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FACULTY OF BIOLOGY ANNUAL SCIENTIFIC MEETING
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SECTION OF MOLECULAR INTERACTIONS IN THE LIVING WORLD

ABSTRACTS

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Moderators:
Senior Researcher I dr. Pincu ROTINBERG
Lecturer dr. Daniela NICUTA

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A PARTICULAR DIFFERENCE BETWEEN HEAG AND HERG CHANNELS COULD PROVIDE A SPECIFIC WAY OF TARGETING ION CHANNELS IN CANCER THERAPY

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The voltage-dependent hEAG and hERG ion channels are members of the same family of potassium channels involved primarily in cellular excitability, having important and potential different roles in regulating cancer cells functions. Due to the high structure similarity of their transmembrane pore-forming domains, hEAG blockers also reduce the hERG conductance, leading to cardiac side effects. A specific blocker of hEAG has not yet been found, although such a compound should have important antitumor activity, as hEAG experimental blockade leads to the inhibition of tumour cells growth and functions. In this study we present a correlation found between two different independent studies which shows that clofilium, an antiarrhythmic agent, has a high potential of being used as a lead compound to find a specific hEAG blocker. The correlation shows that a particular region between the S6 and P helices could provide an alternative binding mode for clofilium derivatives in order to specifically target the hEAG ion channel

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INVESTIGATION OF ANTIOXIDANT POTENTIAL OF SOME PROANTHOCYANIDIN EXTRACTS OBTAINED FROM *VITIS VINIFERA* SEEDS

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Vitis vinifera grape seeds - waste from wine industry - with their high polyphenolic content, represents a generous source for recovery of some bioactive phytocompounds with biomedical, agricultural and ecological capitalization. Among polyphenolic compounds, the proanthocyanidines are chemical structures useful in the detoxification of carcinogenic metabolites, in the scavenging of free radicals, their antioxidant capacity being more effective than other oxidative stress scavengers, as vitamins C, E and beta-carotene. The high throughput technology developed at SCDVV Iasi has led to the separation (by fraction the polyphenolic crude extracts obtained from grape marc after oil removal), the biological characterization of two final bioactive proanthocyanidin phytoproducts and, consequently, waste valorification. The interaction of the fractionated proanthcyanid in phytoproducts with the cell viability, cell apoptosis and cell cycle progression in neoplastic HeLa and Vero normal cells was evaluated in our previous investigations, proving their cytotoxic and/or cytostatic effects with a higher impact on the cancerous cells. Antioxidant property of proanthocyanidin biopreparations ProF-l.f. (laboratory form) and ProF-m.f. (micropilot form) was evaluated in neoplastic HeLa and normal Vero cells by DCFH-DA assay (flow cytometry) and by biochemical assessment of activity levels of catalase, glutathione peroxidase and superoxiddismutase scavenging enzymes. The proanthocyanidin extracts have reduced the levels of the reactive oxygen species, antioxidant potential of the ProF-l.f. beeing slightly higher that of ProF-l.m. both on neoplastic and normal cell cultures. Also, ProF-l.f. was more efficient on neoplastic cells as compared with the normal cells, but the differences weren’t very high. Investigation of the antioxidant enzymes status has revealed a significant increase in the activity of GPx and SOD in HeLa cell cultures with a minor decrease of CAT, while in the normal cells activity of all enzymes was amplified. Amplitude of the enzymatic activity was higher in Vero cells as compared with HeLa cells as well as ProF-l.m. impact on the enzymatic activity was slightly higher than ProF-l.f. Tested biopreparations have exerted an antioxidant effect both in neoplastic and normal cells. By modulating of the ROS scavenging enzymes activity, ProF-l.m. and ProF-l.f. were behave as antioxidant optimizers.

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A GENETICALLY ENGINEERED *ARTHROBACTER NICOTINOVORANS* STRAIN FOR IMPROVED PRODUCTION OF 6-HIDROXY-NICOTINE

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Introduction. The aerobic soil bacterium *Arthrobacter nicotinovorans* is able to utilize D L-nicotine as the sole growth substrate. The ability of this microorganism to metabolize the highly toxic tabacco alkaloid nicotine is linked to the presence in the bacterial cells of the 160 kb catabolic plasmid pAO1. The metabolic intermediate 6-Hydroxy-Nicotine (6HNic) produced by *Arthrobacter nicotinovorans* pAO1 when grown on nicotine containing medium was shown to bind to nAChRs, and by modulating their function, to sustain spatial memory formation in a rat model of Alzheimer’s disease. This paper presents data on the first attempts to produce and isolate 6HNic using a genetically engineered *A. nicotinovorans* pAO1 strain.

