



CONFERENCE BOOK

Martin, Kraków, Szeged

24-26th September 2020

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College & ZOZ Ośrodek UMEA SHINODA-KURACEJO

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PART I

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LETTERS

PROGRAMME

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Polish Pharmaceutical Society (the Kraków Branch)

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**JURY FOR THE BEST PRESENTATION OF YOUNG
SCIENTISTS**

Dr hab. Joanna GDULA-ARGASIŃSKA, dr Henrieta ŠKOVIEROVÁ,
dr Viktoria SZŰTS

The jury consisting of dr hab. Joanna Gdula-Argasińska, dr Henrieta Škovierová and dr Viktoria Szűts awarded the 1st prize in the session of young scientists to Ms. Eliza Matuszewska (Levels of selected heavy metals in Polish bee pollen, royal jelly and propolis). The honorary distinctions were granted to Ms. Karolina Nowak (Regulation of estrogen receptors and NF-κB in methylparaben-stimulated human neutrophils) and Mr. Jan Lazur (Bioelements accumulation by *Agaricus bisporus* fruiting bodies during cultivation process).

Registered participants voted on the Internet for the best poster. The first place went to Ákos Fazekas, Dávid Major, Elias Jigar Sisay, Gábor Veréb, Zsuzsanna László for Possibilities of applying modified polymer membranes for wastewater treatment.

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Krakowski Holding Komunalny SA, as a subsidiary company of the city of Kraków, provides services associated with the municipal management sector, inspires and works towards increasing the performance of Kraków's municipal companies.

KHK SA owns 100% of the shares of Miejskie Przedsiębiorstwo Energetyki Ciepłej SA, Miejskie Przedsiębiorstwo Komunikacyjne SA, Miejskie Przedsiębiorstwo Wodociągów i Kanalizacji SA and Arena Kraków SA. Together, these companies form a tax capital group. KHK SA is the largest municipal holding in Poland.

The KHK SA's priority direction of action for the next five years is to ensure the energy independence of the companies belonging to Krakowski Holding Komunalny's capital group based on renewable energy sources.

These tasks are performed both within the capital group's companies themselves as well as in cooperation with universities and scientific institutions. Krakowski Holding Komunalny SA is also expanding its cooperation network to include international contacts which will enable greater access to modern and innovative technologies that can be adopted in the municipality of Kraków.

Since June 2016, KHK SA has been the owner and operator of The Eco-incinerator Thermal Waste Treatment Plant, which is one of Europe's most modern plants of its type. The Eco-incinerator can treat a maximum of 245 thousand tons of non-recyclable waste per year and use it to generate over 91 thousand MWh of electrical energy and close to 1 million GJ of thermal energy. Around 44% of the electrical energy produced at the Eco-incinerator is considered green, i.e. renewable energy.

The grounds of the Eco-incinerator are used for educational projects. We invite organised groups of children and school youth as well as

specialists in municipal management to tour the plant. The Eco-incinerator has become one of Kraków's architectural hallmarks and a model plant visited by local government and business representatives from all over Poland and abroad to gain knowledge and experience. As a part of ecological education, the plant is visited by almost 7 thousand people every year. One can register for touring the plant's educational path using our website www.khk.krakow.pl.





**A LETTER FROM DEAN OF THE FACULTY OF
PHARMACY JAGIELLONIAN UNIVERSITY
MEDICAL COLLEGE**

Dear Colleagues,

I would like welcome you all to the 3rd International Conference on Pharmaceutical and Medical Sciences.

The year 2020 surprised us with new challenges as well as many limitations. Due to the current pandemic situation and the fact that the health and the security of all our participants are the most important, the conference will be carried out remotely. I am convinced that despite a different format of conference, we will be able to create a very good platform for discussions about many scientific problems in pharmacy and medicine.

We must remember that the Faculty of Pharmacy at the Jagiellonian University is one of the oldest faculties in Poland as well as in Europe.

Today in our Faculty of Pharmacy there are over 1200 students studying pharmacy, medical analytics and cosmetology at Master's and PhD studies and industrial pharmacy, medical analytics, clinical studies of pharmaceutical products and enology at postgraduate studies. We have very professional group of scientists and modern teaching laboratories.

The Faculty of Pharmacy has been involved in the European Association of Faculties of Pharmacy. Our Faculty also cooperates with the District Chamber of Apothecaries and Pharmaceutical Inspectorate in the scope of pharmaceutical training. The Faculty of Pharmacy has achieved a high position in the parametric evaluation and possesses accreditation of specializations.

In this year, we were recognized again as the best field of study – pharmacy – all over the country.

I wish the fruitful discussions.

*Sincerely,
Prof. dr hab. Jacek Sapa*



**A LETTER FROM DEAN OF THE JESSENIUS
FACULTY OF MEDICINE IN MARTIN,
COMENIUS UNIVERSITY IN BRATISLAVA**

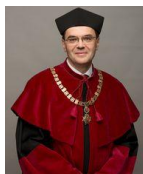
Dear Participants,

Let me welcome you on behalf of Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava. We have been decided to welcome you all in the middle Slovakian city – Martin, which has a very international environment due to hundreds international students. It was also played a very important cultural role in the history of Slovak nation.

Unfortunately because of pandemia we are not able to organized this conference in person, and now it seems that decision to put it into a virtual space was a good idea. I hope that in the future we can meet together here, in this beautiful city.

