed per karyotype.

Conclusions: Cytogenetic alterations represent an importante cause of pregnancy loss and its detection can help couples with genetic counseling. In addition, researches in miscarriage material, it is appropriate the inclusion of other molecular techniques as a complementation to the conventional cytogenetic analysis.

Keywords: Pregnancy loss, Chromosome abnormalities

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Design and construction of expression plasmids for the production of olivetolic acid in Candida utilis

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Due to their diverse medical potential, cannabinoids have garnered significant interest of the scientific community. Scientific research is however limited due to the psychotropic effect of tetrahydrocannabinol. Scientific groups are therefore driven to create an alternative pathway for the production of cannabinoids in microbial systems. This study outlines the construction of expression plasmids pGC2 and pGC3, which harbor optimized genes essential for the biosynthesis of olivetolic acid (OA) in Candida utilis. Within the biosynthetic pathway of cannabinoids, production of OA is necessary for the later formation of the main precursor of cannabinoids - cannabigerolic acid (CBGA).

For the correct production of OA, two main enzymes are needed - tetraketid synthase and olivetolic acid cyclase. Therefore, we designed two optimized genes named CsTKS (responsible for the production of tetraketid synthase) and CsOAC (responsible for the production of olivetolic acid cyclase). Designed genes were introduced into the expression vectors, under the control of a strong constitutive promoter PGAP, by using basic cloning techniques. Verification of the correct integration of expression plasmids into the chromosomes will be performed by PCR as well as qPCR, which ensures the most accurate results regarding gene dosage and expression.

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Design and construction of expression plasmid responsible for the production of prenyltransferase in Pichia pastoris

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Cannabinoids represent the most studied group of compounds originating from the plant Cannabis sativa. A highly relevant issue in the context of cannabinoids is their biosynthesis in microbial systems. For the successful reproduction of the cannabinoid biosynthetic pathway, it is crucial to thoroughly understand the process itself, which consists of three key steps: production of olivetolic acid (OA) through the polyketide pathway, production of cannabigerolic acid (CBGA) through the isoprenoid pathway in presence of prenyltansferases and lastly, cycling of CBGA into acidic forms of cannabinoids. This study outlines the construction of expression plasmid pGP1, which contains optimized gene PPT4-T, responsible for the production of prenyltransferase in Pichia pastoris. We utilized a specific expression vector pGZ1, which possesses an important feature of placing our gene of interest under the control of a strong constitutive promoter PGAP. Verification of correct integration of expression plasmids into the chromosomes of Pichia pastoris was performed by PCR method. Subsequently, these clones were analyzed by qPCR, which ensured the most accurate results regarding gene dosage and overall transcriptional activity. Clones with correctly integrated plasmids into the chromosome, and with confirmed gene expression levels will be further examined functionally.

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Phenolic compounds in mavrud grape marc and rose wines

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Mavrud is one of the most valuable Bulgarian varieties. It develops and reaches the best maturity of fruits in the warmer micro- regions of the country. Grapes matures late and is characterized by good techno-stocks of phenolic compounds, due to which it produces highly dyed juice extract and aging-friendly wine The aim of this work was to determine the amounts of different classes of phenolic compounds in an ethanolic extract from red grape marc and its components, peels and seeds, and to compare the results obtained with wine musts, and rose wine of the same variety of grapes. The results showed that rose grape marc was rich in polyphenol compounds Grape seeds demonstrated the highest contents of proanthocyanidines, (a type of flavonoids with high antioxidant activity) in comparison with other grape pomace components. It can be concluded that the winemaking by-products are a promising source of valuable products with high antioxidant activity.

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Fok1 polymorphism and persistent smell disorders after COVID-19 infection during pregnancy

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Single nucleotide polymorphism Fok1 for VDR gene was associated with the susceptibility to different pathologies as neuropsychiatric disorders, cancer, inflammation and Covid-19 infection. It is known that one of the effects of Covid infection is the persistence of disturbances in the sense organs even after some time. Therefore, 23 Umbrian women who gave birth during the Covid period were recruited. The presence/absence of smell disorders was assessed after 1 year. A saliva sample was taken from all women for the analysis of the Fok1 polymorphism. The results showed that 26% of the women had a smell disorder after 1 year. In these patients, 83% were GG+AG and 17% AA versus 83% GG+AG and 32% AA in women who did not have taste disorders. The results showed an increase in the recessive G allele in women with smell disorder compared to controls as a sign of long-Covid.

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Evaluation of diamine oxidase (DAO) in HT-29 human cell subjected to probiotics

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It is known that mixtures of different probiotic strains provide health benefits, including effective regulation of dysmetabolism caused by a specific diet or irregular food intake. Adverse reactions can occur after ingestion of food-containing histamine that the body cannot degrade, leading to poisoning or, more commonly, intolerance. Our study is based on the established idea that probiotics can function as treatment/preventive agents to regulate altered metabolic states. We tested the hypothesis that certain bacteria could play a role in modulating the cellular DAO enzyme. The DAO enzyme is an homodimeric glycoprotein of approximately 200 kDa. After being synthesized in the mucosa of the small intestine, it is stored in intracellular vesicles of enterocytes and secreted when necessary to contribute to extracellular histamine degradation. HT-29 human colon adenocarcinoma cell line were treated for 18 hours with a single supplementation of a mixture of Lactobacillus plantarum, Lactobacillus acidophilus and Bifidobacterium breve in three different ratios (1:1, 1:10, and 10:1) relative to the number cells. The mixture contained the microorganisms in the form of secretomes, heat-inactivated or viable bacteria. Results from western blotting did not show an increase of the expression of DAO enzyme.