Materials and Methods. The growth, the nicotine consumption and the 6HNic accumulation in a nicotine containing medium were compared for two strains: *A. nicotinovorans* pAO1 wild type strain (wt) and a genetically engineered *A. nicotinovorans* pAO1 strain (pART2NDH) containing the genes nicotine-dehydrogenase (NDH) cloned in the nicotine inducible pART2 expression vector. The bacterial growth curves were fallowed spectrophotometrically. The consumption of nicotine and accumulation of 6HNic in the growth medium was quantified by HPLC using a reverse phase column and as mobile phase 1 mM sulfuric acid:methanol 75:25 at a flow rate of 1 ml/min.

Results and Discussions. In the wt strain, the nicotine is quickly depleted from the medium and only low amounts of 6HNic are observed. In case of pART2 NDH, the overexpression of NDH allowed a 5 fold accumulation of 6HNic in the growth medium. From the Brenda database, several inhibitors for 6HLNO – the downstream enzyme in the nicotine metabolic pathway - were selected: methylene blue (MB), $HgCl_2$ and $ZnSO_4$. As the later compound gave the best results in terms of 6HLNO inhibition, different concentrations of $ZnSO_4$ were tested in order to identify the best conditions for a higher accumulation of 6HNic.

Conclusions. The genetically engineered *A. nicotinovorans* pART2NDH strains provides a feasible biotechnological method for 6HNic production. As expected, the usage of chemical inhibitors for the downstream enzyme in the nicotine metabolic pathway increased the 6HNic levels in the growth medium of the strain.

Acknowledgements. This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS – UEFISCDI, project number PN-II-RU-TE-2014-4-0106.

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INTERACTIONS OF ROUNDUP PESTICIDE UPON SOME PHYSIOLOGICAL PROCESSES IN RYE SEEDLINGS

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Extensive use of pesticides might be unreasonable risk to humans, animals, non-target plants, and the environment. Myers et. al. stated that, since the late '70s until 2016, production of glyphosate-based herbicides (GBHs) was more than 100-fold increase, in response to the unprecedented global emergence and wide spread of glyphosate resistant weeds. To understand the phytotoxic effects of some pesticides on crop plants, in this study we aimed to determine the effects of roundup, an organophosphonate herbicide, upon the main physiological processes in rye seedlings. The study was conducted to determine the germination indicators (energy and germinal faculty), the foliar photosynthetic pigments (chlorophyll a and b, and also carotenoids), the intensity of photosynthesis and respiration, dry matter content and total mineral elements, assessed in the fourteenth day of ontogenetic development. *Secale cereale* cv. Flora caryopses were treated for 6 and 12 hours with 0.1%, 0.2%, 0.5%, and 1.0% Roundup solutions (v/v), containing 0.36 mg/ml, 0.72 mg/ml, 1.8 mg/ml, and 3.6 mg/ml glyphosate (a phosphanoglycine active ingredient). At low concentrations of Roundup (0.1% and 0.2%), energy and germinal faculty showed an insignificant decrease, both after 6 hours and 12 hours of treatment. The highest concentration of herbicide (3.6 mg/ml glyphosate) has induced a decline of germination approximately 1.9-2.2 times compared to the control. The amount of the assimilating pigments decreased, regardless of concentration or duration of treatment, and the ratio of them (a/b and a+b/c) was lower than control, proving the existence of an effect that generate reactive oxygen species. There is a direct dose-response relationship regarding the photosynthesis and respiration processes. Inhibition of photosynthesis reported in case of other fungicides is associated with reduction of stomatal conductance and transpiration, prove affecting of photosystem II activity (Bigot et al., 2011). Similar manifestation presents transpiration activity that declined after Roundup treatment. Photosynthesis and transpiration are directly interlinked affecting plant growth and productivity. Regarding dry matter, the inhibitory effect was maintained in all dilutions and pesticide exposure time, being correlated with the corresponding decrease in the biomass production. Also, the rate of mineral elements synthesis increased and total nitrogen content decreased.