Now please let me to wish you a good conference, a lot of interesting lectures and posters and have a good time.

Prof. dr hab. med. Andrea Čalkovská



**A LETTER FROM DEAN OF THE MEDICAL
FACULTY JAGIELLONIAN UNIVERSITY
MEDICAL COLLEGE**

The 3rd International Conference of Pharmaceutical and Medical Sciences on pathophysiological, pharmacological, and clinical aspects of the environmental pollution, which is organized collectively by the scientific university centers in Kraków (Poland), Martin (Slovakia), and Szeged (Hungary) is a proof that our scientific communities are deeply interested and truly willing to cooperate in this area of science and knowledge related directly to crucial issues of the health, social well-being, and quality of life. Especially noteworthy and commendable is multidisciplinary aspect of the 3rd ICPMS.

COVID-19 pandemic, which complicated very much our life and work, (among others including the organization of 3rd ICPMS itself), makes us even more aware of how fragile and precarious is our environment and what is not only related to the nature itself, but also to social and economic "environment".

The Faculty of Medicine, Jagiellonian University Medical College is profoundly interested in regional and supraregional international scientific investigations on clinical and more general medical aspects and consequences of the degradation of the environment.

Therefore in the name of our Faculty I would like to express our best wishes that 3rd ICPMS will be a success and will provide good opportunity to exchange scientific experiences and opinions and will encourage especially the young generation of scientists to venture in ambitious goals and projects.

Prof. dr hab. med. Maciej Matecki



A LETTER FROM DEAN OF THE FACULTY OF PHARMACY UNIVERSITY OF SZEGED

Dear Colleagues,

It is my pleasure to welcome all the attendees of our conference, the 3rd International Conference of Pharmaceutical and Medical Sciences on behalf of Faculty of Pharmacy, University of Szeged, Hungary. I am convinced that this kind of online conference is the most appropriate answer for the current challenge determined by the epidemic-related restrictions. This way the scientific community may keep contact, establish new connections, and share ideas. When I checked the program of the event and I had the impression that it is really versatile, valuable and colorful. The presentations will give us a cross section of the research direction currently determining the pharmaceutical and medical sciences. Based on this I am convinced that we are going to have a useful and enjoyable two-day conference. I wish all of us thought-provoking discussions, new ideas, new professional connections, altogether a memorable conference.

Prof. dr hab. István Zupkó



A LETTER FROM VICE-PRESIDENT OF THE SUPREME MEDICAL COUNCIL

Distinguished Guests, Ladies and Gentlemen, Dear Colleagues!

On behalf of the President of the Supreme Medical Council, prof. Andrzej Matyja and the Polish medical government, associating all doctors and dentists, I would like to thank the organizers from Poland, Slovakia, and Hungary for their considerable effort to organize the next edition of the conference in this special period of the SARS CoV-2 virus spread around the world.

The Supreme Medical Council is the patronage of the Conference, had in mind not only its scientific program but above all its interdisciplinary character. The transformations of the contemporary world bring a lot of good, but there are also many threats and negative phenomena. One of them is the encapsulation of many environments and the lack of a debate between them, so necessary for social development. In medicine, in addition to the enormous technological progress, the expanding area of telemedicine and those willing to use it, or the wide availability of drugs, we observe in Poland a large fragmentation of specialization. We have nearly 100 medical and dental specializations. At the same time, there is a growing conviction in society that proper treatment can only be carried out by a specialist, bypassing a family doctor, often the best health advisor is "Dr. Google" or the world of overwhelming advertising, presenting various wonderful products that we really know little about.

Yet telemedicine is for medicine, not the other way around. Direct contact between the doctor and the patient was and should be the basic and most important relationship, regardless of the growing possibilities of telecommunications and the Internet world. Man as the subject of treatment requires a holistic view, which is also argued by geneticists, pointing to the need to personalize the methods of treatment.

The threat is not only polypharmacy, but also polytherapy, caused by treatment by many specialists, without mutual communication between them, bypassing the family doctor. And if we add to this the availability of over-the-counter drugs, prescribed in many retail outlets, we know why the patients we asked about how they take drugs, respond "handfuls". And yet it is well known that a person can take 4 or at most 5 medications at the same time, because consuming more of them generates a new disease, which we recognize after years. Of course, the growing problem of drug counterfeiting is not without significance.

To young conference participants who are just at the beginning of their professional career or education, I want to address the words of my Masters and Teachers who taught me the principle that there are things in life that are really worthwhile, and that there are things that are only profitable. I know well that it is not easy for you in a world dominated by commerce, in a world where profit is the primary selection criterion.

I am deeply convinced that the interdisciplinary debate initiated today will be an important step for sharing my experiences and sharing my observations. I hope that soon in the public debate, full of emotions and screaming, there will be a place for competent people who will displace those who supposedly know everything, and speak mainly because they want to be noticed above all.

The task is not easy, because democracy has a lot of good, but it also has its negative face in the form of equating competence with ignorance.

Our worst mistake, however, would be the sin of omission and the feeling of inability to meet difficult challenges.

So I wish you intellectual and civil courage in a difficult journey, but such successes are the gladdest.

All the best to you and your loved ones!