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Study of metabolite production by mixo/heterotrophic microalgae using different carbon sources

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The use of microalgae biomass or produced substances is already widespread in the food industry. It serves as an excellent source of carbohydrates, proteins, lipids and, to a lesser extent, other substances such as those with antioxidant properties. However, the disadvantage of microalgae production is the long cultivation time due to their autotrophic nature. In this work, mixo/heterotrophic microalgae Galdieria sulphuraria CCALA 1067 and Euglena gracilis CCALA 349 were used, whose production speed is several times higher than that of autotrophic microalgae. Microalgae were cultivated under mixo or heterotrophic conditions on different carbon sources. The profile of selected metabolites was observed depending on the given organic substrate and the type of trophic cultivation. The samples were tested for lipophilic pigments and fatty acids produced. The use of mixotrophic or heterotrophic microalgae appears to be a suitable option for the utilization of food industry waste materials, while producing a range of valuable metabolites. Due to their faster growth rate compared to autotrophic microalgae, their use in co-cultivations with other heterotrophic organisms can also be considered.

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Impact of plant essential oils on probiotics production and activity

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Probiotics have been part of the human diet for centuries and are currently a popular topic. While probiotics slow the growth of pathogenic microorganisms in the digestive tract, essential oils destroy them. Combining plant extracts and probiotic strains may result in a synergistic effect. This study observed the effect of media enrichment with some plant essential oils (thyme and oregano) on growth and metabolism of some probiotics (Lactobacillus, Bifidobacterium). The production of lactic acid and the antimicrobial activity against selected bacterial strains were analysed. Some combination of probiotics, prebiotics and essential oils were encapsulated into microparticles, and their stability and probiotics release were tested. The results showed that thyme oil has a positive effect on probiotics growth both in free and encapsulated form. Gradual release of LAB from particles into the medium over 15 days and their antimicrobial activity were confirmed. In conclusion, due to their synergistic effect, the combination of essential oils and probiotics appears promising strategy in the field of active nutritional supplements.

Keywords: Probiotics, essential oils, encapsulation, lactic acid, antimicrobial activity.

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The success of corpse morphology to asses Apoptotic-Like PCD (AL-PCD) rates - A review

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The 21st-century onset has been marked by significant global challenges such as water resource depletion, rise in temperatures, increased salinity in both water and soil and environmental pollution. Simultaneously, the earth's population is growing while land available for agriculture is reducing. Against this backdrop cereals specifically soft wheat cultivars are important for global consumption hence there is a need to evaluate their resistance to abiotic and biotic stresses. In plants such as xylogenesis, embryogenesis, root and leaf development, and senescence PCD(Programmed Cell Death), programmed cell death represents an extremely critical physiological process that occurs during the course of plant development. PCD functions protectively under specific stressful conditions. The cytoplasm

of a plant's cells may undergo condensation when subjected to stress, causing separation of cell walls thus creating a specific structure used to assess AL- PCD(Apoptotic Like-PCD) rate in whole plants or cell cultures. These root hairs can be visualized using light microscopy or stains like propidium iodide or acridine orange, TUNEL assay also would be appropriate for use in its analysis. These cells provide an excellent rapid model for assessment of AL-PCD rates in vivo. Electrophoretic DNA characterization and metabolomics are additional techniques used to identify metabolites involved in plant response.

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Development of nutritionally enhanced foods with microalgae extracts

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Microalgae and cyanobacteria are a promising sustainable source of functional food and nutraceuticals with many potential health benefits. Microalgae are rich in high-value bioactive compound like carotenoids, proteins, vitamins, essential amino acids and fatty acids that offer antioxidant, antimicrobial, anti-inflammatory, antitumoral, anti-glycemic and cholesterol-lowering properties. Due to these reasons, this work was focused on the preparation, characterization and comparison of extracts prepared from selected microalgae and cyanobacteria. The selected microalgae for optimization of extraction methods were Chlorella and Spirulina. A number of extraction procedures using different solvents and extraction methods were performed. The amount of active substances, including antioxidants, phenolic compounds, fatty acids, carotenoids and chlorophylls, was measured. The MTT test of cytotoxicity was used to confirm the safety of produced extracts. To confirm the possibility of using the obtained extracts, enriched cereal porridges were prepared and analyzed. The results showed significant amounts of active compounds were present in the prepared extracts. All of the examined extracts showed no cytotoxic effects. It was discovered that enhanced products had an increase in bioactive compounds and antioxidant activity. The findings indicated that produced extracts are potential for further uses. The gained knowledge can be applied to the creation of fortified food or dietary supplements.

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Red yeast as an alternative beta-glucan source

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Beta-glucans are polysaccharides known for their diverse biological activities, particularly their immunostimulatory properties, making them promising candidates for various therapeutic applications. The primary focus has been on beta-glucans derived from mushrooms. However, yeast could serve as a viable alternative offering several advantages, including higher and more consistent yields and simpler extraction processes. Four carotenogenic yeast strains from the Cystofilobasidium and Rhodotorula genera were evaluated as a potential source of beta- glucans. The cultivation process was optimized in terms of cultivation temperature and C/N ratio. Moreover, chemical stress factors were introduced into the culture medium, specifically tannic acid and sodium chloride, which were able to further enhance the beta-glucan content. Effect of these conditions was simultaneously determined on other metabolites, such as lipids or carotenoids. For the isolation process of beta-glucans, the alkali-acid extraction method with milder conditions and subsequent removal of the lipid content using organic solvents, proved to be the most promising. Finally, the bioactive triple-helical conformation of beta-glucan extracts was verified using Congo red dye. This research contributes to a more thorough understanding of the cultivation conditions of red yeast and possible isolation methods leading to a high yield of biologically active beta-glucans.

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Phytochemical study and pesticidal potential of three Tunisian Eucalyptus essential oils

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Forest species are characterized by their abundance of essential oils (EOs). They play a key role in the search for alternative methods to control weeds, fungi and pests. The present study reports the chemical composition and emphasizes the antifungal and phytotoxic properties of EOs obtained from Eucalyptus populifolia desf, Eucalyptus woolsiana and Eucalyptus exserta. Gas chromatography coupled to mass spectrometry (GC/MS) was used to analyze the EOs. The antifungal and phytotoxic activity was evaluated. All EOs showed a particular abundance of eucalyptol (75.94-7.01%), camphene (6.97-0.4%), globulol (5.20-5.83%) and alpha-pinene (0.71-12.82%). Against four phytopathogenic fungi belonging to the genus Fusarium, the EOs showed significant antifungal properties. Phytotoxic activity against five weeds demonstrated variable efficacy on seed germination and seedling growth, with greater efficacy than glyphosate. Eucalyptus EOs have potential for use in biocontrol formulations.