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EVALUATION OF DNA SINGLE- AND DOUBLE-STRAND BREAKS INDUCTION IN FIBROBLAST CELLS AFTER PHOTON IRRADIATION USING ALKALINE AND NEUTRAL COMET ASSAY

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Single-Cell Gel Electrophoresis – SCGE or Comet Assay (CA) is a sensitive, very cheap and rapid technique for quantifying and analyzing DNA damage in individual cells, being one of the most used methods in the cancer research area for evaluation of genotoxicity and chemoprevention effectiveness (Srinivasan et al., 2007). One of the main objectives of this study is establishing of the genetic apparatus reactivity in mammalian cells to the photons action. This goal was achieved by assessing the degree of DNA damage, as a consequence of photon irradiation, using alkaline and neutral comet assay. Alkaline version is suitable for radiobiological studies because it has high sensitivity for detecting several types of lesions: single-stranded breaks (SSBs), double-strand breaks (DSBs), alkali-labile sites (ALSs) and incomplete excision repair sites (IERSs). On the other hand, the neutral comet assay reflects mainly double-strand breaks (DSBs). In our experiment, the cells were seeded in 25 cm2 cell culture flasks at an initial density of 3 x 105 cells /flask. After 24 hours from the initiation of the cultures, when the cells have 85-90% confluence realizing the monolayer, the cells were counted and distributed in Eppendorf tubes (1x106 cells/tube, 3 tubes/variant), and then, exposed to the photon flux (1Gy, 2 Gy, 3Gy and 5 Gy). Sham irradiated cells were used as control. The comets were stained with ethidium bromide dye. One hundred comets were analysed from each of three slides/variant using CASP 1.2.2 software (CASP or Comet Assay Software Project, http://www.casp.sourceforge.net). The percent of the DNA in the head decreased varying between 97.92 (control) to 97.26 (2 Gy), without evidence of a dose-effect relationship. Instead, percent of DNA in the tail presented a linear increase from 1 Gy to 3 Gy (2.35-3.25). "Tail moment" parameter, which is the product of multiplication between tail length and the total DNA fractionated in the tail, increased from 0.50 (control) to 3.29 (5 Gy), revealing accumulation of a large number of fragments (single and double DNA breaks) of information material. This index provides the most stable estimates for DNA damage because it has a large degree of uniformity in the fragments dispersion. As expected, the highest mean of tail moment, evaluated using neutral Comet assay, was shown at 3Gy and 5Gy, respectively indicating a large percent of double strand breaks.

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ARTHROBACTER PLASMIDS: MOLECULAR CLASSIFICATION AND CONSERVED GENE CLUSTERS

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Members of the *Arthrobacter* genus are ubiquitous in polluted and toxic soil samples. Despite their potential in environmental biotechnologies, theirs practical applications are hampered due to the scarce availability of useful tools for genetic engineering. More than a decade have past since the sequencing of the most known *Arthrorobacter* plasmid – pAO1, but very little in known about the core functions - replication and partition of *Arthrobacters* plasmids.

In this study, the available *Arthrobacter* plasmids sequences were analyzed by BLAST in order to identify their putative replication origin. Gene syntheny and genome wide comparisons were performed and visualized with progressiveMauve. Evolutionary relationships were inferred using the Maximum Likelihood method. Proteome wide comparations for core-genome plot analysis was performed with CMG biotools.

Based on parA homologs sequence, the *Arthrobacter* specific plasmids have been classified into 4 clades. Iteron like sequences were identified on most of the plasmids indicating the position of the putative *Arthrobacter* specific ori's. A cluster of 12 ORFs predicted to encode the components of a T4-secretion system involved in bacterial conjugation was identified as highly conserved and syngenic among a subset of 14 *Arthrobacter* plasmids. Also, a DNA repeat of about 370 nucleotides was found to be present 5’ to the ORFs of DUF4192-, DprA- and ParB-like proteins on 12 additional *Arthrobacter* plasmids. The DNA repeats contain alternating GC and AT reach sequences, potential protein DNA-binding sites and purine reach stretches. A core-genes common for all the *Arthrobacter* plasmids could not be identified, indicating that the plasmid diversity within this genus exceeds what can be inferred from the study of the available sequences.

It is hoped that the findings presented here will stimulate further experimental work aimed at the elucidation of the ORFs implicated in the regulation of the life cycle of these plasmids.