Warszawa 24.09.2020

Vice-President of the Supreme Medical Council
Dr med. Jacek Kozakiewicz



**A LETTER FROM PRESIDENT OF THE REGIONAL
CHAMBER OF PHYSICIANS AND DENTISTS –
MAŁOPOLSKA DISTRICT (KRAKÓW)**

Dear Colleagues,

On behalf of the Regional Chamber Of Physicians And Dentists in Kraków let me welcome you to the 3rd International Conference of Pharmaceutical and Medical Sciences co-organized by distinguished academic centers in Kraków, Martin and Szeged.

First, I would like to congratulate the organizers. It was no small feat to organize this third edition of the international conference in these extremely challenging times. Despite COVID-19 pandemic, rather than postponing or cancelling the conference, they successfully organized the whole endeavour online. That's the scientific spirit!

Second, the conference's themes and interdisciplinary approach – involving experts in the field of medicine, pharmacy, and health sciences more broadly – are very timely indeed. It focuses on issues related to health consequences of pollution and environmental degradation, as well as potential ways and methods to protect human health and treat related illnesses. These issues affect more and more people and require considerable effort on the part of international scientific community to find new and creative solutions. Research, publication and exchange of findings and ideas via conferences like this are much needed.

I would like to thank all distinguished lecturers and wish every participant an engaging conference with many interesting presentations and fruitful discussions.

Lek. stom. Robert Stepień



A LETTER FROM PRESIDENT OF THE POLISH PHARMACEUTICAL SOCIETY

Dear Colleagues,

On behalf of the Polish Pharmaceutical Society I would like to congratulate the organizers on this year's edition of the International Conference on Pharmaceutical and Medical Sciences (3rd ICPMS): "PATHOPHYSIOLOGICAL, PHARMACOLOGICAL AND CLINICAL ASPECTS OF THE ENVIRONMENTAL POLLUTION".

My congratulations on the initiative to raise such important and current issues in the international pharmaceutical environment. I would like to wish you fruitful and creative discussions and successes in integrating the scientific community.

Throughout its history, our Society has always supported scientific initiatives. The beginnings date back to the late 19th century. Even though professional issues were the Society's main concern, it did recognise the importance of development of pharmaceutical science. In order to publish the news on the latest scientific developments and achievements the Society's own newspaper "Wiadomości Farmaceutyczne" was established in 1874. The Society also published textbooks and organised lectures for pharmacists. In 1901 it developed a series of pharmacy courses for women.

After the end of the First World War, when Poland regained independence, the Society integrated all pharmacists from the whole territory of the country and was the patron of the second Polish Pharmacopoeia.

After the Second World War, in 1947, the Polish Pharmaceutical Society (Polskie Towarzystwo Farmaceutyczne) was created, whose roots, however, go back to the previous organization. Our Society laid the foundations for organizational work, but above all, it has been taking

care of the high level of academic education and the close relationship between science and pharmaceutical practice.

I am looking forward to the scientific results of the research that will be discussed at the conference.

Yours faithfully,

Prof. dr hab. Janusz Pluta



A LETTER FROM VICE-PRESIDENT OF DISTRICT PHARMACY COUNCIL IN KRAKÓW

Ladies and Gentlemen!

On behalf of the President of District Pharmacy Council in Kraków mgr farm. Elżbiety Rząsa-Duran it's an honour to welcome participants of the 3rd International Conference on Pharmaceutical and Medical Sciences. Due to the pandemic situation remote organization of conference is a big challenge, but the health and the security of participants is very important.

The first General Assembly of the Regional Pharmaceutical Chamber in Kraków was held in Kraków on November 9, 1991 and this day should be considered as the official date of the creation of the Regional Pharmaceutical Chamber in Kraków. Currently the Pharmaceutical Chamber in Kraków is a functioning entity of the Polish pharmaceutical self-government. At present it has more than 3,000 members. The Regional Pharmaceutical Chamber in Kraków consist of the Presidium which is originated from the Regional Pharmaceutical Council, the Regional Pharmaceutical Court, Regional Professional Liability Commissioner and six committees. The Regional Pharmaceutical Chamber in Kraków carries out the tasks imposed by the state authorities and participates in the activities primed by the Polish Pharmaceutical Chamber. The Regional Pharmaceutical Chamber in Kraków works closely with territorial authorities, i.e. the pharmaceutical inspection, a division of the National Health Fund, Regional Medical Chamber, Polish Pharmaceutical Society and the Faculty of Pharmacy Jagiellonian University Medical College. The Pharmaceutical Chamber in Kraków intervenes in everyday problems of pharmaceutical life, like the cases of breaches of ethics, or crossing rules and legislations for the pharmacy professions in Poland. The Regional Pharmaceutical Chamber together with the Faculty of Pharmacy Jagiellonian University Medical College runs Internet

portals "e-duk@cja" (e-duk@tion) and "e-pharmacy". It makes possible regular professional training scheme conducted by e-learning. It's very useful in time of the pandemic. Furthermore, the Pharmaceutical Board in Kraków acts as an interface between the Faculty of Pharmacy Jagiellonian University Medical College, and pharmacies in whose the student internships are organized. The Pharmaceutical Chamber has published the journal "Farmacja Krakowska" ("Kraków Pharmacy") and organizes cultural and sport events and meetings for pharmacists.