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Production and characterization of extracellular polymeric substances from carotenogenic yeasts

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Extracellular polymeric substances (EPS) have garnered significant attention in research due to their bioactive properties and wide range of potential applications. Despite the increasing demand for exopolysaccharides from various sources, the use of yeast-derived exopolysaccharides remains limited, primarily due to relatively low production yields. Carotenogenic yeasts are well-known for their synthesis of antioxidant pigments and intracellular polysaccharides, proteins, and lipids. However, their capacity to produce EPS and the characteristics of these compounds are relatively unexplored. These EPS are also attractive because they enhance the overall yield of the cultivation process by utilizing the supernatant. The objective was to identify the optimal carbon source for EPS production and to determine its effect on the synthesis of various EPS fractions, as well as carotenoids and lipids. The production and properties of these fractions were studied using gravimetric, chromatographic, and microscopic analyses. These analytical methods revealed a strong dependence of EPS output on the carbon source, with the strain Rhodotorula kratochvilovae achieving the highest EPS yield (5.88±0.36 g/L) when grown on sucrose. This study highlights the potential of optimizing carbon sources to enhance the production of valuable extracellular substances in carotenogenic yeasts, offering promising implications for industrial applications.

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Cultivation of carotenogenic yeast on various combination of waste nitrogenous and carbonaceous substrates

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Carotenogenic yeasts are renowned for their capability to synthesize a range of bioactive compounds, such as beta-glucans, lipids, carotenoids, and various antioxidants. They also hold significant industrial potential as biotransformation agents, capable of converting by- products from diverse food industry sectors—including coffee grounds, whey, poultry fat, feathers, glycerol, and waste oils—into valuable products. This research focuses on repurposing waste poultry feathers as nitrogenous substrates, waste poultry fat and frying oil as carbon sources and whey as combined substrate for the cultivation of carotenogenic yeasts. The initial cultivation was conducted in Erlenmeyer flasks for laboratory screening purposes. Among several strains of red yeast tested, Cystofilobasidium macerans exhibited notable biomass growth (18.8±0.3 g/L). Further investigation is required to assess the growth performance of these carotenogenic yeast strains in laboratory bioreactors, which could potentially serve as models for industrial application.

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Study of the continuous electric field on the RD cancer cell line by response surface methodology

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The application of the electric field is considered to be a very promising method in cancer therapy. Indeed, cancer cells are very sensitive to the electric field although the cellular response is not entirely clear. The tests carried out consisted in subjecting the RD cell line under the effect of the continuous electric field while varying certain parameters (voltage, exposure time and cell concentration). The response surface methodology (RSM) was used to assess the effect of the chosen parameters, as well as the existence of interactions between them. The results obtained showed that the voltage, the cell concentration as well as the interaction between voltage and exposure time have an influence on the mortality rate of the RD cell line.

Keywords: Continuous electric field, RD cancer cell line, RSM, voltage.

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Candida utilis as an efficient heterologous protein production system

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Candida utilis is a yeast used in the food and feed industries that has held GRAS (Generally Recognized As Safe) status for several decades. It combines the advantages of prokaryotes, such as rapid growth and easy genetic manipulation, with those of eukaryotes, including post-translational modification and proteolytic processing. Among its other benefits are growth on inexpensive substrates and rapid adaptation to various carbon sources, including maltose. In our work, we focused on exploring and utilizing the cluster for maltose processing. Specifically, we focused on the promoters PGLC and PAKT, which seem suitable for use in recombinant protein production. The aim of the study was to minimize the rate of catabolic repression in the presence of glucose and increase sensitivity to the inducer maltose. Plasmid constructs with the PGLC promoter were integrated into the chromosomes of yeast strains CuF and CuA and subsequently tested. The level of expression in the presence of both repressive and inductive carbon sources was measured using the ONPG substrate. The PAKT promoter was bioinformatically analyzed and cloned. Additionally, a plasmid vector pΔAkt was constructed, which will enable the creation

of a new yeast strain, CuK, to facilitate the testing of the PAKT promoter.

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Enhanced mucosal delivery of mRNA vaccines using maltodextrin-coated lipoplexes

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Messenger ribonucleic acid (mRNA) offers a promising platform for the development of novel therapeutics, particularly for unmet medical needs. However, its efficient delivery, especially to mucosal surfaces, remains a significant challenge. This study aimed to develop novel mRNA delivery systems using maltodextrin (MDX)-coated lipoplexes (LXs) for enhanced mucosal vaccination. The developed nanoparticles characterized by a size of 100-120 nm and a zeta potential between -20.58 and 9.11 mV. Confocal and cryo-electron microscopy confirmed MDX incorporation into the LX structure. Notably, MDX significantly improved LX stability and mucoadhesive properties, enhancing cellular uptake. In vitro studies using airway epithelial cells demonstrated a significant increase in transfection efficiency and gene expression with MDX-coated LXs compared to commercial LXs. Up to 80% of cells were transfected and protein expression was elevated 4.3-fold for up to 48 hours. We propose that MDX reduces LX hydrophobicity, facilitating mucus penetration and internalization. These findings suggest that MDX-coated LXs represent a promising strategy for mucosal delivery of biopharmaceuticals. The enhanced stability, mucosal passage and cellular uptake of these formulations hold potential for developing effective vaccines against respiratory diseases. Further studies will focus on elucidating the precise mechanism of MDX-mediated enhancement and immunogenicity of MDX- coated LXs in vivo.