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PAIN THERAPY PHARMACOGENOMICS: GENETIC POLYMORPHISMS THAT INFLUENCE THE RESPONSE TO PAIN ANALGESIC MEDICATION

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Genetics and genomics are certain to have a large impact in drug development and proper pharmaceutical treatment of subgroups of patients with many specific diseases. We should be able to increase the therapeutic margin for many agents. Genetic variation will also be important in refining estimates of risk from all kinds of environmental agents and in choosing more effective and more cost-effective risk reduction strategies. The linkage of information about genetic variation and information about environmental, nutritional, behavioral, metabolic, medical, and healthcare factors will be necessary to interpret the variation in clinical and public health terms. However, there is a great risk that present efforts to protect confidentiality and privacy of individual genetic information may make such research infeasible. The response of human organism to the treatment with opioid and non-opioid analgesics is genetically determined and is varying from individual to individual; the source of this variation is studied by the pharmacogenomics of pain. The genetic polymorphisms that explain the variability of the analgesic effect of opioid and non-opioid drugs is discussed in the present paper emphasizing the role of cytocrome P450 CYP2D6 polymorphisms for morphine and codeine. Microarray chips are new research tools in pharmacogenetics opening new perspectives for an individualized drug therapy. A microarray chip can be described as a gene expression assay which consist in a glass slide or a micromembrane spotted with DNA from hundreds or even thousands gene probes. Microarray technology is used in pharmacogenetics research to scan the whole human genome for polymorphisms that can alter the normal individual response to drugs; many thousands of polymorphisms can be scanned simultaneously with a single microarray chip. In the present paper, we will try to demonstrate that microarray technology is one of the most important recent advances in molecular genetics and to expose its applications in pain therapy pharmacogenetics.

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INFLUENCE OF PHOTON IRRADIATION UPON V 79 CELLS PROLIFERATION AND GENOMIC STABILITY – PRELIMINARY DATA

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The main objective of this study is to assess the effect of ionizing radiation on the growth and development of V79 cell cultures, both in the first days after irradiation (expression time) and to the descendants of several generations (P0 - P12), in order to establish how occurred mutations are perpetuated and changed from one generation to another. The Chinese hamster lung fibroblasts (V79) were exposed to photon irradiation (1, 2, 3, and 5 Gy, with a flow source at 260.88 cGy min) using VARIAN CLINAC® 2100SC particle accelerator from "St. Spiridon" Hospital Iasi. The control version was maintained under the same conditions, less radiation exposure. The cell viability (made by tripan blue assay) decreases from one generation to another, both in control (from 97.05% - cell culture initiation – P0 to 90.33% – P12) and irradiated variants, without a direct dose-effect relationship. Worthy of mention is the fact that after reducing the number of living cells until P4 level, there was a slight revival of viability maintained throughout the experiment. Regarding the aneuploidy level, the number of chromosomes was ranging from 18 to 22, without a link between the prevalence of a certain number of chromosomes and radiation dose, however, metaphases with 20 chromosomes had the highest frequency. Also, we count triploid and tetraploid metaphases and chromosomes with single or double chromatid breaks. Accordingly, preliminary results of this complex study shows both cytotoxic effect of the photons irradiation on the V 79 cells (expressed by the cell viability and clonogenic assays), and genotoxic effects evidenced by changes in the number of chromosomes and by identification of chromosomal aberrations (mono- and double strand breaks) in metaphase. Although there is no direct dose-effect relationship, corroborating of processed data allow, for this moment, to establish that the cell viability decreases in the first generation after irradiation, finding a restoration of this parameter in subsequent progeny, but without reaches the control values. This process can be attributed to the intervention of cellular repair mechanisms which, up to a particular level, are able to repair or remove (by apoptosis or another cellular death mechanism) alterations occurred in the DNA. Also, increased of aberration rate and DNA structural damages prove harmful action of photon irradiation upon genetic apparatus, alterations that can persist over several generations.

POSTERS

Central hall, 1st floor: 15^{00} – 16^{00}

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TREATMENT RESISTANCE OF TYROSINE KINASE INHIBITORS INDUCED BY THE PRESENCE OF MUTATIONS IN THE TYROSINE KINASE SITUS OF ABL GENE INVOLVED IN TRANSLOCATION WITH BCR-ABL IN CML

