



New trends in Biology: *from molecules to complex systems*

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ABSTRACTS





ORGANIZERS:

Faculty of Biology

in partnership with:

"Anastasiu Fătu" Botanical Garden and Museum of Natural History in Iași

SECTIONS:

Taxonomy and Ecology

Experimental and Molecular Biology

ASSOCIATED EVENTS:

Round tables: *Educational Resources in Biology* in collaboration with the Romanian Academy - Iași Branch, Subcommission for Nature Monuments

Round table: *Pollution and its effects on organisms* in collaboration with the Romanian Academy - Iași Branch, Commission for Combating Environmental Pollution

Autumn Flowers Exhibition, "Anastasiu Fătu" Botanical Garden of Iași, 45th edition, dedicated to the 165th anniversary of the first botanical garden founding in Romania

Exhibition of 3D molecular models

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SECTION *TAXONOMY AND ECOLOGY*

ORAL COMMUNICATIONS

EDIBLE AND MEDICINAL PLANTS FROM THE UPPER PALEOLITHIC TO BRONZE AGE ON THE TERRITORY OF GEORGIA ACCORDING TO PALYNOLOGICAL STUDY

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Nutrition ration and medication of a man of Upper Paleolithic, Neolithic and Bronze Age of Georgia have been revealed for the first time relying on factual data and using palynological research. Material of 13 archaeological sites was studied, which comprises 153 samples obtained from cultural layers.

The study has shown that a man of the Upper Paleolithic mainly ate meat, as bone material is richly presented in all studied caves. As for vegetative food, he used fruits, sprouts, leaves, seeds and bulbs of many plants. Man of Stone Age already knew 32 species of medicinal plants.

Improvement of man's nutrition took place in the Neolithic because he started cultivating cereals and other plants at that time. Horticulture and viticulture were developed. Flax oil and wine appeared. Folk medicine made progress in the Neolithic period. In comparison with Upper Paleolithic, the Neolithic man studied and used far more medicinal plants. Pollen of 56 medicinal plants was defined from Neolithic layers in all, that is, approximately twice more than in Paleolithic ones. According to palynological analysis, cereals and other kinds of vegetative food were of fundamental importance in the nutrition ration of the Bronze Age population, which was also confirmed during the isotopic analysis of bones of man's skeleton.

Palynological spectra of grain-grinders of Kura-Araxes culture showed that, besides cereals, dry leaves, seeds and fruits of numerous medicinal plants (48 taxa) were rubbed and ground on them. Those medicinal plants, which were found on grain-grinders, were also discovered in the abdomens of deceased persons in burial grounds of settlements. That means that these remedies were prepared in the same place.

A new tradition of funeral appeared in the period of Bedeni culture, according to which some medicines, put in baskets or wooden boxes, were placed in burial alongside with deceased. These are first aid kits and they are found in three burial mounds of Bedeni plateau.

The first great leap in the development of folk medicine during the last 35 thousand years happened in the Neolithic. The second most important change in folk medicine occurred in the period of Bedeni culture. Man used 61 medicinal plants during that time.

According to palynological research, 97 medicinal plants were revealed in all, in the period from Upper Paleolithic to Late Bronze.





A NEW SITE WITH *COCHLEARIA BORZAEANA* (COMAN ET NYÁR.) POBED. IN NORTHERN ROMANIA

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Studii de teren, efectuate între 2017 și 2020, au condus la identificarea unei noi populații de *Cochlearia borzaeana* (Coman et Nyár.) Pobed., o specie rară la nivel național, considerată un endemism Est-Carpatic. Populația este localizată pe valea Cârlibaba, în apropiere de satul Iedu (Jud. Suceava). Fitocenozele identificate au fost încadrate în asociația *Carici flavae*–*Cratoneuretum Kovács et Felföldy 1958 cochlearietosum borzaeanae* (Ștefureac 1972) corr. Kochjarová et al. 2006.





ASPECTS REGARDING THE ECOLOGY AND IMPORTANCE OF *ECHIMUM VULGARE* L. AND *ECHIMUM ITALICUM* L. SPECIES

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This study brings to attention aspects of ecology, and the importance of the species *Echium vulgare* L. and *Echium italicum* L. (family Boraginaceae). The study is based on the analysis of data from the literature.

The genus *Echium* is represented worldwide by 68 species, and in Romania there are 3 species (*Echium italicum*, *Echium russicum*, *Echium vulgare*).

Echium vulgare is a biennial plant species. This species is an Eurasiatic geoelement. It is spread in Europe and Asia, but according to Plants of the World Online (2021) it is also present in other regions of the globe. From an ecological point of view, it is a heliophilic, eurythermic, xero-mesophilic, oligo-mesotrophic, euriionic species.

Echium italicum is a biennial plant species. This species is a southern Eurasiatic geoelement. From an ecological point of view, it is a heliophilic, subthermophilic, xero-mesophilic, neutrophilic and eurinitrophic species.

In Romania, *Echium vulgare* is spread from the plain area to the mountain floor (through meadows, ruderal places, shrubs, gravelly places) and *Echium italicum* from the plain to the oak forests area (through meadows, vineyards, ruderal places, shrubs).

Echium vulgare is a species that has the following potential uses: melliferous (medium economic-beekeeping weight), tinctorial, edible, fodder (in the young stage), ornamental, medicinal. Some parts of the plant (flowers, leaves, stem, root) have uses in traditional medicine. Recent studies have highlighted its antioxidant, antimicrobial, analgesic and antidepressant potential.

Echium italicum has a potential use as edible (leaves) and medicinal, some parts of the plant (flowers, leaves, stem, root) have uses in traditional medicine. Recent studies have highlighted its anxiolytic, sedative, antimicrobial, antioxidant, analgesic, anti-inflammatory and wound healing potential.

Echium vulgare and *Echium italicum* also have the ability to extract heavy metals from polluted soils.





DISTRIBUȚIA ÎN ROMÂNIA A PLANTELOR ALOGENE INVAZIVE DE ÎNGRIJORARE PENTRU UNIUNEA EUROPEANĂ

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În conformitate cu Regulamentul (UE) nr. 1143/2014 al Parlamentului European și al Consiliului din 22 octombrie 2014 privind prevenirea și gestionarea introducerii și răspândirii speciilor alogene invazive, România, ca stat membru, are obligația de a actualiza permanent datele de distribuție pentru speciile alogene invazive de îngrijorare pentru Uniune (Art. 24, 1b). Pentru a răspunde acestei obligații, în țara noastră se derulează un amplu proiect de inventariere și cartare a tuturor speciilor alogene (*Managementul adecvat al speciilor invazive din Romania, în conformitate cu Regulamentul UE 1143/2014 referitor la prevenirea și gestionarea introducerii și răspândirii speciilor alogene invazive – POIM 2014+:120008*), astfel încât să poată fi furnizate hărți actualizate pentru acestea. Un prim pas în acest demers l-a reprezentat realizarea de hărți bazate pe date din literatura de specialitate. Astfel, în această lucrare prezentăm hărțile de distribuție actualizate pentru nouă din cele 36 de specii de plante de îngrijorare pentru Uniune, specii raportate din România: *Ailanthus altissima*, *Asclepias syriaca*, *Cabomba caroliniana*, *Elodea nuttallii*, *Heracleum sosnovskyi*, *Humulus scandens*, *Impatiens glandulifera*, *Ludwigia peploides*, *Myriophyllum aquaticum*. Datele de distribuție sunt însoțite de comentarii privind căile de pătrundere și habitatele invadate. Se remarcă faptul că, în timp ce unele specii au o răspândire extinsă pe teritoriul României (ex., *Ailanthus altissima*, *Asclepias syriaca*, *Impatiens glandulifera*), altele nu au mai fost regăsite de zeci de ani și probabil sunt dispărute (ex., *Cabomba caroliniana*, *Myriophyllum aquaticum*). Cea mai recentă pătrundere este *Ludwigia peploides*, până în prezent cu distribuție la nivelul unui singur bazin hidrografic din sudul țării, afluent al Dunării.





HABITATS OF COMMUNITY AND NATIONAL INTEREST FROM EASTERN PART OF THE NATURA 2000 SITE “SĂRĂTURILE JIJIA INFERIOARĂ – PRUT” (ROSCI0222)

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Humid areas of the lowland river courses have been altered through draining, damming and channel digging and in order to render them suitable for agriculture, grazing and fish farming. These profound modifications result in natural habitat loss with further negative consequences on local fauna. Conservation of biodiversity through habitat diversity preservation is one of the most modern and successful approaches on the matter. Humid areas include a great variety of habitats and favour faunistic concentration. The study site is such an area, encompassed between the valleys of Prut and Jijia. Field studies were conducted in the vegetation seasons of the years 2018 and 2019 and employed the method of Zürich-Montpellier School for vegetation study. In total, 21 associations have been identified. Habitat analysis revealed the presence of six habitats of community interest and another six, of national interest.

A little less than 30% of the studied area is transformed and 59% of the area is covered by habitats of European and national interest (23.5% and 35.5%, respectively). Thus, the conservative value of the study area is quite high given the absence of any kind of management, and therefore it could be easily improved with minimal effort.





THAUMATORYMUS NOTANISOIDES FERR. & NOV. (HYMENOPTERA: TORYMIDAE) NEW TO ROMANIA, A FAUNISTIC ELEMENT WITH MEDITERRANEAN AFFINITIES, WITH ADDITIONS TO ITS BIOLOGY

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On leaves of *Pilosella piloselloides* (Vill.) (Asteraceae) the cynipid wasp *Aulacidea pilosellae* (Kieff.) (Insecta: Hymenoptera: Cynipidae) is forming galls on the mid vein. *Aulacidea pilosellae* is parasitized by *Thaumatorymus notanisoides* Ferr. & Nov. (Insecta: Hymenoptera: Torymidae). The data presented here were gathered by studying the biological material collected in South-Eastern Romania, in Comorova forest near the Black Sea coast. The material consisted of 57 unilocular galls of *P. piloselloides*, from which 5 ♀♀ and 6 ♂♂ specimens of *T. notanisoides* emerged, with a parasitism rate of 19.3%. Of the trophic relations found in this complex the following are new: the relation between *T. notanisoides* and *P. piloselloides*, as well as the whole complex mentioned above; the relation between *A. pilosella* and the host plant *P. piloselloides* as well as the presence of *T. notanisoides* (genus and species) are new to Romania.





ENDOSIMBIONȚII INSECTELOR DIN GENURILE SPALANGIA ȘI EUPELMUS (HYMENOPTERA: CHALCIDOIDEA)

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Muștele sinantropice sunt dăunători asociați activităților umane precum zootehnia și producția de lactate. Controlul acestora utilizând substanțe chimice este problematic, întrucât muștele dezvoltă rezistență la insecticide într-un timp foarte scurt. O alternativă la controlul chimic este cel biologic care utilizează dușmanii naturali ai dăunătorilor. Combaterea biologică este o componentă importantă a agriculturii ecologice, România înregistrând recent cea mai mare creștere a numărului de bovine și ovine crescute ecologic din Uniunea Europeană. Plecând de la rezultatele proiectului PARASYN (PNII) intenționăm să creăm linii de femele telitoce de *Spalangia*, *Trichopria* sau eventual alți parazitoizi ai dipterelelor, adaptate condițiilor locale. Modalitatea de generare a acestora este prin transinfecție cu endosimbionți din genul *Wolbachia* sau alte bacterii endosimbionte. Vom prezenta date privind bacteriile prezente la parazitoizii dipterelelor sinantropice și alte calcidoide.