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Biotechnological production of carotenogenic yeast metabolites with application of complex waste substrates from the food industry under combined stress

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This research is focused on biotechnology of selected strains of carotenogenic yeasts, cultivated using waste substrates. This type of microorganisms are known for its ability to use a wide range of nutritional resources, including waste substrates. The carotenogenic yeasts themselves excel in the production of many biologically active substances (carotenoid pigments, ubiquinone, ergosterol, fatty acids, etc.) that have a wide range of applications in the pharmaceutical, cosmetics and food supplement industries. The most important aspects for the cultivation of these microorganisms are the nutritional ratios of carbon and nitrogen, which are present in some industrial wastes. The waste substrates selected for this study include brewery spent grain hydrolysate, yeast extracts prepared from sedimented brewer's yeast and waste whey from the dairy industry. The pretreatment of these waste substances is also included in this study. The research examines also the impact of different microbiogenic element concentrations on yeast biomass production using whey waste substrate. Results indicate that processed brewer's waste and whey substrate is effective for cultivating carotenogenic yeasts and can serve as complex nutritional source. This research provides valuable insights into the industrial biotechnology applications of carotenogenic yeasts and offers economically viable methods for utilizing selected industrial wastes.

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Wellness tourism trends in hospitality industry on the example of Adjara

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The popularity of wellness tourism is growing in many countries. It is worth noting that the economic benefits of wellness tourism are growing significantly. Due to global challenges, wellness tourism is considered as one of the priority segments of the tourism market, which helps to maintain a healthy life. Especially considering the pandemic conditions of the last 2 years, the direction of medical, healing and wellness tourism has become a priority. The development of wellness tourism in mountainous regions is a priority of many countries. The purpose of our research was to study the aspects of wellness tourism. In particular, the development of services trends. In particular, what wellness services does the institution offer to tourists. We have studied 30 hotels. Thus, proper management of wellness tourism among them should be a major challenge, which in turn will guarantee the attraction of more visitors. The tourism opportunities of the Adjara region, including the development of wellness tourism with complex approaches and taking into account international standards, will create a unique opportunity for the development of Adjara's economy and will give the region the opportunity to join international health organizations.

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Methylmercury Impact and Bioaccumulation in Extremophile Microalgae Strains Coccomyxa Onubensis and Dunaliella Salina from Andalusia (Spain)

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Since the last century, the anthropogenic activities are steadily increasing the atmospheric concentrations of mercury (Hg). The possibility of long-range dispersion in the atmosphere, coupled with high toxicity of Hg and ability to form methylated organic forms with high affinity to biological materials makes Hg a serious threat to human health. The methylated organic form of Hg (MeHg) is bioconcentrated and biomagnified across the entire marine food webs and eventually ends up in human diet. The primary entry point for MeHg in these food webs is phytoplankton predominantly composed by various microalgal species. Thus, understanding the processes of MeHg intake by microalgae, effects on growth, cell toxicity, bioaccumulation and eventual biotransformation is of great interest. This study was conducted on the extremophile microalgae strains local to southern Spain Coccomyxa onnubense (acidophile freshwater alga) and Dunaliella salina (halophile seawater alga). The exposure experiments were conducted at laboratory scale during 72 h cultivation. Additionally, samples from the cultivation with the highest exposure were tested subjected to transmission electron microscopy (TEM) and the determination of superoxide dismutase (SOD) enzyme activity.

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Study of possible mixotrophic cultivation of selected strains of autotrophic microorganisms on alcohol substrates

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In the biotechnology industry, microalgae are often used by microorganisms, especially for the production of important metabolites such as chlorophylls, carotenoids (which are important antioxidants), but also fatty acids, sterols or ubiquinone. This study focuses on the mixotrophic mode of nutrition of autotrophic microalgae. Different types of alcohols were added to the culture medium during cultivation, as a carbonaceous substrate, in order to increase the production of the mentioned metabolites. Desmodesmus quadricaudea (CCALA 464), Chlorella minutissima (CCALA 916), Desmodesmus subscapitus (CCALA 688) and Scendesmus pleiomorphus (CCALA 460) strains were selected for the experiments. Alcohols - methanol, ethanol, isopropyl alcohol and glycerol were chosen as carbon source. These carbon substrates were added to the culture medium at different concentrations. Optical density and quantum yield were observed during cultivation. Afterwards, the biomass as well as the medium was analyzed by different chromatographic methods. It was shown that the autotrophic microorganisms used were able to survive in the environment of added alcohol or use alcohol for mixotrophic mode of nutrition. These findings could be used in the future, for example, for cocultivation with other heterotrophic microorganisms.

Keywords: Microalgae, stress factors, chlorophyll, carotenoids, optical density

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Development of monoclonal antibodies to detect different epitopes of immunoreactive trypsinogen for newborn screening of cystic fibrosis

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Cystic fibrosis (CF) disease is a rare and hereditary disease seen in one out of every 2,500-3,000 babies in Turkey.It affects the lungs and digestive system the secretory glands of many organs from birth onwards, causing dysfunction. Early detection of CF is critical to increase patients' life expectancy and quality of life and initiate an effective treatment strategy. Different epitopes of Immunoreactive trypsinogen (IRT1,IRT2) are important markers which used in the early diagnosis of cystic fibrosis. In this study, we used different immunization and antibody screening protocols to develop new monoclonal antibodies against different epitopes (IRT1,IRT2) of trypsin and evaluate their diagnostic potential.For this purpose, besides trypsin-1 and trypsin-2 immunizations to the Balb/c mice, trypsin-1 and trypsin-2 mix immunizations were applied to boost (or enhance) the common regions of trypsin-1 and trypsin-2 molecules. Trypsin-specific antibodies were determined by indirect ELISA and inhibitor-capture ELISA.As a result of 9 fusions, the monoclonal antibodies obtained which were names 2C1, 4B2, 11F9, and 6G7, were tested for both activity and cross-reactivity. They were characterized, purified, and labeled with HRP enzymes to be used in a sandwich ELISA.Monoclonal antibodies were shown to be efficiently used as capture and/or detection antibodies for the detection of IRT1 and IRT2.