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The presence of Philadelphia chromosome resulted from a mutual translocation between chromosomes 9 and 22 is a genetic marker for CML (Chronic Myeloid Leukemia). At molecular level the translocation involves a break point inside the BCR (Breakpoint Cluster Region) gene on the chromosome 22 , and respectively ABL (Abelson) oncogene on the chromosome 9 resulting the BCR-ABL fusion gene. The resulting fusion gene produces a tyrosine kinase (TK) which can be inactivated by administrating tyrosine kinase inhibitors (TKI). The highlight rate of the occured mutation in patients with resistence to treatment is quite low because there are many other possible causes for treatment resistance. The molecular analysis was performed on a lot of 12 patients selected from ones with CML which initially responded to treatment, but then progressed to the accelerated phase and showed early signs of relapse. The method consisted of semi-nested PCR analysis followed by Sanger sequencing. We found two different mutations at 2 patients (24% of pacients): T315I (which causes resistance to all TK inhibitors) and F317L. Our findings are in accordance with other research that showed that in roughly 33% of cases, mutations in BCR-ABL gene, clonal evolution and amplification of the BCR-ABL gene are common causes of treatment resistance. The molecular technique used has an increased sensitivity because the sequenced gene is ABL involved in translocation with BCR-ABL, thus the result is not tainted by the wild type ABL gene.

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“IN VITRO” MULTIPLICATION OF *MENTHA PIPERITA* L.

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The regenerative potential of meristematic explants from *Mentha piperita* L. was evaluated for the establishment of a clonal propagation protocol, as an alternative for biomass production. *Mentha piperita* L. (Lamiaceae) is one of the most economically important aromatic and medicinal plants ,that contains various classes of compounds (volatile oil rich in menthol and flavonoids, phenolic acids) with multiple pharmacological, cosmetic applications.

Meristematic explants, taken from seedlings of *Mentha piperita* L.,germinated in aseptic conditions were tested for their regenerative potential.

The procedure involved shoot tip cultures, followed by rapid shoot multiplication, rooting and finally establishment of plantlets in soil.

Murashige-Skoog medium has been diversified according to hormonal balance, using benzylaminopurine (BAP) in combination with α-naphthaleneacetic acid (NAA). The agar solidified MS medium containing 0,5 mg/l benzylaminopurine and 0,2 mg/l α-naphthaleneacetic acid (NAA) was optimum for shoot proliferation at *Mentha piperita* L. and allowed the development of large number of cloned shoots.

The regeneration of whole plants was obtained in two steps: the shoots were excised and transferred to fresh medium and then rooting of these shoots was achieved on the same medium with 0,5 mg/l benzylaminopurine and 1 mg/l α-naphthaleneacetic acid.

In each variant of MS basal medium studied it has worked on every 10 samples. The samples were kept in growth chamber at 23±1o C and a photoperiod of 16 hours.

The excised shoots were subcultured for roots induction. Regenerated plants were transferred to ex vitro conditions for an acclimatisation period.

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POPULATION GENETIC INFERENCE FROM HSP70 SEQUENCES POLIMORPHISM IN FISHES

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Heat shock proteins (HSPs) or stress proteins, a subset of molecular chaperones, are a superfamily of, intracellular proteins forming a part of the cellular defence. HSPs have an unusually high degree of identity at the amino acid level, among diverse organisms. Molecular chaperones are major cell constituents in all organisms under nonstress conditions and they are essential to ensure proper folding and intracellular localization of newly synthesized polypeptides. The need for molecular chaperones is higher under stressful conditions, as the rate of damage to cell proteins or problems with proper folding increases markedly (Sorensen et al., 2003; Padmini et al., 2008). The heat-shock protein 70 (HSP70) stress protein family consists of several members with similar molecular sizes, some of which are heat inducible and others are constitutively expressed (Morimoto et al., 1990). Both HSP70 and heat-shock cognate protein 70 (HSC70) are cytosolic. HSP 70 have evolutionarily diverged and have been classified by phylogenetic analysis into four distinct clusters corresponding to their intracellular localization, i.e., in the cytoplasm, endoplasmic reticulum, mitochondria, or chloroplasts (Boorstein et al., 1994; Morimoto et al., 1990); however, the evolutionary relatedness among HSP70 and HSC70 proteins in vertebrates has not been fully elucidated (Yamashita et al., 2004).

Despite the intense interest that has been given to HSPs, a few studies have addressed questions about the nucleotide structure and the evolution of these genes. As a result, the aim of the present study was to clone and analyse the stress-associated HSP70 gene structure in *Cyprinus carpio* by direct sequencing and a phylogeny inferring.

Phylogenetic analysis including neighbour-joining (NJ), maximum-parsimony (MP) and maximum-likelihood (ML) trees of DNA sequence alignments analysis were conducted using Paup 4.0b10 (Swofford 2000) using PaupUp graphical interface (Calendini & Martin, 2005). Genetic distances used in NJ trees are Kimura two-parameter model distances with a transition : transversion ratio of 2 : 1. Non-synonymous/synonymous substitutions distances (dN/dS) ratios, as described by Nei & Gojobori (1986), were calculated using MEGA7 software (Tamura et al., 2013). Bootstrap analysis was made with 1000 replicates except in ML where only 100 replicates were generated. Hierarchical likelihood ratio tests were conducted using a batch file supplied with MODELTEST 3.7 (Posada & Crandall, 1998) to provide the evolutionary models used in ML and Bayesian analysis.