**SPECIILE PALEARCTICE ALE GENULUI FIDIOBIA (HYMENOPTERA, PLATYGASTROIDEA)****Ovidiu Alin Popovici^{1,*}, Lubomir Masner², Zachary Lahey³, Elijah Talamas⁴**¹Facultatea de Biologie, Universitatea „Alexandru Ioan Cuza” din Iași, Iași, România²Agriculture & Agri-Food Canada, Ottawa, Ontario, Canada³The Ohio State University, Columbus, OH, U.S.A.⁴Florida State Collection of Arthropods, Department of Plant Industry, Florida, USA*Autor corespondent: popovici_alin_ovidiu@yahoo.com

Speciile palearctice aparținând genului *Fidiobia* sunt revizuite, redescrise și ilustrate. Au fost elaborate chei de determinare pentru a facilita identificarea speciilor aparținând acestui gen. Lucrarea conține descrieri de specii noi pentru știință, dar și date noi referitoare la distribuția, respectiv biologia unor specii cunoscute.





RELAȚIILE DINTRE AGENTUL DE COMBATERE BIOLOGICĂ *TORYMUS SINENSIS* ȘI SPECII INDIGENE DIN GENUL *TORYMUS* (HYMENOPTERA: *TORYMIDAE*)

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Una dintre cele mai cunoscute specii din Chalcidoidea utilizată în combatere biologică, *Torymus sinensis* Kamiyo (Ts) a fost introdusă în ultimii 30 de ani în numeroase țări pentru a controla populațiile de *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae). Originar din China, *D. kuriphilus* a fost introdus accidental pentru prima dată în Japonia în 1941, unde a devenit un dăunător major al castanilor comestibili. Câțiva ani mai târziu, în 1975, *T. sinensis* a fost introdus pentru prima dată în Japonia. Odată cu colectarea galelor în vederea stabilirii gradului de parazitism a mai fost identificată o altă specie morfologic foarte apropiată de aceasta, *Torymus beneficus* Yasumatsu și Kamiyo (Tb).

Primele semne de hibridizare între Ts și Tb au fost observate în laborator unde au fost produse femele hibride fertile. Hibridi au fost detectați câțiva ani mai târziu și în teren. Cu un ovipozitor mai scurt, reprezentând un dezavantaj reproductiv, *T. beneficus* a fost eliminat de *T. sinensis* prin competiție și introgresie.

Bazat pe eșantionarea în decurs de 8 ani în Franța și în regiunea de origine (China, Japonia), acest studiu are mai multe obiective printre care: studierea relațiilor filogenetice și ecologice între agentul de combatere biologică introdus și speciile indigene de *Torymus* pentru a detectarea eventualelor evenimente de hibridizare între acestea. Caracterizarea morfologică în vederea discriminării fără echivoc între *T. sinensis* și alte specii *Torymus asociate* cu *D. kuriphilus* în Europa.

Pentru aceasta, am folosit o parte a subunității I din Citocrom Oxidaza C (COI), marker mitocondrial, pentru a examina relația dintre Ts și parazitoizii nativi din genul *Torymus*. Au fost utilizați și alți markeri nucleari precum: Wingless, ITS2 și Enolase, deoarece oferă capacități mai bune de reconstrucție filogenetică fiind deja utilizați în multe studii privind insectele.





REVIEW OF *SCOLELEPIS* (POLYCHAETA: SPIONIDAE) FROM THE BLACK SEA

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Currently, four species of *Scolecopsis* have been mentioned in the Black Sea: (1) *S. squamata* (as *Nerine cirratulus*), (2) *S. (Parascolecopsis) tridentata* (as *Nerinides tridentata* or as *Pseudomalacoceros tridentata*), (3) *S. cantabra* (as *Nerinides cantabra* or as *Pseudomalacoceros cantabra*), and (4) *S. foliosa*. The comparison of specimens from the Black Sea identified as *S. squamata* with specimens of *S. cirratulus* from the Mediterranean, of *S. squamata* from the North Sea and the Atlantic coast of Spain, and with the syntypes of *S. mesnili* from the Atlantic coast of France did not revealed significant morphological differences to treat them as distinct species. Slight morphological differences that were evidenced ranged within a size-related variability of taxonomic characters. However, the analysis of sequences of the mitochondrial cytochrome oxidase subunit 1 (COI) of small- and large-bodied specimens of *S. squamata* from the Black Sea revealed the presence of two genetically distinct species, but none of them was identical to *S. squamata* whose sequences exist in GenBank. The only way to ascertain to which species of *Scolecopsis* do belong specimens from the Black Sea is to collect specimens of *Scolecopsis* from their type localities and undertake a comprehensive study of their morphology and information on molecular markers. For the moment, only collection of *Scolecopsis squamata* from its type locality (island of Helgoland, North Sea, Germany) was successful and as the result a neotype was designated and deposited in the collection of the Zoological Museum at Hamburg University. Examination of the available material in the collection of the “Grigore Antipa” National Museum of Natural History, Bucharest, identified as *S. cantabra*, revealed the presence of two species in the same vial, one of them corresponding to *S. cantabra*, and the second one, a previously unrecognized species, is under way to be described as a new species.





EFFECTUL APLICĂRII GUNOIULUI DE GRAJD PROCESAT PRIN COMPOSTARE ASUPRA EVOLUȚIEI ȘI TRANSFERULUI BACTERIILOR REZISTENTE LA ANTIBIOTICE ȘI A GENELOR DE REZISTENȚĂ LA ANTIBIOTICE LA NIVEL DE FERMĂ

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Gunoii de grajd reprezintă unul din principalele rezervoare ambientale de rezistență la antibiotice (RA), iar aplicarea directă favorizează dispersarea în mediu a factorilor RA.

Studiul de față are ca scop investigarea prezenței unor bacterii rezistente la antibiotice în timpul compostării gunoii de grajd în vederea identificării efectelor aplicării acestuia asupra emergenței RA și corelarea prezenței RA cu diferiți parametri fizico-chimici din probele analizate.

Au fost luate în studiu probe de compost/gunoii de grajd, izolarea tulpinilor bacteriene s-a realizat prin metode bazate pe cultivare, iar identificarea izolatelor s-a realizat cu ajutorul metodei automate Maldi-Tof. Măsurarea parametrilor fizico-chimici s-a realizat utilizând metodele electrometrică și spectrofotometrică.

Analiza probelor utilizând metode bazate pe cultivare (directă/îmbogățire) a permis izolarea tuturor fenotipurilor de rezistență investigate, respectiv *Enterococi* rezistenți la vancomicină (VRE), *Staphylococcus aureus* rezistent la meticilină (MRSA), *E. coli* producătoare de beta-lactamaze de spectru extins (ESBL) și *Enterobacteriaceae* producătoare de carbapenemaze (CRE). Identificarea izolatelor bacteriene cu ajutorul metodei Maldi-Tof a evidențiat că tulpinile cu aspect de VRE s-au dovedit a fi tulpini de *Pediococcus* sp., iar unele dintre cele identificate ca fiind MRSA au fost confirmate ca *Staphylococcus sciuri* sau *Staphylococcus warneri*.

Inspecția valorilor parametrilor fizico-chimici măsurați arată o variație a pH-ului între 6,9 și 8,23, și un conținut ridicat de apă, de peste 90%. Valorile nutrienților evidențiază o creștere clară a amoniului și a azotului anorganic total în fermele de bovine și o fermă de suine (F3P).

Studiul evidențiază colonizarea mediului cu tulpini rezistente, densitatea acestora prezintă o tendință descrescătoare începând cu a doua lună de compostare, și creșterea clară a valorilor nutrienților, mai ales în fermele de suine, aspect care întărește necesitatea evaluării strategiilor de intervenție și elaborarea unui set de recomandări care să conducă la diminuarea riscurilor de expunere și o reducere ulterioară a impactului asupra sănătății umane.





AMPHIBIANS FROM THE NATURA 2000 SITE “SĂRĂTURILE JIJIA INFERIOARĂ – PRUT” (ROSCIO222)

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Amphibians are one of the most threatened groups of animals in Europe. Because most of them are protected, amphibians may act as umbrella and flagship species in certain areas. The Natura 2000 site “Sărăturile Jijia inferioară – Prut” (ROSCIO222) is such an area that, despite its conservation status, is largely exposed to human activities and alterations. Thus, the aim of this study is to update the knowledge on the local amphibian fauna. Therefore, we carried out field surveys (visual diurnal and aural nocturnal) in the site area from April to August, 2018 and from March to July, 2019. We identified seven amphibian taxa (*Triturus cristatus*, *Lissotriton vulgaris*, *Bombina bombina*, *Bufo viridis*, *Hyla orientalis*, *Pelobates fuscus* and species of *Phelophylax esculentus* Complex) that are typical for the major relief forms of the area. Five of the identified species are strictly protected according to European and national regulations, whereas the standard data form of the area lists only two species. Thus, the management plan of the site should consider all the strictly protected species as the standard data form fail to reflect the actual richness of the amphibian assemblages.





MIGRATION OF BIRD FAUNA IN THE PERIMETER OF THE ROSPA0072 SIRET MIDDLE FLOODPLAIN (ROMANIA)

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Our ornithological study presents data on the diversity of bird species recorded during the migration time on the territory of ROSPA0072 Siret Middle Floodplain, starting from July 2016 until June 2017. We identified 150 bird species during the spring migration, and 153 bird species in the autumn migration. We notice the presence of some bird species only during the spring, respectively autumn migration in the area, 14 of them included in the Romanian Red Book of Vertebrates. We also present quantitative data for observed bird species, and we recorded bigger populations during the autumn migration than in the time of spring migration but smaller than appear in the official standard forms of the Natura 2000 site. The typical forest species are dominant by diversity but some bird species related to the wetland habitats present significant populations. We notice the relevance of this territory for the migration of the white stork (*Ciconia ciconia*), diurnal raptor bird species, and some waterfowl species in the Eastern Romania.





PATHOLOGICAL EVALUATION OF THE SKELETON M190 DISCOVERED IN THE NECROPOLIS OF THE “VULPE” CHURCH (17TH-19TH CENTURIES) FROM IAȘI (ROMANIA)

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In this study, the authors present a possible case of multiple myeloma, identified in a sample of skeletons discovered in the necropolis of “Vulpe” Church in Iași (Romania) during 2019-2020 archaeological campaign. According to information provided by the archaeologists, the limits of use of the necropolis over time are set between the 17th and 19th centuries.

Multiple myeloma is characterized by an abnormal accumulation of malignant plasma cells in the bone marrow, which causes damage, fragility, and bone destruction, caused by the local imbalance between osteoblasts and osteoclasts. Cases reported in several regions of the world, dating from the Neolithic to the Middle Ages, have increased knowledge about this pathology. Lesions of multiple myeloma are described as uniform and circular in size, unless they join, without the formation of new bone or bone reaction, giving them a “hit” appearance.

The case of multiple myeloma, described in this work, was identified in a skeleton (M190) belonging to a male aged 30-35 years (young adult). There are several perforations typical of multiple myeloma in the cranial segment (frontal, parietal and maxillary bones) and postcranial (femur, tibia, clavicle, sternum, and sacrum). The macroscopic, stereomicroscopic, and histological analyses were followed by imaging analysis (i.e. radiology and computed tomography).

For histological analysis, bone samples were cut using a Dremel 3000 variable speed multi-tool. Samples were cleaned in multiple sonic baths and embedded in Buehler Epothin 2 epoxy resin. The sections were ground and polished using carbide paper and visualized through a transmitted light microscope. The microscopic assessment revealed new periosteal bone formations.





ARCHAEOZOOLOGICAL DATA ON THE BRONZE AGE COMMUNITY OF WIETENBERG CULTURE FROM UROI-SIGHETI (HUNEDOARA COUNTY, ROMANIA)

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The Uroi village, from where the studied site comes from, is located on the right bank of the Mureș river, near the Simeria city (Hunedoara County, Romania), to which it belongs from administrative point of view. The archaeological site is located between 20+280 km and 20+512 km on the highway route, right in the Mureș river meadow, in the flooding area. Archaeological research taken in Uroi-Sighet led to the discovery of 230 complexes belonging to Bronze Age, Iron Age and Middle Ages.

The studied archaeozoological material is dated to the Middle Bronze Age (i.e., phases II and III of the Wietenberg culture).