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Evaluation of Variants of uncertain significance (VUS) in the CDK4 Gene Through In Silico Analysis

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Background: Cyclin-dependent kinase 4(CDK4) is a gene that regulates the cell cycle. Its mutations has been associated with melanoma. breast carcinoma, glioblastoma and sarcoma. CDK4 inhibitors are beneficial for cancer treatment when detected CDK4 mutation is pathogenic/Likely pathogenic. In this study, VUS variants of CDK4 were re-evaluated using 3D modeling and in silico analysis, for the effect regarding the protein structure.

Methods: CDK4 gene variants detected in COMU Medical Genetic Department, and identified by QCI database. Their effects on protein function were evaluated using MutationTester, AlphaMissense, SIFT. Disease association was analyzed with PHD-SNP and SNPS- GO, and protein stabilization by Mupro and I-Mutant2.0. 3D modeling of these variants were also made with Swiss-Model.

Results: We evaluated ten VUS variants in the CDK4 gene. MutationTester classified two (c.661G>A; p.D221N and c. 460G>C; p.V154L) as "Deleterious,", AlphaMissense identified one (c.385G>A; p.D129N) as "Likely Pathogenic.". SIFT classified six as "VUS" and five as "Benign." PHD-SNP associated three with disease, SNP-GO identified all as "Neutral." Mupro indicated that all decreased protein stability, I-Mutant2.0 found one increasing it.

Conclusion: CDK4's VUS variants may increase cancer risk and complicate genetic counseling. MutationTester and AlphaMissen were more effective. Accurate classification by in silico analysis is crucial to determine the right treatment.

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Investigating the expression of age-related genes in the thymus tissue of calorie-restricted mice

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The relationship between diet and aging/cell cycle has been one of the most researched and intriguing topics in recent times. Studies have shown that calorie-restricted diets and certain antioxidants increase life expectancy in various models of aging and cancer development. In a previous study, 3 RNA-seq data were obtained from thymus tissues of Mouse Tumor Virus Transforming Growth Factor-α (MMTV TGF-α) mice which were fed 2 different calorie restricted diet. Accordingly, mice were divided into 3 feeding groups (AL (Ad Libitum), CCR (Chronic caloric restricted), ICR (Intermitted caloric restricted)). In this study, firstly, 8 genes related to aging and cell cycle determined from gene data banks were be investigated in three different RNA-seq DEG data and their expression levels have been determined. The validation of the expression of the genes with different levels of expression according to the difference in nutrition was performed by Real Time PCR. For Real Time PCR, cDNA was synthesized from total RNA obtained from mouse thymus tissues at 18, 50 and 80 weeks. Thus, it was aimed to observe the differences in expression levels of different nutrition style applications in the expression of genes associated with aging, early, mid and late times of life.

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Determining of phytoplankton biomass and composition at Butrinti Lake- An overview of methods efficiency

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Butrinti Lake located in southern Albania is a typical Mediterranean lagoon, which, thanks to the hydrological, geological, and changing climatic factors constitute an anoxic water basin where the phytoplankton biomass varies in quantity and taxonomic composition in spatial and temporal extent. This variation affects the quality of the waters and consequently the composition of the organisms of the food chain, and the quality of fishing and aquaculture. The study of biomass and taxonomic composition of phytoplankton in Butrinti Lake with different methods (microscopy, CARD-FISH, PCR-specific, sequencing, chemotaxonomy), and the results obtained to date are summarized in this study with the aim of their evidence and giving opinions on the advantages and disadvantages of each. Also, the study discusses the need for the combination of methodologies to obtain the most reliable and representative results, as well as gives explanations on the impact of human activities (agriculture, aquaculture, etc) on the alteration of physical-composition of waters, which in turn are responsible for changes in biomass quantity and composition.

Keywords: anoxy, biomass, taxonomy, PCR, sequencing

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Chemical composition and evaluation of the biological activities of essential oils from two species of Eucalyptus

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Medicinal plants and in particular Eucalyptus are known for their ability to produce natural substances with biological activities of great agronomic and pharmaceutical interest. The ob-

jective of this work was to determine the chemical composition of the essential oils of two species of Eucalyptus and to evaluate their antioxidant and antifungal potential against fungi isolated from the seeds of three varieties of wheat. The essential oils studied showed good antioxidant potential but significant antifungal activity against four strains of phytopathogenic fungi. Thus, these oils allowed the protection of wheat seeds against attacks by storage fungi. These results suggest that this treatment is an effective solution for the food industry to improve the storage conditions of wheat seeds.

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Genetic distribution of BDNF rs6265 polymorphism in turkish patients with autistic disorders

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The brain-derived neurotrophic factor (BDNF) gene, which is essential for neurodevelopment, has been found to have numerous variants. One of the most significant variants is the BDNF rs6265 polymorphism, located in exon XI of the BDNF pro-domain region. The alteration of 'G' allele to 'A,' eliminates a CpG methylation site and allows for epigenetic and genotypic interaction, which could partially explain environmental phenotypes such as depressive disorders. The BDNF rs6265 polymorphism is believed to be linked to anxiety features and the etiology of disorders associated with depression and anxiety, considered phenotypic variants of autism spectrum disorders (ASD). Our aim in this study is to determine the genotype distribution of the BDNF rs6265 polymorphism, which is thought to be associated with depression and anxiety in autistic patients in the Turkish population. Within the scope of our study, 20 Autistic disorder patients were included who were applied to Istanbul NP Brain Hospital. As a result, phenotype distributions of BDNF rs6265 polymorphism were determined as 45% (n=9) normal (CC), 35% (n=7) heterozygous risk (CT), and 20% (n=4) homozygous risk (TT). When assessing Turkish individuals with autistic disorders, the rs6265 polymorphism may be taken into consideration in terms of risk calculation and personalized medicine.

Keywords: ASD, Brain-derived neurotrophic factor, Neurodevelopmental disorders, rs6265 polymorphism, Risk assessment

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Does the homo- or heterochirality of ferrocene conjugates with valine, leucine and phenylalanine have a major impact on their antiproliferative activity?