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PAPANICOLAOU TEST: PERFORMS AND LIMITIES

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Harvesting the smears and the cytodiagnostic interpretation represent the first step in diagnosing the presinvasive pathology of the cervix. The study lot was made of 1476 patients who came for a specialty consult in two medical units in Iasi, in the period of time between 2010 and 2015 and who were harvested Pap smears. The data was statistically processed in order to draw conclusions about the incidence of cervical bening pathology and the usefulness of its detection through this test. If we refer to the whole lot, the cytological results are satisfactory: 83% smears are within normal limits, 10,1% ASCUS, 2,7% L-SIL, 0,8% H-SIL. From all the feminine genital neoplasias, the cervical cancer is the easiest to detect, with low costs, as it benefits from very effective early diagnostic methods: cytology, HPV testing, colposcopy, biopsy. We estimate that 50-75% of the results that are false negative are due to the harvesting errors.

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EFFECTS OF *CRATAEGUS MONOGINA L.*(HAWTHORN LEAVES AND FLOWERS) HYDROALCOHOLIC EXTRACTS ON CELL DIVISION IN *CUCUMIS SATIVUS L.*

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Keywords: Hawthorn, flavonoids, polyphenols, chromosomes mutation, cell cycle, *Cucumis sativus* L. root meristems.

Abstract: Hawthorn (*Crataegus monogyna*) hydroalcoholic extract was prepared by extraction of powdered dried leaves and flowers with ethanol 70% v/v (1:10), by reflux for two hours. Extract was qualitative and quantitative analyzed. This extract was tested for effects on seed germination and cell division cycle of *Cucumis sativus* root meristems.

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DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION VALUES IN PATIENTS WITH SEPSIS WITH ORO-MAXILLOFACIAL PORTAL OF ENTRY

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In patients with risk factors for developing methicillin resistance, first-line treatment options should be reconsidered and antibiotics such as: clindamycin, trimethoprim-sulfamethoxazole, or new cyclins should be administered. In hospital settings, the alternatives include vancomycin, teicoplanin and linezolid. The favorable disease course depended on precocity of etiologic diagnosis and initiation of appropriate therapy. A total of 9036 bacterial strains isolated from patients admitted to the "Sf. Spiridon" Emergency County Hospital during 2013-2016 were tested. Minimum inhibitory concentration (MIC) was determined and CMI 50 and CMI 90 values were calculated for the following antimicrobial agents: gentamicin, oxacillin, rifampicin, kanamycin, erythromycin, trimethoprim-sulfamethoxazole tetracycline, penicillin, ofloxacin, ciprofloxacin, Vancomycin and Tobramycin. Susceptibility categories were established according to the breakpoints recommended by CLSI 2016 (Clinical and Laboratory Standards Institute) for the fully susceptible category (in this study the intermediately susceptible isolates were classified as resistant). The *S. aureus* strains tested in this study demonstrated elevated MIC 90 values (64μg/ml) for kanamycin and tetracycline and high percentage of resistance to kanamycin, erythromycin, trimethoprim-sulfamethoxazole. For penicillin the resistance rate was 94.7%. Resistance to third generation cephalosporins and aztreonam has progressively increased in Romania after their introduction in therapy. The study showed that 45% of all infections could be prevented by vancomycin administration.

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THE NEUROCOGNITIVE EFFECTS OF *HYPERICUM PERFORATUM* L. EXTRACT ON AMYLOID BETA (25-35)-INDUCED MEMORY DEFICITS AND OXIDATIVE STRESS IN LABORATORY RATS