For the phase II of the Wietenberg culture (W II) were identified 103 faunal remains, and for the phase III (W III) 647 fragments. Animal husbandry was the main activity to obtaining food, domestic mammals having frequencies of 93% in W II, and 87% in W III. The identified domestic mammals are: *Bos taurus* (cattle), *Ovis aries* (sheep), *Capra hircus* (goat), *Sus domesticus* (pig), *Equus caballus* (horse) and *Canis familiaris* (dog).

With a frequency of only 7% wild mammals, the hunting was a minor occupation for the inhabitants of the W II settlement, but relatively more important in W III, with 13% of remains. The identified wild species are: *Cervus elaphus* (red deer), *Sus scrofa* (wild boar), to which are added *Capreolus capreolus* (roe deer) and *Lepus europaeus* (hare) in the W III level.

The presence of mollusks in both cultural levels, as well as the identification of a fragment of *Silurus glanis* (catfish) in the W III assemblage both indicate that the prehistoric communities of Uroi-Sighet were also using aquatic resources for food procurement.





DENTAL MICROWEAR PATTERNS – EVIDENCE IN A HUMAN POPULATION OF 17TH CENTURY FROM IASI CITY (ROMANIA)

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Dental microwear is used in attempts to characterize the diet of past human populations and it refers to the microscopic marks left on the teeth hard tissues (i.e., enamel, dentine), usually on buccal and occlusal surfaces. The microwear patterns provides insight into the physical properties of the food and abrasives consumed in the weeks or even months before death. Several studies address dental microwear analysis of past human populations in diachronic and synchronic approach, but the knowledge of within-group microwear variation is still precarious. The aim of this research is to identify, and describe patterns of the within-group dental microwear variation, on M2 molars, depending on sex and anatomic position (i.e., left/right, superior/inferior) in a 17th century archaeological population discovered in Iasi city (Romania). The dental microwear analysis was performed on the “facet 9” of the distobuccal cusp (mandibular molars) and mesiolingual cusp (maxillary molars) through three nonoverlapping consecutive micrographs/molar, using a scanning electron microscope (SEM) Tescan Vega II SBH. The microwear features were identified as linear marks (fine and coarse scratches) and nonlinear marks (small and large pits) using an early version of MicroWear software. The microwear features along with their dimensions (i.e., length and width) were subjected to multivariate analysis. The microwear profile of the analysed archaeological population can be described as “abrasive” due to the high proportion of pronounced traces such as coarse scratches and large pits. Our findings show a within-group microwear variation: two microwear profiles based on molar laterality in females (left and right for both maxillary and mandibular M2 molars), and one less heterogeneous microwear profile in males. The microwear pattern in males is similar to that of the right molars in females.





ASSESSING THE SEXUAL DIMORPHISM IN AN ARCHAEOLOGICAL HUMAN POPULATION OF 17TH CENTURY FROM IAȘI (ROMANIA): GEOMETRIC MORPHOMETRICS OF THE SECOND MOLAR TEETH

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The dental features are highly heritable and evolutionarily conservative, representing an excellent anthropological material in the study of phylogeny, migrations and morphometrical variability. The dental variability is a very debated topics, especially in correlation with the sexual dimorphism. Although the sexual dimorphism has been intensively studied on the molar size (i.e. mesiodistal and buccolingual diameters), the molar shape by sex is not yet a topic addressed at the same scale. This study aims to evaluate the size and shape in correlation, in order to obtain predictable dental models in terms of sexual dimorphism. For this purpose, the Geometric Morphometrics method was applied on a sample of permanent second molars M2 (upper/maxillary – M2, and lower/mandibular – M2). The dental material belongs to human skeletons of 17th century from the “Curtea Domnească” necropolis of Iași city (Romania). The bidimensional data were collected using a set of landmarks placed on the occlusal surface, at groove junctions, and a set of semilandmarks on the periphery of the same surface. Our results highlight how different regions of the tooth are associated, and how patterns of the M2 shape variation are defined in the two sexes. The size is found to be a poor indicator for sexual dimorphism, contrary to the shape which additionally provides a high degree of discriminant role. According to our results, the upper M2 tooth is stronger sexual dimorphic than the lower M2. Discriminatory features for the upper second molar (M2) are described by the variability of buccolingual area, shape variability of hypocone and paracone cusps, while in the lower second molar (M2), the distal cusps have a greater dimorphic role.

The dental phenotypic patterns described in our study could be applied in a complementary method to identify sex in skeletal samples, when classical methods cannot be applied.





CERCETĂRI MULTIDISCIPLINARE PRIVIND INTERACȚIUNILE OM - MEDIU LA CETATEA ROMANO-BIZANTINĂ DE LA IBIDA (DOBROGEA, SUD-ESTUL ROMÂNIEI)

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Situată în partea de nord a Dobrogei, care aparținea provinciei Scythia Minor, Ibida a fost cea mai mare și una dintre cele mai importante fortificații din această regiune în perioada romano-bizantină, între secolele IV-VI. Studiul nostru evidențiază utilitatea cercetărilor multidisciplinare pe teritoriul cetății Ibida, prin integrarea datelor faunistice și a celor obținute din analiza fitolitelor, în strânsă legătură cu caracteristicile solului, pentru a înțelege interacțiunile om-mediu din trecut.

Rezultatele obținute pe baza fitolitelor indică dominanța gramineelor spontane. Faza de locuire corespunzătoare perioadei romano-bizantine a fost identificată la 1,65 m adâncime. Acesta a fost cel mai bogat nivel în artefacte și a înregistrat un procentaj semnificativ de fitolite de tip ELONGATE DENDRITIC, sugerând o acumulare importantă de inflorescențe de graminee în stratul cultural. Acest lucru ar putea fi explicat fie prin prezența cerealelor la nivelul locuinței (depozitare), fie prin prezența gramineelor spontane utilizate ca furaje pentru animale. Prelucrarea datelor faunistice a fost efectuată pentru a evidenția caracteristici din diferite contexte arheologice (adică *intramuros*, zid și *extramuros*). Identificarea a numeroase rămășițe de bovine și ovine / caprine și caracteristicile solului sugerează o utilizare pastorală a terenului din apropierea cetății.





ECOPHYSIOLOGICAL AND LIFE-HISTORY ADAPTATIONS OF *GAMMARUS BALCANICUS* (Schäferna, 1922) IN A SINKING-CAVE STREAM FROM WESTERN CARPATHIANS (ROMANIA)

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Freshwater gammarids are known to colonise occasionally sinking-cave streams, providing contrasting morphological, life-history and ecophysiological adaptations compared to their surface conspecifics. In this study, a subterranean and a surface population of the species *Gammarus balcanicus* was surveyed for one year in a sinking-cave stream from the Western Carpathians (Romania). The results showed that the cave-dwelling population comprised individuals that were significantly larger compared to their surface conspecifics, had larger body-size at sexual maturity and that the females produced fewer, but larger eggs, compared to the population situated outside the cave. The trophic position and the omnivory were significantly higher for the cave-dwelling compared to surface population and the elemental imbalance for C:P molar ratios lower, but similar for C:N. However, the subterranean population did not present troglomorphic characters or longer lifespan as known for other cave-surface paired crustaceans. This, together with the rather extensive hydrological connection of the habitats, suggests active gene-flow between populations and similar response to seasonality for body-size distributions, indicating that the observed ecophysiological and life-history differences are rather the consequence of phenotypic plasticity than the result of genetic adaptation.





RESPONSE OF AQUATIC MACROINVERTEBRATES COMMUNITIES TO MULTIPLE ANTHROPOGENIC STRESSORS IN A LOWLAND TRIBUTARY RIVER

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In the current study the response of aquatic macroinvertebrate communities to multiple anthropogenic stressors in a typical lowland river that crosses pristine sectors situated toward headwaters, as well as densely populated urban areas was assessed. We wished to develop an effective bioassay procedure for assessing water and sediment quality in lotic ecosystems from Romania with the aid of macroinvertebrate organisms correlated with physicochemical parameters and pollutants in both dissolved fractions and material bonded to sediment. A fast scanning approach of the river, from springs to the mouth, was employed. We found significant changes in physicochemical parameters along a longitudinal gradient, the highest values being registered within the urban area and heavily agriculturally developed areas. The macroinvertebrates showed affinities for certain abiotic factors, emphasising their potential use for future studies as reliable ecological indicators, shaped by a synergic combination of urban effects and magnitude of type of land use.





SPECII DE PLANTE CU STATUT DE PROTECȚIE LA NIVEL EUROPEAN PREZENTE ÎN COLECȚIA HERBARULUI UNIVERSITĂȚII “ALEXANDRU IOAN CUZA” DIN IAȘI

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Diminuarea suprafețelor cu vegetație naturală și seminaturală, prin presiunea economică și antropică, pune în pericol efectivele populațiilor de plante din România. La nivel mondial, numărul speciilor de plante periclitare sau în curs de dispariție este în creștere. Pentru a identifica principalii factori de risc cu care speciile se confruntă, este nevoie să se cunoască cât mai exact zonele în care acestea cresc, astfel încât să poată fi luate măsurile necesare pentru protecția lor.

Prezentul studiu și-a propus să centralizeze speciile de plante din România, ce sunt protejate la nivel european și care se găsesc în colecția herbarului Facultății de Biologie a Universității “Alexandru Ioan Cuza” din Iași (acronim I), cât și date privind distribuția acestora în țară. Pe baza documentelor elaborate la nivel european (Convenția de la Berna, Directiva Habitate) și a materialului de herbar existent în colecție s-a întocmit o listă de specii. Specimenele de herbar au fost verificate și revizuite, iar pentru fiecare s-au notat informații despre localizare, data colectării, dar și categoria zoologică. Materialul prelucrat a fost introdus în baza de date a herbarului.





SECTION EXPERIMENTAL AND MOLECULAR BIOLOGY

ORAL COMMUNICATIONS

DIETARY INTERVENTION EFFECT OF DIHYDROMYRICETIN ENCAPSULATED WITH D- α -TOCOPHERYL POLYETHYLENE GLYCOL 1000 SUCCINATE-SAPONIN SELF-MICROEMULSION DRUG DELIVERY SYSTEM

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Dihydromyricetin (DMY) has a good effect on extenuating hyperlipidemia, which is highly related with high fat diet (HFD), but its poor absorptivity in blood and intestines restrained its availability and application.

The present study aims to design a self-microemulsion delivery system (d- α -tocopheryl polyethylene glycol 1000 succinate - quillaja saponin) to enhance the absorptivity of DMY (DMY-S), and to investigate its dietary intervention effect on HFD fed mice.

High-throughput sequencing analysis of intestinal microbiota and UPLC-QTOF/MS-based liver metabolomics were applied to disclose the preventive effect of DMY-S on hyperlipidemia in HFD mice and to figure out the possible mechanism.

DMY-S can inhibit the increase of bodyweight and fat mass, preventing non-alcoholic fatty liver disease. Compared to the model group, the abundance of mice intestinal flora is mainly changed in certain bacterial genera of *Firmicutes* and *Bacteroides*, including *norank_f_Muribaculaceae* and *Blautia*. The result of metabolism analysis indicated that the expression levels of cinnosiol B, creatine, pantothenic acid and aminobutyric acid in the liver tissues of mice treated with DMY-S showed a down-regulation. The DMY-S prevented hyperlipidemia in HFD mice mainly by affecting the metabolic pathways including glycerophospholipid metabolism, sphingolipid metabolism and pantothenate and CoA biosynthesis.

Taken together, the results suggest that TPGS-QS-SMEDDS can improve the biological activity of DMY in the body, and DMY-S can prevent related diseases caused by HFD by regulating lipid metabolism and excretion. These findings may contribute to improving the bioavailability of DMY and understanding the metabolic mechanism of DMY-S.