I wish You fruitful conference and successful cooperation

*Vice-President of District Pharmacy Council in Kraków
Dr n. farm. Przemysław F. Szybka*



**A LETTER FROM A VICE-DEAN (2017-2020) OF
THE HEALTH SCIENCES FACULTY
JAGIELLONIAN UNIVERSITY
MEDICAL COLLEGE**

On September 24–26, 2020, the International Conference on Pharmaceutical and Medical Sciences (3rd ICPMS) will be held in Krakow under the Honorary Patronage of the Vice-Rector of the Jagiellonian University for Collegium Medicum, prof. dr hab. Tomasz Grodzicki and with the support of the Authorities of all Departments of the Jagiellonian University of Medical College, in particular the Faculty of Pharmacy (Dean – prof. dr Jacek Sapa), as well as research centers from abroad: the Faculty of Medicine in Martin (Slovakia) under the direction of Ms. Dean, prof. Andrea Calkovska and the Faculty of Pharmacy of the University of Szeged under the direction of Dean prof. Istvan ZUPKO – all of them with the status of co-organizers, which is a novelty in such projects.

I extend cordial greetings to all participants of the International Conference on Pharmaceutical and Medical Sciences (3rd ICPMS) taking place in Krakow. Organized for the third time, it follows the best Krakow traditions. The theme of this year's conference – the slogan of which is: "PATHOPHYSIOLOGICAL, PHARMACOLOGICAL AND CLINICAL ASPECTS OF THE ENVIRONMENTAL POLLUTION" – are multi-faceted issues related to environmental contamination and its broadly understood impact on health.

The conference is an admirable enterprise for many reasons. This year, due to the pandemic, it is organized online.

The international nature of the conference is also of great importance. Apart from scientists from Poland, Slovakia and Hungary, representatives from Germany, Italy, Iran and Morocco participate in it. Thanks to this formula, scientists from many countries can exchange the

results of research and studies, share experiences, make scientific contacts and make friends.

Therefore, I wish you a successful conference - an interesting debate and a lot of new information about the latest achievements and medical programs. I also wish you successful implementation of your individual aspirations and all personal well-being.

Thanks go to Chair of the Scientific Committee Professor Dariusz Adamek, and Professor Bożena Muszyńska and Professor Zsolt Szakonyi. Special thanks go to Chair of Organizing Committee Professor Włodzimierz Opoka and to the Organizing Committee.

Cograts, The Scientific Triangle" (ST)/Krakow-Martin-Szeged is good idea!

Prof. dr hab. Roman NOWOBILSKI



**A LETTER FROM CO-CHAIRPERSONS OF THE SCIENTIFIC
AND ORGANIZING COMMITTEES
OF THE 3RD ICPMS MARTIN, KRAKÓW, SZEGED 2020**

The pollution and, more generally the degradation of our environment is a hot topic not only interesting representatives of biomedical and life sciences, geosciences (including e.g. climatology) and economics but obviously it directly affects virtually every individual. The attention to the deterioration of the quality of the air we breathe, the water we drink and the food we eat reaches more and more people and therefore becomes also a political issue. Undoubtedly one of the crucial ways to understand true causes and mechanisms of this process or processes runs through the hardship of scientific efforts in the field of pharmaceutical and medical sciences. The 3rd International Conference of Pharmaceutical and Medical Sciences cooperatively organized by scientific university centers in Kraków (Poland), Martin (Slovakia), and Szeged (Hungary) is intended to boost scientific activities in this area of investigations, what emphasizes its title: "PATHOPHYSIOLOGICAL, PHARMACOLOGICAL AND CLINICAL ASPECTS OF THE ENVIRONMENTAL POLLUTION". The concept of the 3rd ICPMS – which originally was to be held stationary in Martin, but due to COVID-19 pandemic will be carried out via Internet – in a sense will be a continuation of the previous – 2nd ICPMS two years ago, which was focused on biomedical security. We are very proud that the 3rd ICPMS received support from the Authorities of Jagiellonian University Medical College in Kraków, from the Authorities of the Jessenius Faculty of Medicine in Martin, Comenius University of Bratislava, and from the Authorities of the University in Szeged. It seems therefore right to think that the 3rd

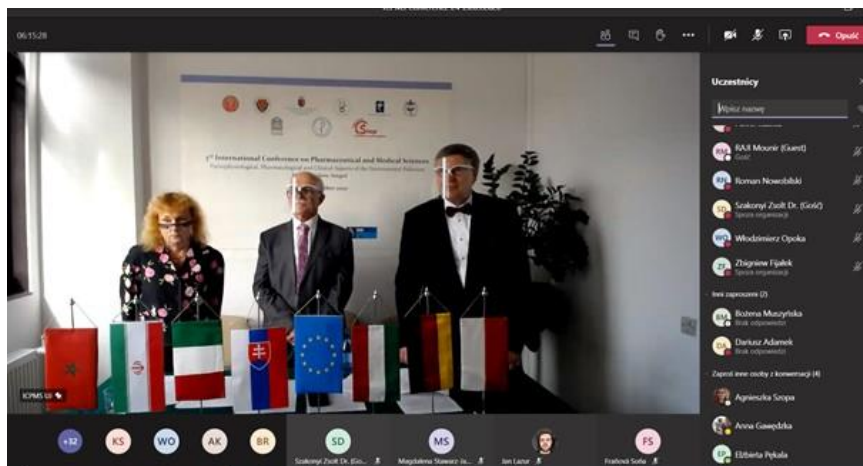
ICPMS may even bring the firstfruits of, let's name it – “triple scientific entente” in the field of medicine, pharmacy, and health technology.