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When the chirality of the peptide backbone is altered in terms of an L– to D–substitution, both self-assembly and biological properties are affected [1,2]. Our recent study on the antiproliferative activity of the symmetrically disubstituted homochiral ferrocene conjugates Ac–AA1–NH–Fn–NH–AA2–Boc (AA1 = AA2 = Val, Leu and Phe, respectively) on the HeLa, HepG2 and MCF-7 cell lines revealed that the HeLa cell line was only sensitive to Leu-peptides, whereas Phe-peptides only affected the MCF-7 cell line. Compared to cisplatin, the IC50 values obtained for Phe-peptides were even lower, clearly indicating their drug candidate's potential [3]. To improve the overall antiproliferative activity, we prepared and tested the heterochiral diastereomers of Val-, Leu- and Phe- conjugates with ferrocene. The IC50 values presented here indicate that the alteration of the peptide backbone chirality from homo- (L, L or D, D) to hetero- (L, D or D, L) allows for a significantly higher potency of the heterochiral peptides against the tested cell lines compared to their homochiral counterparts.

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Renpenning Syndrome: Frameshift PQBP1 Variant in a Patient with Fragile X-Like Phenotype

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Background/Objectives: PQBP1 gene encodes the polyglutamine-binding protein 1 (PQBP1), which is involved in mRNA splicing and transcription. Pathogenic variants in this gene cause X-linked mental retardation disorders collectively referred to as Renpenning syndrome; characterized by microcephaly, short stature, small testes, and dysmorphic facial features.
Methods: Following DNA isolation from peripheral blood; array-CGH analysis, CGG trinucleotide repeat analysis of the FMR1 gene and, whole exome sequencing (WES) analysis were conducted.
Results: A 7-year-old male patient was referred to our clinic due to developmental and language delay with a preliminary diagnosis of Fragile X syndrome. The patient has speech delay, developmental delays, and learning difficulties. In a physical examination, microcephaly, long triangular face, sparse

eyebrows, prominent eyelashes, thin upper lip, long philtrum, upslanted palpebral fissures, and proximally placed thumb were observed. Chromosome analysis and Fragile x, microarray, analyses were found to be normal. In WES analysis (NM_001032382.2) PQBP1 c.450_453delCAGA;p.D150fs*44 pathogenic frameshift deletion was detected.

Conclusion: Renpenning syndrome requires considering Fragile X syndrome as a differential diagnosis. Clinical parameters such as head circumference, testicular volume, and height play a role in distinguishing between the two syndromes during first examination, thereby guiding further diagnostic tests and improving diagnostic accuracy.

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Analysis of etiological, clinical manifestations and gross lesions associated with young pigeon disease in a pigeon loft outbreak

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Young Pigeon Disease Syndrome (YPDS) is a multifactorial condition that poses a significant health challenge to young pigeons, particularly those between the ages of 3 and 12 weeks post-weaning. In some cases, it can also affect older pigeons. This syndrome is characterized by high morbidity and mortality, leading to considerable losses in the pigeon breeding and racing industries. The primary viral agent responsible for YPDS is pigeon circovirus (PiCV), known for its immunosuppressive effects, which increase susceptibility to secondary infections caused by Escherichia coli and other opportunistic pathogens like Candida albicans and Trichomonas gallinae. Additionally, pigeon aviadenovirus (PiAdV) and Columbid herpesvirus-1 (CoHV-1) are associated with the syndrome, contributing to its complex pathogenesis. Clinical signs of YPDS are non-specific and include lethargy, weight loss, vomiting, diarrhea, and respiratory distress. The disease is most severe in juvenile pigeons, with rapid progression often leading to death within 3 to 5 days, while adult birds that develop clinical signs may take up to 8 days to die.

Keywords: pigeon, young pigeon disease syndrome, pigeon circovirus, pigeon aviadenovirus, co-infection.

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Electrophysiological profile of human granulosa cells from follicular fluid of poor and normo- responder patients

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Granulosa cells (GCs) play an essential role in oocyte maturation, providing energy and supporting steroid synthesis, during the process of folliculogenesis. Voltage-gated potassium (Kv) channels are the principal determinants of membrane potential in a variety of cell types, however their functional role in the ovary and reproductive disorders is less understood. Follicular GCs were obtained from twenty patients undergoing in vitro fertilization during assisted reproductive techniques and analyzed by patch clamp technique. Three different types of voltage-dependent K^+ currents in hGCs were characterised: a large-conductance calcium-activated potassium current and voltage-activated outward currents with rapid activation and inactivation (IA), supported by a third underlying ultrarapid slow-activated current (IKur), recorded for the first time in hGCs. Electrophysiological comparison between GCs obtained from poor and normo – responder patients, based on Bologna Criteria, showed a negative shift (~ 6 mV) of steady state inactivation of IA in poor responder and a major inactivation grade of IA current estimated to the end of test pulse at +40 mV. A significant reduction of cell capacitance was observed, indicating that there may be a reduced cell connection in GCs between normo- and poor responder patients. These results can help increasing the knowledge on the physiology of ion.

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Effect of heat stress on the germination of three Mediterranean endemic plant species of the genus Limonium (Plumbaginaceae)

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The climate change has a strong effect in the Iberian Peninsula, where year after year new records of high average and maximum temperatures are recorded. The prolongation of the period of high temperatures affects the biological processes of plants. In this study we analysed the effect of temperatures on seed germination on three endemic species of the genus Limonium: L. girardianum, L. albuferae and L. dufourii, the last two being exclusive of the Valencian salt marshes. Germination was considered, as this represents one of the most susceptible phases of the biological cycle of plants and key for survival of species propagated by seeds. The effects of 4 temperature ranges were tested: 25/15, 30/20, 35/25, and 40/30ºC with a photoperiod of 16 hours of light and 8 hours of darkness. The results obtained indicate that germination of the three species is strongly affected by increasing temperature, optimum germination in L. girardianum and L. albuferae being at 25/15ºC and drastically reduced at higher temperatures. L dufourii had an optimal germination at 30/20ºC but higher temperatures had

also a negative effect on its germination. The results obtained suggest that the population decline of these species registered in recent years may be partly related

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Metabolic and docking simulations of the potential antimicrobial effects of 3-(Phenylimino) oxindole