THE EFFECT OF SECONDARY METABOLITES ON ANTIMICROBIAL ACTIVITY IN WATER TREATMENT

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Microorganisms can be found naturally in nature (soil, air, water). Through our food and air, we are exposed to these species from birth. The greatest health risk from the presence of these microorganisms in water is from drinking contaminated water. Outbreaks of waterborne disease have been associated with inadequate treatment of water supplies and unsatisfactory management of water distribution. The main objective of this work is to study the antimicrobial activity of secondary metabolites on *Escherichia coli* and *Bacillus subtilis*.

The antimicrobial activity of various compounds against *Escherichia coli* and *Bacillus subtilis* was determined by the broth micro dilution method. This method is one of the most fundamental methods for analyzing the susceptibility of microorganisms to antimicrobial agents. To apply this method, it was based on: the preparation of solutions of the compounds to be tested, preparation of the inoculum, micro dilution in broth of the compounds, inoculation and incubation.

The results of the antimicrobial activity against *Escherichia coli* and *Bacillus subtilis* of 12 compounds of natural origin (secondary metabolites and major compounds), showed a good antimicrobial activity of thymol and carvacrol as curative means against the formation of biofilms of these two bacterial species on the channels of water treatment.





WHERE SHOULD THE LINE BE DRAWN WHEN CONSIDERING THE BENEFITS AND DISBENEFITS OF USING PESTICIDES FROM A FUNGAL PERSPECTIVE?

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The introduction of pesticides, and particularly the insecticides, have changed the modern life in many ways. Such commercially available products have a wide range of applications, from crop protection and husbandry up to the domestic use. Modern intensive agriculture has benefited the most from their use.

The industry of pesticides has a long history of big mistakes, with widely marketed compounds that were highly toxic to both humans and wildlife. Negative effects such as carcinogenic, teratogenic, endocrine-disrupting effects, among many others, combined with a strong resilience and persistence in natural ecosystems are subjects of important public concerns. There is a long list of products that have been used in the past but are currently being banned from commercialization due to their toxicity. Today, in most countries, there are governmental bodies and authorities involved in regulation and approval of pesticides before entering the market.

When assessing the negative side-effects of such products, many scholars are focusing on the toxicity over human health or aquatic animals, for practical reasons, while other types of organisms are often being ignored, although very important in environment.

In the present study we assessed the toxicity of currently market-available insecticides over saprotrophic beneficial fungi. Several saprotrophic yeasts, as well as filamentous fungi (Ascomycota and Basidiomycota) were cultured under the influence of insecticides. Insecticides were added in different concentrations as commercial formulation and not as pure compounds, through filter sterilization. Standard media were used according to the species requirements. The toxicity was evaluated through biomass accumulation rate and minimum inhibitory concentration of insecticides, which were verified using both disc-diffusion and serial micro-dilution methods.

The used insecticides containing synthetic pyrethroids presented varied effects over fungal growth and development in our study, suggesting a significant negative impact over such non-target organisms.





COMPARATIVE ANALYSIS OF SOME MORPHOLOGICAL AND BIOCHEMICAL TRAITS IN 37 TOMATO CULTIVARS GROWN IN ECOLOGICAL GARDEN OF THE BOTANICAL GARDEN „VASILE FATI“, JIBOU

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The aims of this study were a brief description of the tomato cultivars collection grown in ecological conditions in Jibou Botanical Garden and a comparative study of the morphological and biochemical features of the fruits. The collection consists of cultivars and varieties of tomatoes from Romania and Europe, obtained by seed exchange with other botanical gardens.

One of the purposes of our botanical garden is to preserve in its collection the seeds of these tomato cultivars and varieties. In order to analyze the impact of the ecological growth on the cultivars and varieties, a comparative analysis of the morphological characteristics and the content of some bioactive compounds (ascorbic acid, lycopene and beta carotene) of the fruits was performed.

Results from this preliminary study will be used for a comprehensive description of our collection during the next years.





STUDIUL PORTAJULUI INTESTINAL DE ENTEROBACTERII PRODUCĂTOARE DE BETA-LACTAMAZE DE SPECTRU LARG LA LUCRĂTORII DIN STAȚIILE DE EPURARE A APELOR UZATE

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Rezistența la antibiotice este o amenințare iminentă la adresa sănătății publice globale, din cauza creșterii alarmante a ratelor de rezistență ale agenților patogeni, fiind necesare măsuri de reducere a riscurilor de transmitere și expunere umană. Stațiile de epurare a apelor uzate (SEAU) sunt puncte critice pentru diseminarea bacteriilor rezistente cu importanță clinică de origine umană, în mediu.

Scopul studiului este determinarea profilurilor de sensibilitate la antibiotice ale unor tulpini de *Enterobacteriaceae* producătoare de beta-lactamaze cu spectru extins (ESBL-E) izolate din probe de materii fecale prelevate de la lucrătorii din SEAU.

Au fost luate în studiu un număr de 29 de tulpini bacteriene aparținând familiei *Enterobacteriaceae*: *E. coli* și grupul KESC (*Klebsiella*, *Enterobacter*, *Serratia*, *Citrobacter*), izolate din probe de materii fecale colectate de la lucrători SEAU, pe medii cromogene selective, suplimentate cu cefalosporine de generația a III-a, care permit recuperarea selectivă a tulpinilor ESBL-E. Testarea spectrului de sensibilitate la antibiotice s-a realizat prin metoda disc difuzimetrică Kirby-Bauer, iar rezultatele obținute au fost interpretate cu ajutorul standardului CLSI, ediția 2019.

Tulpinile de *Enterobacteriaceae* izolate din probe de materii fecale colectate de la lucrătorii din SEAU au prezentat rate înalte de rezistență la antibioticele beta-lactamice, dar și la antibiotice din alte clase, precum trimetoprim-sulfametoxazol și tetraciclină. Toate tulpinile de *Enterobacteriaceae* au manifestat sensibilitate la: cefepim, ertapenem, meropenem, imipenem, amikacină și ciprofloxacina. Un procent de 17.24 % dintre tulpinile analizate a manifestat multirezistență la antibiotice, iar testele de confirmare au evidențiat faptul că majoritatea tulpinilor de *Enterobacteriaceae* (24/29) au manifestat fenotipul ESBL.

Studiul realizat evidențiază colonizarea lucrătorilor SEAU cu tulpini ESBL care au manifestat rate înalte de rezistență la antibiotice, aspect care întărește necesitatea implementării unor măsuri de reducere a utilizării abuzive a antibioticelor la nivel global și elaborarea de politici adecvate de reducere a prevalenței acestora în comunitate.





FIBROUS GELATIN-BASED SCAFFOLDS ENRICHED WITH MNPs DISPLAY CYTOCOMPATIBILITY WITH HUMAN ADIPOSE-DERIVED STEM CELLS

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Human adipose-derived stem cells (hASC) are promising candidates for nervous tissue engineering grace to their ability to transdifferentiate into cells of ectodermal origin, such as neurons or glial cells, considering their mesodermal origin. Moreover, magnetic biomaterials are currently investigated as potential scaffolds to promote nerve regeneration. Therefore, we aim to develop and investigate the cytocompatibility and potential of natural-based materials enriched with magnetic nanoparticles (MNPs) to support hASCs growth, viability and proliferation. Gelatin composites, generated through electrospinning technique, were enriched with different concentrations of MNPs (0%, 0.5%, 1%, 2%), seeded with hASC and maintained in standard culture conditions for one week. In order to assess their biocompatibility, cell viability and proliferation were investigated using MTT test, while cytotoxic profile was evaluated based on lactic dehydrogenase (LDH) release in culture medium. The extent of both live and dead cells was revealed through Live/Dead assay and changes in cytoskeleton arrangement and focal adhesion formation were investigated by immunolabeling and confocal microscopy. All tested composites manifested good biocompatibility when tested in contact with hASCs, without inducing significant cytotoxic effects for up to seven days of *in vitro* culture. However, results indicated increased cell viability and proliferation in the presence of MNPs, with a proportional increase dependent on MNPs concentration. Cytoskeleton and focal adhesion immunolabeling revealed an elongated shape of actin microfilaments and highlighted paxillin distribution, indicating beneficial effects of MNPs-enriched scaffolds. Therefore, materials with MNPs content manifest good interaction with hASC and could be used in further studies of hASC transdifferentiation towards neuronal lineage.

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RESVERATROL-LOADED VESICULAR ELASTIC NANOCARRIERS GEL IN IMIQUIMOD-INDUCED PSORIASIS TREATMENT: IN VITRO AND IN VIVO EVALUATION

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This work aimed to develop a new efficient approach for safe treatment of psoriasis. To achieve that, resveratrol-loaded spanlastics(F1-F12) were prepared and evaluated by complete in vitro characterization. The two optimal formulations (F10 and F11) had their particle size in the nano range with high entrapment efficiency and sustainable drug release. These two formulae were incorporated in carbopol 934 gel formulations (G1-G8) with different concentrations of drug and carbopol 934 polymer. G1 and G5 (1% w/w Carbopol 934 gel and 0.1% resveratrol) showed $40.13\% \pm 2.017\%$ and $73.76\% \pm 2.46\%$, 8 hours drug release, respectively. Their pH was accepted and non-irritant. At a shear stress of 500 s^{-1} , G1 and G5 showed a reasonable viscosity of $1048.5 \pm 2.12 \text{ cps}$ and $954 \pm 2.15 \text{ cps}$, respectively. In the in vivo psoriasis study, mice treated by G5 gel showed significant improvement of erythema and scaling compared to positive control group and they maintained healthy skin as shown in histopathological observations. Moreover, this group showed the least changes in mRNA expression of inflammatory cytokines. Concisely, our results suggest that selected carbopol gel of resveratrol-loaded spanlastics could maximize resveratrol topical anti-psoriatic effect.





MTT AND CLONOGENIC ASSAYS TO EVALUATE THE IN VITRO EFFECT OF BLEOMYCIN IN DIFFERENT TUMOR CELL LINES

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Bleomycin (BLM) is an anti-tumor antibiotic glycopeptide synthesized by *Streptomyces verticillus*, inducing DNA double strand breaks, similar to those obtained by radiotherapy. It is considered a radiomimetic drug indicated, in association with other cytotoxic agents, for slow or stop the growth of cancer cells. The aim of this study is to assess the bleomycin effect using the MTT and the clonogenic assays, both tests being applied in chemoradiation and radiosensitivity analyses. Clonogenic assay is commonly used to investigate survival of irradiated cancer cells, whereas MTT method is performed for the study of chemosensitivity or toxicity of the drugs on different cell lines. Despite its long duration, the colony formation assay is reliable method used in cancer research, allowing the *in vitro* screening to determine therapeutic radiation doses and drug concentrations.

In present study the HeLa cervical cancer cell line and MCF-7 breast carcinoma cells were treated with logarithmic bleomycin doses (0.1, 1.0 and 10 $\mu\text{g}/\text{mL}$), for 24 and 48 h. For the colonies formation experiment, 100 cells/well were seeded in 12-well plates and allowed to grow for two days before treatment. After the expiration of the treatment period (48 hours), the fresh growth medium was added, the procedure being repeated at each 3 days. Experiment was stopped when the number of the colonies at the Control reached over 50 cells/colony (two weeks). At this moment, the colonies were washed with PBS, fixed in ethanol, and stained with Trypan blue. The number and size of the colonies were analyzed using ImageJ plugin.

The cell viability generally had a dose-dependent diminution, the effect being more intense after 48 h of treatment. Regarding the colonies formation, the number of the colonies decreased at high doses (10 $\mu\text{g}/\text{mL}$), and the MCF-7 cell line was more sensitive to the radiomimetic treatment than HeLa cells.