We would like to cordially invite anybody, who represents whichever pharmaceutical, biological or more generally – biomedical sciences, as well as students of Pharmacy, Health Sciences or Medicine, and who are interested in issues related to health consequences of pollution of the environment and the ways and methods to protect our health and treat the related illnesses, to take active part in our Conference, which will be held in the “cloud” on 24-26 September, 2020.

Prof. dr hab. Dariusz Adamek

Prof. dr hab. Bożena Muszyńska

Prof. dr hab. Włodzimierz Opoka



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XXX – Years of activity

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KRS: 0000047746

Statut Szowarzyszenia LEKARZE NADZIEI / The statute of the Association of Hope Doctors

Przekonani, iż działalność humanitarna stanowi nieodłączną część życia społecznego w cywilizowanym świecie, świadomi potrzeby objęcia pomocą charytatywną wszystkich ludzi w zagrożeniu zdrowia i życia – zarówno we własnym kraju jak i poza jego granicami / Convinced that humanitarian activity is an integral part of social life in a civilized world ... conscious of the need to provide charitable assistance to all people, whose health and life are at risk, both within and outside their own country

Misja Szowarzyszenia / Mission of Association

- Niesienie pomocy medycznej ludności w zagrożeniu życia i zdrowia w kraju i poza jego granicami.
- Pozyskiwanie leków i materiałów medycznych.
- Działanie na rzecz mniejszości narodowych.
- Promocję zdrowia wśród dzieci i młodzieży.
- Providing medical assistance to the population in case of life and health in the country and abroad.
- Acquisition of medicines and medical materials.
- Acting for national minorities.
- Health promotion among children and young people.

- Przychodnia lekarska dla bezdomnych i ubogich.
- Punkt charytatywny wydawania leków i materiałów medycznych.
- Szpitalna medycyna – pomoc osobom samotnym, niepełnosprawnym, byłym kombatanom.
- A medical clinic for the homeless and the poor.
- Charity point for the dispensing of medicines and medical supplies.
- Medical volunteering, helping people who are lonely, disabled, former veterans

Funkcje / Activities

- Organizacja wysyłki materiałów medycznych, odżywek do Polaków w kraju.
- Współpraca z organizacjami charytatywno-humanitarnymi w Polsce i zagranicą.
- Organization of sending medical materials and nutritional supplements for Poles in the country.
- Cooperation with charitable and humanitarian organizations in Poland and abroad.



Koncentracja na programach niesienia pomocy w kraju i zagranicą

Concentration on assistance programs inside the country and abroad

Nasi specjaliści / Our specialists

- Neurochirurg / Neurosurgeon
- Internista / Internist
- Choroby zakaźne / Infectious diseases
- Psychiatra / Psychiatrist
- Laryngolog / Laryngologist
- Geriatra / Geriatricist
- Lekarz ogólny / General practitioner

- Chirurg / Surgeon
- Kardiolog / Cardiologist
- Ginekolog / Gynecologist
- Psycholog / Psychologist
- Dermatolog / Dermatologist
- Okulista / Ophthalmologist
- Stomatolog / Dentist

KONTAKT / CONTACT

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AL. POKOJU 7, 31-548 KRAKÓW

TEL (12) 414-30-05

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Mecenat / Patronage



Medical Aid For Poland Fund – wyposażenie gabinetu stomatologicznego.



Rotary Club Kraków Wawel – międzynarodowy projekt - Matching Grant.



Fundacja Polskich Kawalerów Maltańskich – oddział Radomsko-Lubelski wyposażenie gabinetu ginekologicznego.



Okręgowa Izba Lekarska w Krakowie – pomoc medyczna.



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* 10 degree courses * 6000 students * more than 1550 academic teachers and
researches * 160 full professors*

Jagiellonian University Medical College is a part the Jagiellonian University – the oldest higher education institution in Poland and one of the oldest in Europe. It was founded on May 12, 1364 by the Polish king Casimir the Great. It has been an international institution since its inception. Poles, Ruthenians, Lithuanians, Hungarians, Germans, Czechs, the Swiss, the English, the Dutch, the French the Spanish, Italians, and even Tatars studied here in the old days.

JU MC is formed by three faculties: Faculty of Medicine – instituted in 1364, Poland's oldest unit schooling physicians, Faculty of Pharmacy and Faculty of Health Sciences.

All faculties cooperate with numerous European and American universities, currently putting into effect many bilateral agreements with institutions from a number of countries. We are accredited by the U.S. Department of Education and Medical Board of California.

Part of JU MC is the School of Medicine in English. Its rapid development resulted in currently educating nearly 700 students coming from

five continents. SME cooperates with institutions playing an important role in the American and European educational systems, like the National Board of Examiners, the Association of American Medical Colleges, and the European Board of Medical Assessors. SME graduates are completing their residencies and postgraduate training in university hospitals in various countries such as the USA, Canada, Australia, England, Ireland, Norway, Sweden, as well as Poland.

The University also offers doctoral and postgraduate studies for physicians from various regions of the country in all fields of medicine.

Didactic base for clinical teaching includes the University Hospital – one of the most innovative in Europe, University Children's Hospital, University Hospital of Orthopedics and Rehabilitation in Zakopane, University Clinic of Dentistry, and select clinical departments located in Kraków. University Hospitals are leading medical facilities that blend years of experience with state-of-the-art medicine.