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This study investigates the potential antimicrobial properties of the isatin derivative 3-(Phenylimino)oxindole through metabolic and molecular docking simulations. Gram-positive and Gram-negative bacteria exploit host lipids for infection, exacerbating antibiotic resistance issues. Hybrid drug design strategies are being explored to overcome this challenge. Isatin, a naturally occurring compound with known antibacterial properties, is the focus of this study. We utilized the OECD QSAR Toolbox for quantitative structure-activity relationship (QSAR) analysis to evaluate the protein binding and metabolic fate of 3-(Phenylimino)oxindole. Molecular docking was performed using AutoDock4 with Topoisomerase IV from Staphylococcus aureus as the target. Metabolic studies identified 63 metabolites, suggesting diverse biotransformation pathways, including central carbon and aromatic compound metabolism. The docking studies revealed that 3-(Phenylimino) oxindole binds strongly to Topoisomerase IV with a binding energy of -5.13 kcal/mol and favorable interaction profiles, including hydrogen bonds and pi-stacking interactions. The validation of docking results showed high reliability with an average RMSD of 0.00 Å. These findings highlight the potential of 3-(Phenylimino)oxindole as a promising antimicrobial agent, particularly against bacterial Topoisomerase IV, and support its further development as a therapeutic agent.

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Comparison of three exosome isolation techniques based on distinct principles

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Introduction: Exosomes are the smallest extracellular vesicles, ranging from 30 to 120 nm in diameter. They are capable of carrying RNA, DNA, lipids, and proteins, playing a crucial role in intercellular communication. In recent years, they have gained attention for their potential to carry diagnostic and prognostic biomarkers in a wide range of diseases, from cancer to neurodegenerative conditions. Exosomes can be isolated from various biological fluids such as blood, cerebrospinal fluid, saliva, and breast milk. Several isolation methods, including ultracentrifugation, size exclusion chromatography (SEC), and polyethylene glycol precipitation, are available. The choice of isolation method depends on the type of sample, cost-effectiveness, technical complexity, and purity. However, there is a need for studies comparing these methods to determine the optimal approach.

Aim: This study aims to compare the efficiency of different exosome isolation techniques from human blood plasma, focusing on particle concentration and size recovery for diagnostic and prognostic biomarker analysis.

Methods: In this study, exosomes were isolated from blood plasma using three different methods: SEC (Izon qEV 35 nm), ultracentrifugation (Beckman Coulter), and ExoSID. The isolated exosomes were analyzed using Nanoparticle Tracking Analysis (NTA) with the NanoSight Pro.

Findings: The isolation using ultracentrifugation resulted in 47.72% of particles in the 0-200 nm range, with a particle concentration of 2.27E+10. SEC yielded 85.24% of particles in the 0-200 nm range, with a particle concentration of 3.42E+10. The ExoSID method yielded 27.06% of particles in the 0-200 nm range, with a particle concentration of 4.22E+10.

Conclusion: While the ExoSID method achieved the highest particle concentration (4.22E+10), it had the lowest percentage of particles in the desired size range (27.06%). Column chromatography, yielding 85.24% of particles in the 0-200 nm range and a high concentration of 3.42E+10, proved to be the optimal method.

Keywords: exosome isolation, exosomes, nanoparticle tracking analysis

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Phytoremediation potential of Azolla caroliniana Willd. for microplastic pollution

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This study conducted experimental model applications to assess the effects of microplastic (MP) pollution in freshwater and explore potential phytoremediation strategies. The capability of Azolla caroliniana Willd. for MP phytoremediation was investigated using a growth medium containing MPs at varying concentrations (1, 5, and 10 mg/L of fluorescent red polyethylene microspheres, measuring 250-350 µm). The study evaluated A. caroliniana's effectiveness in removing MPs by measuring the

percentage of removal efficiency and bioconcentration factor (BCF) on the 3rd, 5th, 7th, and 14th days. The MPs employed in this research are not taken up by the plant due to their size; instead, they adhere to the roots and leaf surfaces underwater, thus being removed from the environment. After 14 days, the average MP removal efficiencies of A. caroliniana at concentrations of 1, 5, and 10 mg/L were found to be 27.6%, 23.8%, and 21.1%, respectively. The highest BCF values were recorded at MP concentrations of 1 and 5 mg/L, with values of 0.50 and 0.45, respectively.

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The importance of Healthcare Programs and development of the Human Resources in the hospital sector

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HealthCare field belongs to a complex system which is continually changing/adapting according to epidemiological and societal shifts. Moreover, emerging technologies and ongoing environments create new approaches for global health. Human resources has a key contribution to the health system/health service delivery process. Current trends of innovative technologies and globalization have taken the world to a new level of economic development. It should be noted that the development of the healthcare system depends on three main factors: human resources, material capital, and availability of consumables. Also, Proper healthcare programs and human resources strategically enhance the health sector, therefore it is providing quality healthcare services. In the present studies, we aimed to research current trends regarding Healthcare programs and some aspects of human resources in the medical field. We analyzed government programs and their contribution to enhancing the function of hospitals. The survey involved administrative staff, clinical staff and patients (from eight hospitals). Taken all together, the government plays a significant role in clinic operations. The right policy in the direction of human resources has special importance to enhance healthcare systems; Finally, strategically and target improving the healthcare programs, also properly qualified human resources support increasing society's welfare.

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The alteration of the thyroid hormones and lipid profile in menopausal women with breast tumors

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Breast cancer is the most commonly diagnosed cancer among women worldwide. Thyroid hormones and lipids significantly involve cellular metabolism and control numerous pathways in targeted tissues. Venous blood samples were investigated. The samples were collected from patients with benign and malignant breast tumors. The number of patients was 15 in each group (Control, benign and malignant group). We studied the tumor markers (CAE, 125 and CAE), lipid profile (cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and triglycerides (TG)), and thyroid related hormones (thyroid stimulating hormone (TSH), Free thyroxine (FT4) and free triiodothyronine (FT3)). $P < 0.05$ was regarded as statistically significant. The study revealed significant alteration regarding CHOL and LDL levels within tumors compared to the control group ($P<0.0001$, respectively). TSH was significantly elevated in the background of the low FT4. Our study revealed a significant alteration according to the lipid profile and thyroid hormones among tumors ($P<0.0001$). All tumor markers were elevated, among them the CA 15-3 level was significantly high in benign and malignant tumors.