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IN VITRO ASSESSMENT OF CYTOTOXICITY IN HEALTHY CELLS FOLLOWING EXPOSURE TO HEAVY METALS

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Heavy metal pollution is one of the main problems of contemporaneity, difficult to manage, due to their chemical stability and high degree of toxicity. The present study aims to investigate cytotoxic events occurring as a result of direct exposure of mammalian cells to the action of some heavy metals (cadmium and lead), emphasizing the importance of investigation of intracellular damages, for which data from the literature are limited. Thus, the effect of heavy metals, administered in form of salts, respectively cadmium chloride (CdCl₂) and lead nitrate (Pb(NO₃)₂) was *in vitro* tested, on certain cytophysiological processes – cell viability, protein synthesis, enzymes involved in protection against oxidative stress, cell cycle, the complex process of apoptosis, integrity of genetic material – in normal cell lines, kidney cells from the African monkey *Cercopithecus aethiops*, Vero (ATCC® CCL-81) and Chinese hamster lung fibroblasts, V-79 (ATCC® CCL-93™), at different doses and time intervals.

All our *in vitro* research has revealed that both cadmium and lead are cytotoxic and genotoxic, by decreasing cell viability and protein synthesis, as well as by damaging DNA in Vero renal cells and V-79 fibroblasts, the toxicity of these heavy metals can be the result of complex mechanisms of action involving the induction of programmed cell death (apoptosis) and oxidative stress.

These results represent the scientific support of future research directions, which on the one hand, will highlight the effect of heavy metals on other cell lines, healthy or tumor, in order to use *in vitro* cell systems as methods for rapid testing of heavy metal toxicity, and on the other hand, they will contribute to the development of effective protection and treatment methodologies for diseases related to exposure to heavy metals.

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COGNITIVE FACILITATION AND ANTIOXIDANT EFFECTS OF AN ESSENTIAL OIL MIX ON SCOPOLAMINE-INDUCED AMNESIA IN RATS: MOLECULAR MODELING OF IN VITRO AND IN VIVO APPROACHES

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The present study investigated the capability of an essential oil mix (MO: 1% and 3%) in ameliorating amnesia and brain oxidative stress in a rat model of scopolamine (Sco) and tried to explore the underlying mechanism. The MO was administered by inhalation to rats once daily for 21 days, while Sco (0.7 mg/kg) treatment was delivered 30 min before behavioral tests. Donepezil (DP: 5 mg/kg) was used as a positive reference drug. The cognitive-enhancing effects of the MO in the Sco rat model were assessed in the Y-maze, radial arm maze (RAM), and novel object recognition (NOR) tests. As identified by gas chromatography-mass spectrometry (GC-MS), the chemical composition of the MO is comprised by limonene (91.11%), followed by γ -terpinene (2.02%), β -myrcene (1.92%), β -pinene (1.76%), α -pinene (1.01%), sabinene (0.67%), linalool (0.55%), cymene (0.53%), and valencene (0.43%). Molecular interactions of limonene as the major compound in MO with the active site of butyrylcholinesterase (BChE) was explored via molecular docking experiments, and Van der Waals (vdW) contacts were observed between limonene and the active site residues SER198, HIS438, LEU286, VAL288, and PHE329. The brain oxidative status and acetylcholinesterase (AChE) and BChE inhibitory activities were also determined. MO reversed Sco-induced memory deficits and brain oxidative stress, along with cholinesterase inhibitory effects, which is an important mechanism in the anti-amnesia effect. Our present findings suggest that MO ameliorated memory impairment induced by Sco via restoration of the cholinergic system activity and brain antioxidant status.





**VALORIZATION OF ESSENTIAL OILS FROM *TETRACLINIS ARTICULATA* AND *PINUS HALEPENSIS*:
APPLICATION IN AN ANIMAL MODEL WITH ALZHEIMER'S DISEASE
INDUCED BY B-AMYLOID (1-42)**

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Many new drugs derived from secondary plant metabolites have been applied for the treatment and prevention of various diseases. Aromatic plants are often used in traditional medicine. Essential oils are widely used as antibiotics to treat infectious diseases due to bacterial contamination; they are also used as antioxidants to treat neurodegenerative disorders including Alzheimer's disease.

The objectives of this work were therefore to analyze the chemical compositions, to evaluate, *in vitro*, the antimicrobial and antioxidant activities and to examine, *in vivo*, the neuroprotective effects in rats with Alzheimer's disease induced by β -amyloid (1-42) of essential oils from *Pinus halepensis* Mill. and *Tetraclinis articulata* (Vahl) Masters.

The essential oils were extracted by the hydrodistillation technique using a Clevenger type apparatus. The ability of essential oils to inhibit microorganisms was tested using three different methods: the disk diffusion method, the liquid macrodilution method and the solid state dilution method. The antimicrobial activity was variable depending on the nature of the strain and the essential oil. Subsequently, *in vitro* antioxidant activity was evaluated by two methods: the DPPH free radical scavenging test and the ABTS test. Based on the results, both oils tested have an important antioxidant potential. Also, we demonstrated that our essential oils reversed the A β 1-42-induced decreasing of the spontaneous alternation in the Y-maze test and the A β 1-42-induced increasing of the working and reference memory errors in the radial arm maze test. Biochemical estimates of brain homogenates for acetylcholinesterase and biomarkers of oxidative stress were also performed. Furthermore, the oxidant-antioxidant status in the rat hippocampus was recovered after treatment with essential oils. These results suggest that the essential oils of *P. halepensis* and *T. articulata* have neuroprotective activity and can be considered as a therapeutic tool for the mitigation of amyloid A β toxicity and neuronal dysfunction.





AMELIORATIVE EFFECTS OF COTININE AND 6-HYDROXY-L-NICOTINE IN A SCOPOLAMINE-INDUCED ZEBRAFISH MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD), a progressive neurodegenerative disorder, affects over 50 million people worldwide and represents the most common form of dementia and the fifth leading cause of death. AD is associated with memory impairments and cognitive decline, although several neuropsychiatric symptoms, such as anxiety, depression or apathy were also observed in the early stages of the disease. Zebrafish (*Danio rerio*) was successfully used to simulate AD pathology. Cotinine (COT) and 6-hydroxy-L-nicotine (6HLN) are two nicotinic derivatives that possess pro-cognitive and antioxidant properties in different rat models of AD, eluding the side-effects of nicotine (NIC), their precursor. The current study aimed to evaluate the effects of COT and 6HLN on memory deficits, anxiety-like behavior and oxidative stress in the scopolamine (SCOP)-induced zebrafish model of AD. For this, 1 and 2 mg/L of COT and 6HLN were administered to zebrafish that were previously treated with SCOP (100 μM). The memory performances were assessed in Y-maze and novel object recognition (NOR) tasks while the anxiety-like behavior was assessed in novel tank diving test (NTT). The biochemical parameters of oxidative stress were monitored from brain samples and RT-qPCR analysis was used to measure the *egr1* and *nrf2a* gene expression. Data were statistically analyzed using one-way ANOVA and Tukey's multiple comparison tests. Our results indicated that COT and 6HLN mitigated the SCOP-induced memory deficits and anxiety in the specific behavioral tasks. The biochemical analysis revealed that both compounds reduce brain oxidative stress. Also, COT and 6HLN increased the *egr1* and *nrf2a* genes expression in the brain of the zebrafish treated with SCOP suggesting a potential pro-cognitive and antioxidant effect of the treatment. Taken together, our results suggest that COT and 6HLN might represent new therapeutic agents for ameliorating AD-like condition.





IN SEARCH OF BIOMARKERS OF EMOTIONAL BURNOUT (EMOTIONAL EXHAUSTION STAGE)

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The neurophysiological mechanisms of emotional burnout remain insufficiently studied. It was found that EEG changes during burnout differ from biomarkers of depression and chronic fatigue syndrome. To establish the EEG markers of burnout was our aim. 182 volunteers, first-third year students from the Taras Shevchenko National University of Kyiv aged 18 to 24 years participated in this study. The EEGs were recorded during the resting state (3 min, closed eyes condition) monopolarly using EEG 23-channel system Neurocom. To establish EEG correlates of emotional burnout during rest state we used a special software written in Python 3.6 to implement Power Spectral Density calculation, the interhemispheric and intrahemispheric average coherence and Detrended Fluctuation Analysis (DFA). We analyzed separate artifact-free EEG segments in all frequency bands from 0.2 to 45 Hz. Psychological testing was performed before the registration of EEG. To determine the formation of emotional burnout Boyko's “Syndrome of emotional burnout” Inventory was used. The Exhaustion phase of emotional burnout was formed in 13 participants, and it was under development in 47 participants. In background EEG activity during the development of the exhaustion variations in EEG spatial synchronization were observed in low- and high-frequency EEG components. Development of exhaustion includes breaking of parietal-occipital links (alpha2,3-subbands) and formation of interhemispheric prefrontal, anterior frontal, and frontal links (alpha1,2-subbands). There were interruptions of coherence in the beta1 (interhemispheric frontal-frontal and parietal-parietal links) and beta2 subbands (right interhemispheric frontal link). DFA describes the long-term temporal correlations in the cortex, which are involved in different aspects of brain functioning. We detected a high resting state DFA scaling exponent values (up to 0.90-0,95) under exhaustion development in the theta1 (left prefrontal area), theta2 (right frontal area), alpha3 (posterior regions), beta2 (left temporal region). Obtained values of DFA exponent and average coherence suggest the exhaustion formation is accompanied by the changes in visual and verbal processing, emotional processes (discretion and analysis).





A LINK BETWEEN BRAIN CANCER AND NEURODEGENERATION

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Aging may be defined as a time-dependent accumulation of cellular damage, requiring an equilibrium between the promotion of tissue renewal and the suppression of cell cycle. Advanced age is recognized as the dominant risk factor for most human diseases, including malignant neoplasms and neurodegenerative disorders. A growing body of evidence indicates a negative association between carcinogenesis and neurodegeneration, suggesting that the predisposition for one family of diseases may decrease the risk for the other. Therefore, the focus of this study has been to assess the impact of β -amyloid peptide, a key molecule in Alzheimer's disease pathogenesis, on glioblastoma within an in vitro system.

U87 cells were grown in RPMI-1640 media supplemented with 10% foetal bovine serum. 24 h from seeding a β -amyloid peptide suspension in different concentrations has been administrated to the cells and incubated for different periods of time, as follows: 6h, 12h, 24h, 48h and 72h. After treatment removal, cell viability and the level of reactive species of oxygen (ROS) were evaluated using standardized kits. Moreover, on fixed cells, immunofluorescence protocols were applied in order to assess different molecular markers.

Cellular viability has been found impaired by β -amyloid administration in a time-dependent manner. Also, a time-dependently increase in ROS level was detected as response to β -amyloid peptide suspensions. Additionally, the exposure of U87 cells for 72h to β -amyloid resulted in downregulation of AKT3 and BDNF expressions.

β -amyloid peptide presents the potential to impair U87 cells survival capacities and to ameliorate their characteristic aggressive behavior.





SWEROSIDE CAN PREVENTS SCOPOLAMINE-INDUCED MEMORY IMPAIRMENT AND BRAIN OXIDATIVE STRESS IN ZEBRAFISH (*DANIO RERIO*)

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Alzheimer's disease (AD) is the most common neurodegenerative disorder, being associated with memory disorders and cognitive decline that ultimately affect thought, reason, video-spatial orientation, and behavior. AD is associated with mitochondrial dysfunction and a significant difficulty in cholinergic transmission, therefore, there is a special interest in the selection of different compounds capable of regulating acetylcholinesterase (AChE) activity. Recently, it has been discovered that several plant iridoids have significant neuroprotective effects, being able to slow down the process of neurodegeneration. The mechanisms of action of iridoids have been shown to be mediated by the inhibition of cholinesterases including AChE. We focused on one natural iridoid, Sweroside (Swe) which is described in studies as an iridoid with beneficial effects on the SNC, without adverse effects. The Swe was administered to zebrafish, chronically by immersion, immersion in concentrations of 1 µg/L, 3 µg/L and 5 µg/L. The zebrafish dementia model was established by treating the zebrafish with Sco (100 µM), 30 min before behaviorally tested. As a positive control we used Galantamine (Gal, 10 mg/L). Anxiety was measured using the Novel Tank Test (NTT), spatial memory was assessed using by Y-maze test, and recognition memory was assessed using by the Novel Object Recognition Test (NOR). We also evaluated the impact of the Swe on the cholinergic and oxidative status of this animal model. Our results show that Swe can effectively restore the antioxidant defense mechanism by increasing the level of antioxidant activity in the brain and can improve cognitive dysfunction of amnesic fish by inhibiting AChE, which is also correlated with improved memory parameters, as shown in behavioral approaches (NTT, Y maze and NOR).