Our prestige in Poland and abroad is illustrated by widely recognised research achievements and rise of the number of patent applications and patents year by year. The scientists and physicians from the JU Medical College carry out pioneer studies, e.g. in cardiac surgery, urology, and neurology, often leading to development of novel treatment methods. Their findings have been published in some of the most prestigious international journals like European Journal of Cardio-Thoracic Medicine, New England Journal of Medicine, Journal of Medicinal Chemistry, and Lancet.

JU MC is an heir to the long tradition in educating highly-specialized medical personnel for the whole of the healthcare system. The status of the oldest educational institution in Poland obligates us to maintain the highest standards in teaching, research, and clinical activity.

Jagiellonian University Medical College continue the work of our renowned graduates and professors in order to meet the new challenges of modern times.



Collegium Nowodworskiego fot. Anna Wojnar



Collegium Novum fot. Anna Wojnar



The University Hospital fot. archiwum Warbud

PROGRAMME

Thursday, 24th September 2020

15.00–15.30 Opening Ceremony – welcoming speeches

Prof. dr hab. med. Tomasz GRODZICKI, Vice-Rector of Jagiellonian University Medical College (Poland)

Prof. dr hab. Andrea ČALKOVSKÁ, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Dean of Faculty of Medicine (Slovakia)

Prof. dr hab. István ZUPKÓ, University of Szeged, Dean of Faculty of Pharmacy (Hungary)

Prof. dr. hab. Jacek SAPA, Jagiellonian University Medical College, Dean of Faculty of Pharmacy (Poland)

Dr med. Jacek KOZAKIEWICZ, Vice-President of the Polish Supreme Medical Council (Poland)

Prof. dr hab. Janusz PLUTA, President of the Polish Pharmaceutical Society (Poland)

Mgr Elżbieta PIOTROWSKA-RUTKOWSKA, President of the Polish Supreme Pharmaceutical Council (Poland)

Dr Robert STĘPIEŃ, President of the Regional Chamber of Physicians and Dentists – Małopolska District (Poland)

Dr Przemysław SZYBKĄ, Vice-President of District Pharmacy Council in Kraków (Poland)

15.30-15.40 Technical break, sponsor presentations

15.40–16.40 Inaugural – honorary lectures

Presidents: Prof. dr hab. Bożena MUSZYŃSKA (Poland)

Prof. dr hab. Dariusz ADAMEK (Poland)

15.40–16.00 Prof. dr Soňa FRAŇOVÁ, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin (Slovakia)

Pharmacotherapy of allergic asthma in experimental conditions

16.00–16.20 Prof. dr Zsolt SZAKONYI, Vice-Dean Faculty of Pharmacy, Institute of Pharmaceutical Chemistry, University of Szeged (Hungary)

Synthesis, chemical and biological application of steviol- and isosteviol-based diterpenoids, obtained from Stevia rebaudiana L.

16.20–16.40 Prof. dr hab. Tomasz BRZOZOWSKI, Head of the Department of Physiology Faculty of Medicine, Jagiellonian University Medical College, Kraków (Poland)

Physiological and pharmacological treatment of inflammatory bowel disease (IBD), the real problem in gastroenterology of XXI century. Evidence from experimental and clinical medicine

16.40–17.00 Technical break, sponsor presentations

17.00–18.00 Session I

Presidents: Prof. dr hab. Zsolt SZAKONYI (Hungary)
Prof. dr hab. Erika HALAŠOVÁ (Slovakia)

17.00–17.20 Dr Janka PÁLEŠOVÁ, Comenius University in Bratislava Jessenius Faculty of Medicine in Martin (Slovakia)

Effect of simvastatin on human dental stem cells

17.20–17.40 Prof. dr hab. István ZUPKÓ, University of Szeged, Faculty of Pharmacy (Hungary)

In vitro antimetastatic properties of estrane and androstane analogs on human cancer cells

17.40–18.00 Dr Urszula DOBOSZEWSKA, Jagiellonian University Medical College, Faculty of Pharmacy (Poland)

Pathophysiological role of zinc (deficiency) – focus on coronavirus disease 2019

18.00–18.10 Discussion

18.10–18.30 Election of the jury for the best Young Scientists' presentation

Friday, 25th September 2020

10.00–12.30 Session II – Session of Young Scientists

Presidents: Dr Viktória SZÚTS (Hungary)

Prof. dr hab. Zbigniew FIJAŁEK (Poland)

10.00–10.15 Mrs Eliza MATUSZEWSKA (Poland)

Levels of selected heavy metals in Polish bee pollen, royal jelly, and propolis

10.15–10.30 Mrs Noemi NICOSIA (Italy)

LSD-microdosing as a new potential strategy in psychiatric illnesses

10.30–10.45 Mr Jan LAZUR (Poland)

Bioelements accumulation by Agaricus bisporus fruiting bodies during cultivation process

10.45–11.00 Ms Magdalena SKALSKA (Poland)

Evaluation of lipophilicity and admet parameters of 10-triazolylmethyl-1,8-diazaphenothiazines

11.00–11.15 Technical break, sponsor presentations

11.15–11.30 Ms Ewelina GAPYS (Poland)

New azidothymidine derivatives of dipyridothiazines – synthesis and properties

11.30–11.45 Ms Kamila STACHYRA (Poland)

Comparison the action of selected air pollution components on atherosclerosis development in apoE-knockout mice

11.45–12.00 Ms Anna FURGAŁA-WOJAS (Poland)