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Hypericum perforatum L., also known as perforate St John's-wort, is one of the most ancient medicinal herbs commonly used for many human ailments. *Hypericum perforatum* L. presents anti-depressant, anxiolytic, anti-inflammatory, antiseptic and sedative effects in the nervous system. In the present study, the effects of the hydroalcoholic extract of *Hypericum perforatum* L. administration (25 mg/kg and 75 mg/kg, i.p., for 7 days) on spatial memory performance were assessed on amyloid beta (25-35)-treated rats. The amyloid beta (25-35)-induced memory impairments were observed, as measured by the Y-maze and radial arm-maze tasks. Decreased activities of superoxide dismutase and glutathione peroxidase were observed in the rat hippocampal homogenates of amyloid beta (25-35)-treated animals as compared with control. Also, production of malondialdehyde significantly increased in the rat hippocampal homogenates of amyloid beta (25-35)-treated animals as compared with control, as a consequence of impaired antioxidant enzymes activities. Additionally, the administration of the hydroalcoholic extract of *Hypericum perforatum* L. at amyloid beta (25-35)-treated animals improved short term memory performance in Y-maze test, but did not exhibit significant improvements in long term memory in radial arm maze test. These findings associated with a decreased oxidative stress level, suggest the antioxidant effects and memory-enhancing proprieties of the hydroalcoholic extract of *Hypericum perforatum* L.. The obtained results indicate that the hydroalcoholic extract of *Hypericum perforatum* L. may be a potential candidate for the development of therapeutic agents to manage memory impairment and oxidative stress associated with Alzheimer’s disease.

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AMELIORATIVE EFFECTS OF *MATRICARIA CHAMOMILLA* L. EXTRACT ON SCOPOLAMINE-INDUCED MEMORY IMPAIRMENT IN RATS: A BEHAVIORAL AND BIOCHEMICAL STUDY

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Matricaria chamomilla L. is one of the most ancient medicinal herbs commonly used for many human ailments, including nervous disturbance. In the present study, the effects of the hydroalcoholic extract of *Matricaria chamomilla* L. administration (25 mg/kg and 75 mg/kg, i.p., for 7 days) on spatial memory performance were assessed in scopolamine-treated rats. Scopolamine-induced memory impairments were observed, as measured by the Y-maze and radial arm-maze tasks. Decreased activities of superoxide dismutase, glutathione peroxidase and catalase along with increase of acetylcholinesterase activity and decrease of total content of reduced glutathione were observed in the rat hippocampal homogenates of scopolamine-treated animals as compared with control. Production of protein carbonyl and malondialdehyde significantly increased in the rat hippocampal homogenates of scopolamine-treated animals as compared with control, as a consequence of impaired antioxidant enzymes activities. Additionally, in scopolamine-treated rats treated with the hydroalcoholic extract significantly improved memory formation and decreased oxidative stress, suggesting memory-enhancing and antioxidant effects. These findings suggest that the hydroalcoholic extract of *Matricaria chamomilla* L. may be a potential candidate for the development of therapeutic agents to manage memory impairment and oxidative stress associated with Alzheimer’s disease through decreasing the activity of acetylcholinesterase and anti-oxidative mechanism.

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EFFECTS OF *HYPERICUM PERFOLIATUM* (ST JOHN`S WORT AERIAL PARTS) HYDROALCOHOLIC EXTRACTS ON CELL DIVISION IN *CUCUMIS SATIVUS L.*

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Key words. St John`s Wort, flavonoids, polyphenols, chromosomes mutation, cell cycle, *Cucumis sativus* root meristems.

Abstract. St John`s Wort (*Hypericum perforatum*) hydroalcoholic extract was prepared by extraction of powdered dried flowering aerial parts with ethanol 70% v/v (1:10), by reflux for two hours. Extract was qualitative and quantitative analyzed. This extract was tested for effects on seed germination and cell division cycle of *Cucumis sativus* root meristems.

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MOLECULAR PHYLOGENY OF *SCARDIUNIUS* GENUS INFERRED BY NUCLEAR RAG1 (RECOMBINATION ACTIVATING GENE 1) GENE ANALYSIS