The authors declare no conflict of interest.





INVESTIGATION OF THE ACTION OF ROIFOLINE ON COGNITIVE PROCESSES IN ZEBRAFISH

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Alzheimer's disease (AD) is a global priority according to the World Health Organization. As the average age of the population increases, the rate of AD also increases (Sinyor et al., 2020). AD affects people from the age of 65, being a neurodegenerative disorder involving language, memory, understanding, judgment, and reasoning (Kumar and Tsao, 2018). According to reports of AD, the histopathological characteristics associated with Alzheimer's dementia are represented by extracellular accumulations of beta-amyloid leading to the formation of neuritic plaques or senile plaques, but also by internal accumulations of neurofibrillary tangles formed by hyperphosphorylated microtubules associated with tau proteins. Rhoifolin (Rho), a flavone glycoside, exerts many biological activities such as anticancer, antidiabetic, hepatoprotective, antirheumatic, antibacterial, and antiviral properties. The goal of this study was to investigate the improvement impact of Rho (1, 3, and 5 $\mu\text{g/L}$) on scopolamine (Sco, 100 μM)-induced zebrafish amnesia. Zebrafish have been used as an animal model because it has a high degree of similarity to the human brain model. The behavioral tests used were the Y-maze test that examines the spatial memory of the animal model and their response to novelty, but also the new object recognition test (NOR) - through which we evaluate the memory of zebrafish (*Danio rerio*) recognition of a new object.

Our study data highlighted the beneficial effects of Rho treatment on the spatial memory in the Y maze test and recognition memory in the NOR test, thus suggesting the beneficial effects of Rho on cognitive processes, but also on the amelioration of neuropsychic symptoms of AD in the animal model of dementia represented by zebrafish (*Danio rerio*).

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EVALUAREA EFECTELOR ROIFOLINEI ASUPRA STATUSULUI OXIDATIV DIN CREIERUL PEȘTELUI ZEBRĂ

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Cea mai frecventă tulburare degenerativă a creierului este boala Alzheimer (AD). Manifestările clinice ale AD sunt disfuncțiile emoționale și intelectuale (Casado et al., 2008). AD se caracterizează prin pierderea celulelor neuronale și acumularea fibrelor de β -amiloid în plăcile neuritice și în pereții vaselor de sânge, prin acumularea în timp a filamentelor elicoidale pereche sub formă de „ghemuri” neurofibrilare în neuroni (Chauhan & Chauhan, 2006). Roifolina (Rho, apigenin 7-O- β -neohesperidosida) este o flavonglicozidă care prezintă numeroase activități dintre care amintim: anticanceroasă, antidiabetică, hepatoprotectivă, antireumatică, antibacteriană și antivirală (Brinza et al., 2020). Acțiunea antioxidantă a Rho (1, 3 și 5 μ g/L) a fost evaluată din omogenatele de creier ale unui model de pește zebură (*Danio rerio*) tratat cu scopolamină (100 μ M) pe baza determinării activității specifice a unor enzime antioxidante precum: superoxid-dismutaza (SOD), glutationperoxidaza (GPX) și catalaza (CAT), precum și prin determinarea conținutului total de glutation redus (GSH) și a nivelurilor de proteine carbonilate și de malondialdehidă (MDA). Administrarea Rho a determinat scăderea nivelului de MDA și de proteine carbonilate induse de scopolamină și a restabilit activitatea antioxidantă a SOD, CAT, GPX, și GSH.

Administrarea de Rho peștilor zebură tratați cu scopolamină a arătat ca poate produce efecte neuroprotective diminuând stresul oxidativ. De aceea, Rho poate fi utilizată pentru reducerea degradărilor oxidative produse în cursul AD.

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EVALUAREA EFECTELOR BAICALINEI ASUPRA STATUSULUI OXIDATIV DIN CREIERUL PEȘTELUI ZEBRĂ

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80% din totalul cazurilor de demență este reprezentat de boala Alzheimer (AD), iar la nivel mondial există aproximativ 30-35 de milioane de oameni afectați de această boală (Boiangiu *et al.*, 2020). AD o afecțiune neurodegenerativă caracterizată de pierderea progresivă a memoriei, acompaniată de formarea plăcilor senile de β -amiloid, a ghemurilor neurofibrilare și de moartea neuronală (Boiangiu *et al.*, 2020). Baicalina este o flavonă extrasă din rădăcina plantei *Scutellaria baicalensis* Georgi. Baicalina intervine în prevenirea apoptozei celulare și prezintă efecte antioxidante și antiinflamatoare (Wu *et al.*, 2018). Acțiunea antioxidantă a baicalinei (1, 3 și 5 $\mu\text{g/L}$) a fost evaluată din omogenatele de creier ale unui model de pește zebură (*Danio rerio*) tratat cu scopolamină (100 μM) pe baza determinării activității specifice a unor enzime antioxidante precum: superoxid-dismutaza (SOD), glutatationperoxidaza (GPX) și catalaza (CAT), precum și prin determinarea conținutului total de glutatation redus (GSH) și a nivelurilor de proteine carbonilate și de malondialdehidă (MDA).

Rezultatele studiului sugerează că baicalina protejează creierul împotriva acțiunii stresului oxidativ indus de scopolamină prin reglarea activității enzimelor antioxidante concomitent cu reglarea proceselor de oxidare a proteinelor și lipidelor. Ca urmare a prezentului studiu se poate afirma că administrarea baicalinei a demonstrat efecte de stimulare a activității antioxidante și protecție împotriva stresului oxidativ în cazul peștelui zebură. Astfel, baicalina poate fi utilizată cu succes în tratamentele de ameliorare ale degradărilor provocate de stresul oxidativ în cazul pacienților ce prezintă AD.

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THE NATURAL NEUROPROTECTIVE COMPOUNDS USED IN THE 6-HYDROXYDOPAMINE INDUCED PARKINSON'S DISEASE IN ZEBRAFISH: THE CURRENT APPLICATIONS AND PERSPECTIVES

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by a degeneration of the dopaminergic neurons in the *substantia nigra pars compacta*, resulting in the loss of dopamine in the striatum, leading thus to the PD classic movement symptoms: resting tremor, rigidity, and bradykinesia/akinesia. Furthermore, Levodopa's efficacy declines with long term use, generating therefore serious motor complications. Neuroprotection implies the use of different agents exhibiting various neuroprotective strategies: iron chelators, antioxidant, anti-inflammatory, anti-lipid peroxidation, monoamine oxidase inhibitors, dopamine receptor agonists, neurotrophic factors...

The aim of the present study is to summarize and analyze the neuroprotective activities of natural compounds, including extracts, formulations, fractions and bioactive drugs investigated using the 6-OHDA induced lesions in zebrafish model, providing thus future applications in the clinical trials. All the natural drugs tested were retrieved from PubMed Database, via pre-set searching strings. The relevant compounds were included and analyzed according to their biological activities.

Several natural compounds were analyzed, they were reported to modulate different signaling pathways involved in the pathogenesis of PD; the main compounds found were bioactive drugs, as acteoside, berberine, chrysin, the derivatives from Danshensu, Hesperidin, Naringenin, Panaxatriol saponins, Protocatechuic, quercetin, Schisantherin A, Tetramethylpyrazine, Theacrine and Xanthotoxin...; the plant extracts investigated were the aqueous and methanolic extracts of leaves from *Ceratonia siliqua*, the ethanolic extract of *Fructus Alpinia oxyphylla*, and its bioactive compound: oxyphylla A, *Ginkgo biloba* L., preparations, the Hexane fraction from *Spondias mombin* L.

The herbal medicines studied deserve further consideration as examining the optimum concentrations, bioavailability and the ability of these agents to cross the blood brain barrier to exert their effects against PD. A complete understanding of the molecular mechanisms involved in PD, and larger epidemiologic and randomized clinical trials in humans are also required.





IMPORTANȚA VITAMINEI D ÎN CONVULSIILE EPILEPTICE

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Prezenta lucrare este importantă, deoarece direcționează atenția asupra importanței semnificative a vitaminei D, în epilepsie, prin analiza datelor deja existente în literatura de specialitate. Vitamina D, în forma sa naturală, este procesată în ficat, respectiv în rinichi cu ajutorul precursorilor vitaminei. 24-hidroxilază (CYP24A1) ajută organismul să poată cataboliza 1,25 (OH) 2D numit și calcitriol în 1,24,25 (OH) 3D prin acest proces se leagă receptorul de vitamina D (VDR), el fiind procesat în produse de excreție numit acid calcitroic.

Astfel, vitamina D în cantități deficitare, în organism, este din ce în ce mai studiată, având în vedere că poate indica o înrăutățire a stării de sănătate mai ales la persoanele care au fost diagnosticate cu diferite boli neurologice. Această vitamină, împreună cu receptorii ei joacă roluri importante în creier cum este reglarea creșterii celulare, procesele de diferențiere celulară, dar și neuroprotecția acestora prin reglarea neuronală a calciului, modularea imună, mecanismul antioxidant, activitatea crescută a conducerii electrice a nervilor motori și senzitivi, respectiv mecanismele de detoxifiere ale organismului.

Este studiat și cunoscut faptul că anumite medicamente anticonvulsive, în special cele inductoare enzimatică, scad cantitatea de vitamina D din organism, astfel încât pot să apară ca efecte adverse convulsii noi sau modificarea tipului de convulsie. Vitamina D ajută la modularea pozitivă a neuromediatorilor și receptorilor cerebrali prin intermediul receptorilor GABA α , dar ea are importanță și în sintetizarea calcitriolului care va conduce la creșterea plasmei, dar și la scăderea concentrațiilor de calciu din creier, astfel încât se reduce hiperexcitabilitatea neuronală și convuliile epileptice. De asemenea, această vitamină are importanță în reglarea interleukinei 6 și în anumiți factori de creștere anticonvulsivanți cum este factorul neurotrofic derivat din celulele gliante și neutrofină 3.





APPLICATIONS OF PROTEOMICS IN LIFE SCIENCES

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The central dogma in biology is that DNA directs transcription of the coding information to RNA, which is then translated into proteins. So, one gene can lead to one protein. However, combination of various exons from the same gene can lead to formation of protein isoforms, which sometimes are tissue or organ specific. Individual proteins are also post-translationally modified by methylation, acetylation, phosphorylation, myristoylation, etc., which leads to formation of modified proteins, sometimes with various functions. Lastly, unmodified or modified proteins also interact with other proteins, to form stable or transient protein-protein interactions. Here, we show how the use of mass spectrometry-based proteomics can investigate the structure and function of proteins and entire proteomes.





INVESTIGATION AND CHARACTERIZATION OF THE JUMPING TRANSLOCATION BREAKPOINT (JTB) PROTEIN USING MASS SPECTROMETRY BASED PROTEOMICS

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Human JTB (hJTB) is a gene located on the human chromosome 1 at q21 which is involved in the unbalanced translocation in various types of cancer. JTB protein is ubiquitously present in normal cells and is found to be overexpressed in various types of cancer including prostate and breast cancer. Hence this protein could be a biomarker for tumor malignancies and a potential target for their treatment. However, the biological function and the pathway through which this protein causes increased cell growth and proliferation is not entirely clear. Investigation and comparison of the proteomes of cells with upregulated and downregulated JTB can be a good approach to understand the function of the protein and also its contribution to tumorigenesis. In this study, MCF7 breast cancer cell lines were transfected with the sense orientation of the JTB cDNA in HA, His and FLAG tagged CMV expression vector as well as with shRNA plasmids. Proteins extracted from transient and stable transfected cells were separated using SDS-PAGE. The expression of JTB was confirmed by western blotting technique. In gel digested peptides were analyzed by a Nano Acquity UPLC coupled with QTOF Xevo G2 Mass Spectrometer. Data processing was done using Mascot 2.4 server and Scaffold 4.1 software. We found several proteins that were dysregulated. Furthermore, we will do Immunoprecipitation to look at JTB protein interacting partners. These studies could help us elucidate the mechanism through which JTB induces cell proliferation and test the JTB protein as a potential drug target for malignancies.