Analgesic, antidepressant-like and anxiolytic-like properties of ropinirole in the reserpine-induced fibromyalgia-like model in mice

12.00–12.15 Ms Karolina NOWAK (Poland)

Regulation of estrogen receptors and NF- κ B in methylparaben-stimulated human neutrophils

12.15–12.30 Mr Raji MOUNIR (Maroco)

Synthesis and investigation of pinane-based chiral tridentate ligands in the asymmetric catalytic reactions

12.30–14.40 Technical break, sponsor presentations

14.40–18.00 Session III

Presidents: Prof. dr hab. Ján ŠVIHRA (Slovakia)

Prof. dr hab. Roman NOWOBILSKI (Poland)

14.40–15.00 Prof. Keyhan SAYADPOUR ZANJANI (Iran)

Platelet inhibition in children receiving Aspirin after BT shunt or PDA stent by impedance aggregometry: A prospective study

15.00–15.15 Dr hab. Anna W. SOBAŃSKA (Poland)

Micellar thin layer chromatographic approach to studies of the soil-water partition coefficient of pharmaceuticals

15.15–15.30 Dr Agnieszka KLUPCZYŃSKA (Poland)

Determination of illicit drugs in urban wastewater in Poland by liquid chromatography-tandem mass spectrometry method

15.30–15.45 Dr hab. Grażyna CHŁOŃ-RZEPA (Poland)

8-alkylamine derivatives of purine-2,6-dione as PDE inhibitors and TRPA1 antagonists: a novel approach for the treatment of chronic respiratory diseases

16.00–16.15 Discussion

16.15–16.30 Technical break, sponsor presentations

- 16.30–16.45 Dr Katarína LEŠKOVÁ (Slovakia)
Double-hit and triple-hit DLBCLs: possibilities of their identification using significant prognostic and predictive markers
- 16.45–17.00 Dr Ewa SURÓWKA (Poland)
Differences in ROS-scavengers and osmoprotectants patterns in Arabidopsis thaliana plants with variable tocopherols composition growing under salt stress
- 17.00–17.15 Mr Jan LAZUR (Poland)
Utilization of medicines and environmental protection
- 17.15–17.30 Dr Przemysław SZAFRAŃSKI (Poland)
Chemical derivatization with polycyclic aromatics: development of reagents and methods

17.30–17.45 Discussion

17.45–18.00 Sponsor presentations

Saturday, 26th September 2020

10.00–11.15 Session IV

Presidents: Prof. dr hab. István ZUPKÓ (Hungary),
Prof. dr hab. Juraj MOKRÝ (Slovakia)

- 10.00–10.15 Dr Adrian PODKOWA (Poland)
Assessment of zinc and magnesium levels in teeth of rats fed with different diets
- 10.15–10.30 Mr Karol JĖDREJKO (Poland)
Unintended doping and anti-doping over-the-counter (OTC) medications
- 10.30–10.45 Mr Krzysztof KONIEWICZ (Poland)

*Presentation of the exhibition in the Museum of Pharmacy
Jagiellonian University Medical College in Kraków*

10.45-11.00 Dr Agata KRYCZYK-POPRAWA (Poland)
Tuberculosis among the homeless in Cracow

11.00–11.15 Discussion

11.15–11.30 Technical break, sponsor presentations

11.30–12.00 Closing ceremony

Announcement of the results of the competition for the
best Young Scientist's presentation and the best poster

Speeches of the co-organizers from Slovakia, Hungary
and Poland

PART II

HONORARY LECTURES

PHARMACOTHERAPY OF ALLERGIC ASTHMA IN EXPERIMENTAL CONDITIONS

Prof. dr hab. **Soňa Fraňová**

Comenius University in Bratislava, Jessenius Faculty of Medicine, Department of Pharmacology, Martin, Slovakia

Asthma is now viewed as a heterogeneous inflammatory airways disorder which gives rise to several different clinical phenotypes. Eosinophilic inflammation is generally considered to be the main feature of asthmatic airways. It is generally accepted that the Th1/Th2 balance is responsible for the development of allergic asthma. Th2 cells secrete IL-4, IL-5, and IL-13. IL-4, together with IL-13, is required for B-cell maturation and IgE production. IL-5 activates eosinophils and attracts them to the lung, where they secrete numerous inflammatory cytokines and chemokines. IL-4, IL-9, and IL-13 regulate mast cell activation. IL-13 also directly affects the airway epithelium, increases the goblet cell differentiation, activation of fibroblasts, and bronchial hyperresponsiveness. These cytokines may also, in turn, affect the Th1/Th2 balance.

The trend in asthma pharmacotherapy is to either identify new anti-asthmatic drugs with simultaneous bronchodilatory, anti-inflammatory and anti-remodelling effects or to optimise the current pharmacotherapy.

Current asthma therapy is based on the combined administration of bronchodilatory relievers and asthma controllers with an anti-inflammatory effect. Treatment is conducted in five stages according to the severity of asthma and the appearance of symptoms. In terms of optimisation of current therapy, a combination of low doses of inhaled corticosteroids (ICS) with long-acting beta2 receptor agonists (LABA) is recommended. These drugs potentiate each other's effect. ICS increase the expression of beta2 receptors and the bronchodilatory effect of LABA. LABA, on the other hand, increase the anti-inflammatory action of ICS. The results of our experiments of 21 days of inhalation administration of budesonide and salmeterol under conditions of experimental allergic inflammation showed that the above combination of drugs com-

pared to monotherapy given at half doses potentiates the resulting anti-tussive, bronchodilatory, cilio-modulatory and anti-inflammatory effect.