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Generation of brain organoid from embryonic hippocampal cells in simulated microgravity

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Microgravity is the characteristic condition in which objects and people appear weightless, as happens in the space environment. On the ground, the microgravity is simulated by different approaches including so-called clinostats. A clinostat with one rotation axis (2D clinostat) is operated constantly in one direction resulting in a static change of the gravity vector and in the prevention of sedimentation. 3D clinostats and random positioning machines (RPM) are further machines used for ground-based microgravity experiments aiming to simulate microgravity conditions. 3D clinostats are commonly rotating continuously in a random positioning machine not only the velocity, but also the direction of rotation is altered in a real random mode. The aim of the work was to use the 3D clinostat to generate organoid from embryonic hippocampal cells. The yuri-RPM 3D clinostat was used. Embryonic hippocampal cells (HN9.10e cell line) cultured in the flask was placed on the rotary table for 3 days. The results showed the formation and progressive enlargement of the organoid. Future studies will be useful to test the effect of vitamins or nutraceuticals or drug on the cerebral organoids.

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Privacy-preserving machine learning inference for clinically actionable models

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Introduction and Aim: This study explores machine learning (ML) algorithms that require integration of privacy and security measures due to regulatory concerns. We aim to demonstrate how privacy-preserving ML inference methods can protect the intellectual property of models and ensure the privacy of sensitive data. Our approach includes conducting a security analysis to establish query limits.

Method: We developed privacy-preserving tree-based ML inference model and conducted two security scenarios, each with four parts increasing in synthetic data points. Using XGBoost algorithm, we generate a target model for each scenario and create substitute models through rigorous cross- validation to evaluate the target model's security. Our analysis involves comparing performance metrics between the substitute and target models, assessing feature gain values, and comparing outputs of public and privacy-preserving models.

Findings: The performance differences between the best-performing substitute models and target models for scenario A are 0.27, 0.18, 0.25, 0.26, and for scenario B are 0.04, 0, 0.04, 0.03 in terms of accuracy, sensitivity, precision, and F-score. In scenarios tested, the privacy-preserving XGBoost model matches the plaintext model in prediction accuracy.

Conclusion: The study confirms the effectiveness of the implementation with a query threshold (100) which is sufficient for 14 years.

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GMO products in Cyprus market?

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Due to the significant growth in the presence of genetically modified crops on the world market, despite the advantages of agronomic traits acquired through biotechnology such as resistance to insects and viruses, it has therefore been essential to establish in place reliable genetically modified organisms genetically modified organism (GMO) detection methods. The objective of this study was to be able to identify the presence or absence of GMOs) from samples obtained from food products sold at local markets in Northern Cyprus. The samples were specifically collected from two local markets and an organic shop in Nicosia, mainly banana, apricot and corn between May and June 2024.

Multiplex real time PCR was used for the detection of common GMO markers, notably the promoter 35S, the tNOS terminator and the pFM promoter. The experimental objective of this study was to detect these genetic components in samples collected from local markets in order to assess the presence of GMO.

The targeted GMO markers were not present in any sample tested. Internal and positive controls were used to verify the accuracy of the results, ensuring their validity.

No genetically modified organisms were detected in the food product samples that were tested. Further studies must be conducted by accredited laboratories to maintain the market surveillance.

Keywords: GMO, Cyprus, PCR, banana, apricot, corn, berry

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Relation of post-operative atrial fibrillation after coronary bypass surgery with vitamin D deficiency and vitamin D supplementation: A meta-analysis

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The correlation between vitamin D (VD) deficiency and post-operative atrial fibrillation (POAF) after coronary artery bypass grafting (CABG) surgery and the possible prophylactic effect of VD supplementation before the operation have not yet been clarified. We conducted a meta-analysis according to the PRISMA guidelines on studies containing data on the pre-operative levels of 25- hydroxyvitamin D (25(OH)D) and the incidence of POAF after CABG as well as on studies containing data on the incidence of POAF after CABG with and without VD supplementation before surgery. Pubmed, Scopus and Web of Science were searched, and 15 articles were selected. 7 articles with 1025 patients contained POAF data in patients that received VD supplements before CABG versus placebo. POAF occurred in 79 (15.7%) of 502 patients that received VD and 131 (25.0%) of 523 patients that received placebo ($p<0.001$). 8 studies with 1126 patients contained data about 25(OH)D in patients that developed POAF after CABG vs patients that did not. The concentrations of 25(OH)D were not significantly different between the two groups. Our data suggest that VD supp-

lementation should be examined as an option for the reduction of POAF after CABG surgery.

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Experimental Validation and Characterization of in silico Predicted FDA-approved Drugs as Antibacterial Agents

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Antibiotic resistance has become a global problem, therefore, there is an urgent need to develop new-generation antibiotics. In this project, systematic approaches combining computational and experimental studies were developed for the inhibition of the bacterial ribosome, one of the main targets of antibiotics, to reduce the cost and period for drug design. Virtual libraries were prepared with FDA-approved drugs, and the affinities of the compounds to different functional regions of the bacterial ribosome were predicted by molecular docking. Following *in silico*-prediction, confirmation and characterization of the drug candidates with high docking scores were aimed within the scope of this study. Firstly, the antibacterial activity of these candidates was investigated against *E. coli*. Then, the toxicity of the selected drugs on human cells was evaluated. Next, IC_{50} values were determined by *in vitro* translation inhibition analysis based on luciferase enzyme synthesis with *E. coli* S30 extract. The findings from this project and the developed systematic approach will provide very useful information in solving the problem of antibiotic resistance. This work was supported by TUBITAK 1001 (121Z330) and ITU-BAP (TDK-2022-44130).

Keywords: Bacterial ribosome; antibiotics; protein synthesis; *E.coli*