PROTEOMIC ANALYSIS OF HUMAN BREAST MILK TO REVEAL POTENTIAL PROTEIN BIOMARKERS FOR BREAST CANCER

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Breast cancer (BC), one of the most common cancers, is a leading cause of death for women in the United States. An estimated 1 in 8 women in the United States will develop BC in their lifetime. Early diagnosis and treatment of BC is crucial, and protein biomarkers for this disease could make this possible. Mass spectrometry (MS)-based proteomic methods are ideal for the investigation of protein biomarkers. This study employs MS-based proteomics to study the protein differences in human breast milk from women with BC and matched controls. If significant protein dysregulations are revealed, they could be considered potential future protein biomarkers of BC for diagnosis and treatment. In this study, six human breast milk samples were analyzed by performing a 2D-SDS-PAGE and further analyzed via nanoLC-MS/MS. The human breast milk samples consisted of three comparison pairs of BC vs. control. There were one Coomassie blue-stained gel (6 total gels) and two replicate silver-stained gels (12 total gels) for each sample of breast milk. The silver-stained gels were scanned using a laser densitometer and the images were analyzed using Progenesis Same Spots and Progenesis PG240 software. The spot percentages were measured for each dysregulated spot. An in-gel trypsin digestion was performed for each spot with statistically significant dysregulation. This was followed by nanoLC-MS/MS, data processing, database search and statistical analysis. The dysregulated proteins found in this study can be investigated as BC biomarkers for future clinical methods for early diagnosis and treatment of BC.





MASS SPECTROMETRIC CHARACTERIZATION OF THE ZEIN PROTEIN COMPOSITION IN MAIZE FLOUR „ECO- FRIENDLY” EXTRACTS

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Zein proteins, first identified in 1821, belong to the prolamine protein class that in cereals represents more than 60% of the total endosperm protein. Zein proteins are a family of related polypeptides encoded by a multigene family whose structural genes are located in maize in three main clusters on chromosomes 4, 7, and 10 (Dong *et al.*, 2016). Zein proteins have found wide application in refining food products, such as candies, nuts, and apples. For agricultural applications, microspheres are produced out of zein proteins which are filled with pheromones and/or pesticides for controlled release of the biologically active substances to diminish odor and to provide a safer working environment (Postu *et al.*, 2019). Zein proteins have been applied for coating of tablets and in zein-coated chitosan/tripolyphosphate nanoparticles for oral delivery of selenite (Postu *et al.*, 2019). Zein-coating has also been shown to protect peptides with antioxidant and antihypertensive activities.

In this study, a procedure for the extraction of corn grain zein proteins, which is considered environmentally friendly and which aimed at producing a highly homogeneous zein protein product, was applied to investigate the izoforms composition. To verify homogeneity of the extracted and defatted protein material from maize flour we applied direct ESI- QTOF mass spectrometric measurements.

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POSTERS

MICROPROPAGATION FROM CALLUS CULTURES OF *CAPSICUM ANNUUM* L.

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Capsicum annuum L. (Solanaceae) is a well known source of natural compounds with high antioxidant, antimicrobial, anticancer activities. One of the most important secondary metabolites, with medicinal properties is capsaicin synthesized in fruits. Plant tissue cultures are a better alternative for producing secondary metabolites. Indirect micropropagation through callus cultures has become a biotechnological approach for the improvement of economically important plants.

The initiation of “in vitro” cultures of *Capsicum annuum* L aimed to assess the dedifferentiation capacity, depending on explant origin and growth regulators and to develop a multiplication protocol, based on indirect regeneration through shoots, followed by roots development induction. The proliferative capacity was tested on plantlets explants, cultivated on Murashige-Skoog basal medium, testing two auxins: 2,4 dichlorophenoxyacetic acid (2,4 D) and indolylacetic acid (IAA) and a cytokinine: benzylaminopurine (BAP).

The MS medium with 1.0 mg/l IAA and 1.0 mg/l BAP proved to be the best for callus induction from hypokotyl explants. Shoot regeneration was achieved after subculturing the calli on MS medium, supplemented with 1 mg/l BAP and 0,1 mg/l IAA. It was found to be the best for multiple shoot regeneration from callus through organogenesis. Root system development was achieved on MS medium without growth regulators. The rooted shoots were transferred for acclimatization to pots, containing sterile mixture of perlite-soil.





ANALYSIS OF THE LAKE TROUT HEART AND BLOOD PROTEOME USING EVOLUTIONARY PROTEOMICS

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Salvelinus namaycush (lake trout) is a top-predator fish in the Great Lakes region. The Great Lakes Fish Monitoring and Surveillance Program (GLFMSP) uses lake trout as bioindicators of the presence of persistent, bioaccumulative and toxic (PBT) chemicals. Elevated levels of PBTs can cause changes in transcribed genes, translated mRNAs, proteins produced, and post-translational modifications of these proteins in aquatic species. Though lake trout is used as a bioindicator of PBTs, there is currently no well-developed proteome database for the species that has been monitored for five decades. In this study, heart and blood samples from lake trout were analyzed by SDS-PAGE, followed by in-gel trypsin digestion and analysis by nanoLC-MS/MS. The data was searched against different NCBI and UniProtKB databases in Mascot Daemon and the output was analyzed by Scaffold 4.3 software. Databases used include *Actinopterygii*, *Salmonidae*, *Salvelinus*, as well as the highly studied species *Oncorhynchus mykiss* and *Danio rerio*. Using these better developed protein databases, we were able to identify many novel proteins for the lake trout, as well as explore evolutionary relationships for the lake trout species. This ongoing project will potentially lead to a more developed, comprehensive proteome database for lake trout that can be used for further proteomic studies on the effects of legacy PBT chemicals in the Great Lakes ecosystem.





IDENTIFICATION OF INVERTEBRATES USING DNA BARCODING. STUDY CASE: DANUBE RIVER-DANUBE DELTA-BLACK SEA SYSTEM

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In the last decades, the use of molecular techniques for the identification and classification of life forms has become quite popular. The most widely applied method is represented by DNA barcoding, a modern technique complementing traditional morphology-based taxonomic assignments, that uses a short, standardized fragment of genomic DNA in order to identify an organism up to the species level. Therefore, in the present study, we applied both morphological and molecular approach based on DNA barcoding (cytochrome c oxidase subunit 1 gene sequencing) in order to identify 5 marine and 16 freshwater invertebrate species, respectively, belonging to 9 major taxonomic groups of Porifera (1), Hirudinea (2), Gastropoda (5), Bivalvia (3), Amphipoda (3), Mysida (2), Isopoda (1), Insecta (3), Tunicata (1) inhabiting the Danube River-Danube Delta-Black Sea System. The study was of major interest due to the lack of molecular data on the investigated groups from the study area, which is shelter to many endemic and Ponto-Caspian species. The molecular method proved to be successful for all the analysed individuals, being very helpful to assign the exact taxon to those misidentified through morphotaxonomical criteria. Our results suggest that DNA barcoding represent a valuable and efficient tool for species discrimination and we strongly recommend that further specimens morphologically identified should be sequenced, to test the feasibility of COI gene fragment amplification for species identification. Considering these aspects, we can assume that DNA barcoding is an effective approach for species diagnosis. However, combining phenotypic and molecular criteria increase the accuracy and reliability of results.





NEUROPROTECTIVE AND ANTIOXIDANT EFFECTS OF THE AQUEOUS EXTRACT OF *XIMENIA AMERICANA* STEM BARK ON MEMORY IMPAIRMENT INDUCED BY DIAZEPAM IN MICE

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Ximenia Americana (XA) is a plant traditionally used for the treatment of headaches, rheumatoid arthritis, intestinal diseases and mental illness. The current study was undertaken to investigate the neuroprotective and antioxidant effects of the aqueous extract of *X. americana* on diazepam-induced amnesia. Thirty-six mice were randomly divided into six groups: normal control received distilled water (10 mL/kg), negative control received diazepam on day 14 (DZP 3 mg/kg, p.o.), the positive control received piracetam (PIR 150 mg/kg, p.o.), three tested groups received XA aqueous extract (25, 50 and 75 mg/kg BW, p.o.) for 14 consecutive days. Radial Arm Maze (RAM) and T-Maze were carried out to assess the behavioral response 30 min after treatment. At the end of behavioral tests, superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and malondialdehyde (MDA) levels were assessed in mice brain homogenates. Brain was also isolated for histological studies. The administration of XA aqueous extract significantly ($p < 0,001$) decreased the reference memory errors and the working memory errors in the RAM, compared with normal control group. Additionally, XA aqueous extract-treated groups significantly ($p < 0,001$) increased the time spent in the preferred arm in the T-maze compared to DZP non-treated group. *X. americana* DZP-treated groups significantly ($p < 0,001$) increased CAT and GSH levels, and a decrease in MDA level was observed in brain homogenates compared to DZP non-treated group. The aqueous extract improved memory in behavioral tests and decreased the oxidative stress in the rat hippocampus. These results suggest that XA has protective and antioxidant effects.





THE STIMULATORY EFFECT OF THE ROMANIAN MONOFLORAL HONEY ON KERATINOCYTE PROLIFERATION

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The main constituents of honey are sugars, which represent 95-99% of the dry matter, but it also contains phenolic compounds, proteins, organic acids, lipids, vitamins, water and minerals. Clinical studies have reported the successful use of honey in skin wound treatment (El-Ashram *et al.*, 2021). However, more complex studies are required to identify and understand how certain compounds act on the wound healing process. The aim of this study was to evaluate the effect of different honey varieties on keratinocyte proliferation *in vitro* and to correlate their biological activity to the physicochemical characteristics.

Three honey samples of different floral origin were analysed in this study: linden, sunflower and rapeseed honey. For the melissopalynological analysis, we have used the nonacetolytic method (Louveau *et al.*, 1978). For the physicochemical characterization, the moisture, pH and free acidity were analysed. The total phenolic content (TPC) was determined by Folin-Ciocalteu method and the ascorbic acid content by the DCPIP method (Ferreira *et al.*, 2009). The effect of honey samples on HaCaT keratinocyte proliferation was evaluated by the Neutral Red assay after 24 and 48 h of incubation. Cellular morphology observation was performed in a similar experiment using phase-contrast microscopy.

The results confirmed the botanical origin of monofloral honey samples. The physicochemical characterization proved the quality of honey. TPC values ranged from 450 mg GAE/kg honey for linden honey to 560 mg GAE/kg honey for sunflower honey. The ascorbic acid content was 2.5 mg/kg honey for sunflower honey and undetectable for the other two samples. Cell viability tests indicated a wide range of biocompatibility (0.1-5 mg/mL) of all honey samples. A stimulation of keratinocyte proliferation was observed in case of all honey samples by up to 5%. No alterations in cell morphology were observed.

Based on these promising results we envision further studies to test of the honey potential for wound healing applications.