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“Cyprinid” is the name given to any fish that belongs to the carp family Cyprinidae which includes fishes like the carp, the crucian carp, the zebra fish, the chub, the rudd etc. With over 200 genus and 2000 species, the minnow family is the largest family of freshwater fishes. Within this family, because of the high genetic and morphological variability, the phylogenetic relationships were never completely solved. The rudd, common term used for *Scardinius erythrophthalmus* species is a bentho-pelagic freshwater fish which is most often encountered in nutrient-rich waters with abundant vegetation. It belongs to Scardinius genera which includes a total number of 10 species *(S .erythrophthalmus, S. hesperedicus, S. knezevici, S. plotizza, S. dergle, S. acarnicus, S. elmaliensis, S. graecus, S. racovitzai* and *S. scardafa*) and more than half of them have a status from near threatened to critically endangered on IUCN Red List. This study aims to present the phylogenetic relationships within this genus by analyzing the RAG1 gene, a nuclear protein-coding locus (NPCL) marker that is applicable across diverse taxa and show good phylogenetic discrimination. The total DNA was isolated for the species from Romania (*S. erythrophthalmus* and *S. racovitzai*) using different protocols according to the sample type. RAG1 gene amplification was performed using the RAG1F and RAG9R primers and the amplicons were sequenced using CEQ 8000 Genetic Analysis System (Beckman Coulter) with two other internal primers. The GenBank sequences for *Scardinius* genera species RAG1 gene dataset and *Carassius carassius* as outgroup were used for phylogeny inference. The sequences were aligned using ClustalW method from MEGA 7 software and the phylogenetic trees were constructed using BEAST v1.8 (Bayesian Evolutionary Analysis Sampling Trees). Our results show a complex phylogeny within the *Scardinius* genus and RAG1 gene is a good NPLC marker for the phylogenetic studies of this genera.

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PRELIMINARY DATA ON PHYLOGENY AND PHYLOGEOGRAPHY OF *COBITIS ELONGATOIDES* INFERRED BY CYTOCHROME B GENE ANALYSIS

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Cobitis is a Palearctic genus of ray-finned fish in the Cobitidae family, with more than 80 identified species, however the clasification within family is disputed and remain uncertain. The phylogeny of the genus *Cobitis* remains one of the most interesting problems of the family Cobitidae. The classification within Cobitidae family is still disputed, the number of recognized species varies according to the number of morphological traits considered. Phenotypic species-specific character may be partly modified toward unification due to asexual reproduction, poluploidy and hybridization within the genus *Cobitis*. The most common species for Danube and Dniester River Basins are *Cobitis taenia*, *Cobitis tanaitica*, *Cobitis elongatoides* and belongs to the *Cobitis taenia* hybrid complex. These species have a particular type of reproduction (gynogenesis) that leads to an asexual lineage (diploid, triploid and rarely tetraploid individuals). *Cobitis elongatoides* was exclusively the maternal ancestor of all the *C. elongatoides-tanaitica* hybrids, while the hybridization process was reciprocal within the *C. elongatoides*-taenia complex. *Cobitis elongatoides*, commonly named spined loach, is native to Danube basin, upper Elbe and Odra drainages, and also Dniester River. In the present work, we investigated the variability, phylogenetic and phylogeographic relationships between the *Cobitis elongatoides* individuals from different river basins analyzing the molecular data provided by the mitochondrial marker cytochrome b gene (575bp). Total DNA extraction for the captured individuals from Prut and Dniester Rivers was performed using phenol chloroform isoamyl alcohol protocol. In order to amplify the cytochrome b gene, the L15267 and H15891 primers were used. The amplicons were successfully sequenced using CEQ 8000 Genetic Analysis System (Beckman Coulter). Genetic diversity within and between hydrological basins was assessed in Arlequin (v 3.5) software, computing general molecular indices. Our data reveal a high similarity between *C. elongatoides* individuals and *C. elongatorides* x *C. tanaitica* hybrids that belong to different tributaries of the Danube river basin, pointing that *C. elongatoides* is the maternal ancestor of all *C. elongatorides* x *C. tanaitica hybrids*.

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SERUM SELENIUM AND MAGNESIUM LEVELS VARIATION IN ALZHEIMER'S DISEASE AND MILD COGNITIVE IMPAIRMENT PATIENTS

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The main symptoms of Alzheimer's disease (AD) is memory loss. Due to intense brain degeneration causing memory loss halmark, several metals ions function impairment has been described. In this way, selenium, and magnesium which are both extremely important in brain tissue can be quantified from blood serum. This study aims to quantify and compare selenium and magnesium levels in demented patients. Blood serum samples were collected from Alzheimer`s disease (n=20), and mild cognitive impairment (n=17) patients admitted in Socola Psychiatric Institute, Iasi. Also, samples from age and sex-matched control subjects were collected. Graphite furnace atomic absorption spectrometer with high resolution and continuous source (GF HR CS-AAS) analysis was performed for metal ions concentration determination. Standard statistic analysis was performed (one-way ANOVA). Selenium levels were found increase in AD patients with more than 35%, as compared to healthy controls. Magnesium levels also varied in a significant manner in AD patients, as compared with MCI patients and healthy controls. Thus, serum magnesium and selenium levels significant variation indicates that they may be a useful parameter in AD severity evaluation, but further studies are needed.