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IMPACTUL TRATAMENTULUI CU PLASMĂ NON-TERMICĂ LA PRESIUNE ATMOSFERICĂ ASUPRA EXPRESIEI GENELOR ASOCIATE CONTROLULUI APOPTOZEI

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Osteosarcomul este un neoplasm osos malign ce apare ca o tumoră solidă producătoare de osteoid în metafiza oaselor lungi și este printre cele mai frecvente cauze de deces legate de cancer în rândul copiilor. Ca urmare a efectelor anti-tumorale selective observate, plasma non-termică la presiune atmosferică (CAP) a fost propusă ca potențial nou tratament în terapia cancerului. CAP este un gaz parțial ionizat compus din ioni, electroni, fotoni și elemente neutre, care sunt specii active capabile să inducă diferite fenomene fizice și reacții chimice. Scopul acestui studiu a fost de a evalua impactul expunerii indirecte la CAP a două modele celulare *in vitro* (osteoblaste și celule de osteosarcom) asupra expresiei unor gene asociate apoptozei. Mediul activat cu plasmă (PAM) utilizat a fost obținut prin expunerea la descărcări CAP a unei soluții RPMI 1640, pentru 30, 60 și 90 de secunde. Extracțiile ARN au avut loc la 2 ore după realizarea tratamentului, cuantificările relative ale expresiei genelor BAX, CASP3 și BCL2 fiind realizate prin tehnica RT-qPCR. Eficiența și selectivitatea tratamentului au fost evaluate utilizând testul colorimetric MTT, viabilitatea celulelor tumorale ajungând la valoarea de 25,25%, pe când viabilitatea celulelor normale fiind peste 71,87%. Analizând modificările nivelurilor de expresie genică la celulele normale, s-a observat supraexprimarea genei BAX la varianta de tratament de 60s, a genei CASP3 la probele tratate 30s și 60s și a genei BCL2 pentru toate variantele experimentale. În cazul celulelor tumorale, s-au identificat scăderi ale nivelului de expresie a genei CASP3, la variantele de tratament 30s și 60s, și a genei BCL2, în cazul tratamentului de 90s, și supraexprimarea genei BAX la probele tratate 30s. În concluzie, în cadrul acestui studiu, tratamentul indirect cu CAP a prezentat un efect citotoxic selectiv, observându-se activarea căii apoptotice intrinseci mediată de gena BAX și declanșarea unui mecanism molecular anti-apoptotic.





DRAFT GENOME OF A USEFUL BACTERIA: PAENARTHROBACTER NICOTINOVORANS

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Arthrobacter genus comprises Gram-positive soil bacteria in the form of cocci or rods. Several *Arthrobacter* species have a fully sequenced genome, interest that came from the meaningful role these bacteria have in the bioremediation of soil. *Paenarthrobacter nicotinovorans* is a member of this genus that exhibits the unique ability to degrade nicotine and used it as a C source. This ability is due to the presence of a *nic*-genes cluster on a megaplasmid: pAO1. Thus, *P. nicotinovorans* acquires a pivotal ecological role in detoxification of nicotine-contaminated soils. Also, it has important applications in the pharmaceutical industry: 6-hydroxynicotine, the first intermediate from the nicotine catabolic pathway, has been shown to possess a neuroprotective effect in laboratory rats.

Using *P. nicotinovorans* for biotechnology applications such as producing 6-hydroxynicotine is difficult due to the lack tools to perform metabolic engineering. Hence, we aim to overcome this issue by providing the complete genome of *P. nicotinovorans* pAO1+. Genomic DNA was isolated using the Qiagen DNeasy UltraClean Microbial Kit. The sequencing library was prepared by random fragmentation and 5' and 3' adapter ligation. Paired-end sequencing was performed on an Illumina NovaSeq 6000, read length 150 bp. QC was done with fastQ, while trimming, clipping and filtering were performed with fastp. SPAdes 3.14.1 was used for assembly, and both checkM and Quast was used to evaluate the results. The draft genome of pAO1+ is 4.4 Mbp in size and consists of 31 contigs. The largest contig is 778 kbp, the mean contig length is 143 kbp and N50 is 270 kbp. CheckM indicated completeness of 99.7% and no contamination. Currently, we are working on sequencing high molecular weight gDNA using nano-pore technology in order to provide a scaffold and perform the final assembly.

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CRISPR-CPF1 SYSTEM AND ITS UTILITY IN EDITING THE *PAENARTHROBACTER NICOTINOVORANS* GENOME

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Paenarthrobacter nicotinovorans is a Gram-positive bacterium that is best known due to its ability to metabolize nicotine. The strain has a proven potential for converting nicotine containing waste into useful chemicals. Its applications in biotechnology are hampered by the lack of reliable gene editing systems that would permit rational engineering of the nicotine degradation pathway.

CRISPR systems have been extensively used for genomic editing of eukaryotic cells and proved to be reliable and accurate. The applicability of CRISPR system for genomic editing of *Paenarthrobacter* strains remains elusive. The main goal of this work is to evaluate the applicability and functionality of the CRISPR-Cpf1 system in *P. nicotinovorans*. CRISPR-Cpf1 is a class 2 type V CRISPR system known to work in the closely related *Corynebacterium glutamicum* strains. The draft genome of *P. nicotinovorans* (BioProject accession PRJNA693273) was used to screen for the presence of incompatible CRISPR systems using CRISPRs web server (<https://crispr.i2bc.paris-saclay.fr/>). A number of 4 CRISPRs candidates were found on different contigs, but none were related to CRISPR-Cpf1. Hence, we concluded that the system might work in this strain and the pJYS3_ΔcrtYf plasmid (Addgene 85542) containing a functional a CRISPR-Cpf1 system was electroporated into *P. nicotinovorans*. No transformants were obtained upon selection with kanamycin, indicating that the pJYS3 replicating origin might not be functional in *P. nicotinovorans*. Next, the CRISPR-Cpf1 genes from pJYS3_ΔcrtYf were amplified by PCR and ongoing work aims to clone these genes into a plasmid known to work in *P. nicotinovorans* – pART2.





ANTIBIOTIC RESIDENCE OF PAENARTHROBACTER NICOTINOVORANS – AN IN-SILICO AND IN-VITRO STUDY

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The ability to metabolize nicotine brought the Gram-positive bacterium *Paenarthrobacter nicotinovorans* into the researchers' limelight since the 60's. Many advancements have been made since then and the potential applications of *P. nicotinovorans* nicotine catabolic pathway for the conversion of nicotine containing waste into useful chemicals have been proved. Yet, the applications of this strain in biotechnology are hampered by the lack of a complete genome and data on the physiology of the strain. Knowledge on the antibiotic resistance is especially important when attempting any metabolic engineering approaches that relies on plasmids as expression vectors.

Hence, our aim is to provide insights into the antibiotic resistance of *P. nicotinovorans* using the mixed approach: the draft genome of the strain was submitted to CARD (The Comprehensive Antibiotic Resistance Database) and the hits were tested in the lab using the Kirby-Bauer disk diffusion test. The draft genome was sequenced by NGS using the Illumina NovaSeq 6000 and the reads assambled with SPAdes 3.14.1. CARD gave a total of 255 loose hits (individual antibiotics and major antibiotic classes). All hits were filtered to 14 major hits based on an aminoacid identity level higher then 50%. A total of 15 antibiotics comprising 7 of the CARD major hits and 3 non-hits were tested in the lab. *P. nicotinovorans* was found to be resistant to 7 antibiotics (Kanamycine, Ceftriaxone, Erytromycin, Gentamicin, Neomycin, Spectinomycin, Vancomycin) coresponding to 6 CARD major hits. Although CARD also indicated a hit for tetracycline, the strain is sensible to this antibiotic at a concentration of 30 µg/ml. *P. nicotinovorans* was also found to be resistant to nalidixic acid, although CARD did not identify any hits for this antibiotic. One interesting find is that sensibility of *P. nicotinovorans* to certain antibiotics is modulated by the presence of nicotine in the growth medium.

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STUDY OF THE MECHANISMS OF ANTIBIOTIC RESISTANCE OF BACTERIA ISOLATED FROM THE INFLUENT OF AN WASTEWATER TREATMENT PLANT

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Wastewater treatment plants (WWTP) are important hotspots for the selection of antibiotic resistant bacteria and antibiotic resistance genes. These plants collect wastewater from several industrial production process or from households, which are often polluted with various antimicrobial substances that represent a major factor involved in the selection of resistant microorganisms. Moreover, the reuse of wastewater after the treatment process leads to the dissemination of clinically important bacteria and resistance genes along surface waters (rivers, lakes, seas, oceans) and soil.

In this regard, the main goal of our study was to investigate the spread of resistant bacteria in the influent of an urban WWTP and to analyze the resistance mechanism of the tested bacteria. For this purpose, microorganisms were isolated on different culture media supplemented with some highly used antibiotics in Romania. The drug concentrations used were established according to the Clinical and Laboratory Standards Institute guideline for resistant bacteria. The antibiotic resistance was evaluated using minimum inhibitory concentration (MIC) assay. The presence of bacterial enzymes that hydrolyze β -lactam antibiotics was determined using chromogenic screening plates and phenotypic tests. The biofilm-forming capacity was assessed using crystal violet method.

Following the experiments performed, we obtained 96 resistant strains. The highest values of MIC were noted for ampicillin, tetracycline, chloramphenicol, erythromycin, and vancomycin. Only 55,96% of Gram-negative bacterial strains were carbapenemase-producing organisms. Among 11 strains that were tested for biofilm production, 3 formed a moderate adherent biofilm, while 5 formed a weakly adherent biofilm.

Our results indicate an increased presence of antibiotic resistant bacteria in analysed water samples, and the isolated strains presented several mechanisms of resistance with strong implication in resistance phenomenon.





ORGANIC ACIDS PRODUCTION BY PHOSPHATE SOLUBILIZING BACTERIA

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Microorganisms are involved in a range of processes that affect the transformation of soil phosphorus. Phosphate solubilizing bacteria (PSB) have the ability to convert insoluble phosphorus compounds in soluble forms using different mechanisms. It is generally accepted that the main mechanism of mineral phosphate solubilization by PSB strains is the decrease of soil pH due to the release of low molecular weight organic acids. Therefore, the main goal of this study was to identify the organic acids involved in tricalcium phosphate solubilization. The bacterial strains used were isolated from soils cultivated with maize (*Zea mays* L.) (P1.5S and P3.4S) and municipal wastewater (D12). The ability of the bacterial strains to solubilize tricalcium phosphate was determined at 28°C using phosphomolybdate ammonium method. Also, the pH of the bacterial cultures was measured and the organic acids were identified using HPLC (High Performance Liquid Chromatography). The amounts of solubilized phosphorus by the tested bacterial strains ranged between 8,36 and 14,59 μg/mL, the highest amount being recorded in the case of D12 strain. HPLC analysis of bacterial culture supernatants showed that all bacterial strains produce malic (or formic) acid. The other identified organic acids were tartaric, lactic, acetic, citric, succinic and oxalic acids. In conclusion, one of the mechanisms involved in tricalcium phosphate solubilization by the tested bacterial strains is the acidification of the culture medium due to the production of organic acids.





ANTI-CANDIDA ACTIVITY OF SYNTHETIC FLAVONOID Br-Cl

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Antibiotic resistance is one of the biggest threats to global health, food security, and development today. Therefore, the discovery of new drugs capable of overcoming the resistance mechanisms of microorganisms is crucial for overall health and safety. To discover new "magic bullets", researchers should turn their attention to the development of drugs belonging to a new class of compounds to which microorganisms have not developed resistance. A potential solution would be the use of flavonoids, compounds well known for their antibacterial, antifungal, antioxidant, antitumor and anti-inflammatory properties. In addition to natural flavonoids, synthetic flavonoids are very interesting due to their higher antimicrobial activity. In this context, we investigated anti-Candida activity of a new synthetic flavonoid Br-Cl. The antifungal activity was assessed by minimum inhibitory concentration (MIC) and minimum fungicidal concentrations. The mechanism of action was investigated using cell membrane permeability test, hyphal growth test and scanning electron microscopy (SEM). Our flavonoid showed a very good antimicrobial activity with a MIC of 15.62 $\mu\text{g}/\text{mL}$. After only one hour of incubation, the flavonoid Br-Cl caused significant and irreversible damage to the cell membrane. In the presence of the tested flavonoid, *Candida albicans* yeast to hyphal transition was prevented. Our results showed that flavonoid Br-Cl has a high potential to be used as an efficient antifungal agent.





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