An innovative goal of pharmacological modulation of allergic inflammation is to influence the activity of ion channels, which regulate the activity of immune cells. One of them is the Ca²⁺ CRAC channel, which is involved in the activation of T, B lymphocytes, mastocytes, eosinophils and the synthesis of inflammatory cytokines. In our experimental conditions (allergen-induced asthma model), 21-day administration of the RP3128 CRAC channel blocker demonstrated an excellent effect on respiratory defence mechanisms. This substance significantly inhibited markers of the inflammatory response: eNO, inflammatory cytokines (IL-4, IL-5, IL-13), reduced the number of eosinophils and mastocytes in the homogenate of lung tissue.

At present, there is an increasing number of experimental works describing the critical role of Rho-kinase signalling pathway in the pathogenesis of allergic asthma. Rho-kinases (ROCK) belong to a group of serine-threonine kinases that participate in the control of critical physiological functions such as smooth muscle cell contractility, migration, proliferation, immune cell adhesion and inflammatory stimulation.

In summary, new findings related to the mechanism of allergic inflammation of the respiratory tract offer new possibilities for pharmacological intervention.

Acknowledgments: This work was supported by: the Slovak Research and Development Agency under the contract No. APVV-19-0033, Grant VEGA 1/0165/17, VEGA 1/0253/19.

SYNTHESIS, CHEMICAL AND BIOLOGICAL APPLICATION OF STEVIOL- AND ISOSTEVIOL-BASED DITERPENOIDS, OBTAINED FROM *STEVIA REBAUDIANA* L.

Zsolt Szakonyi¹, Dániel Ozsvár¹, Viktória Nagy², István Zupkó²

¹Institute of Pharmaceutical Chemistry, University of Szeged, H-6720 Szeged, Eötvös utca 6, Hungary

²Department of Pharmacodynamics and Biopharmacy, University of Szeged, H-6720 Szeged, Eötvös utca 6, Hungary

In the recent decade stevia glycosides (isolated from Paraguayan shrub *Stevia rebaudiana* L.), have been proved important natural compounds in the market of artificial sweeteners because of the easy cultivation, the high diterpene glycoside content of the plant, and easy isolation of its glycoside content. Because of the huge volume isolation of glycosides, the aglycon steviol and its isomer isosteviol have come into prominence nowadays as promising starting material for the synthesis of bioactive compounds.

The aim of the present lecture is to give a short review on the chemical and biological applications of this promising diterpenoid type compounds according to the literature and our recent results in this field.

Since the commercially available *Stevia* glycosides, its aglycons, ent-kaurane diterpenoid steviol or ent-beyerane diterpenoid isosteviol have been proved excellent starting material for the synthesis of diterpenoids with wide range of biological activity such as cytotoxic and apoptosis, or glutathione S-transferase inducing activity. Some, at C-4 position COOH→NH₂ substituted derivatives possess inhibitory effects against *Hepatitis B* virus.

Isosteviol derivatives prepared with wide range of chemical modification also have proven interest molecules with remarkable pharmacological activities. Some polioliol type isosteviol derivatives bear antiproliferative activities, evaluated against human gastric carcinoma MGC-803, HepG-2 and breast carcinoma MDA-MB-231 cell lines. Similarly, excellent antiproliferative activity was observed on the wide range of human

cancer cell lines by some structural modifications done at the C-19 of beyerane-skeleton of isosteviol.

A library of steviol-based trifunctional chiral ligands, such as aminodiols and 1,3-heterocycles has been developed and applied as chiral catalysts. The resulted steviol-type aminodiols were tested against a panel of human adherent cancer cell lines (A2780, SiHa, HeLa and MDA-MB-231) with remarkable results.

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**PHYSIOLOGICAL AND PHARMACOLOGICAL TREATMENT
OF INFLAMMATORY BOWEL DISEASE (IBD), THE REAL
PROBLEM IN GASTROENTEROLOGY OF XXI CENTURY.
EVIDENCE FROM EXPERIMENTAL AND CLINICAL
MEDICINE**

Tomasz Brzozowski

Department of Physiology Jagiellonian University Medical College, Kraków,
Poland

Inflammatory bowel diseases (IBDs) pertain to a group of disorders of inflammatory conditions of the colon and small intestine, which display two major phenotypic forms, Crohn's disease (CD) and ulcerative colitis (UC), both characterized by a cyclical nature, alternating between active and quiescent states [Abraham C and Cho J, 2009]. Even though most of CD patients are underweight, the ratio of intraabdominal fat to total abdominal fat is far greater than in control groups when assessed by magnetic resonance imaging (MRI) [Desreumaux P et al., 2012]. In CD the hypertrophied mesenteric adipose white tissue (mWAT) could be a major contributor of the increased circulating pro-inflammatory cytokines and plays a role in the pathogenesis and activity of the disease [Fink et al, 2012, Bilski et al, 2013]. Diet-induced obesity (DIO) by feeding rodents high fat diets is the most common model reported in literature because this approach mimics human characteristics of obesity and leads to the increased mesenteric fat deposition, colonic inflammation and the deterioration of experimental colitis [de La Serre et al., 2010; Liu et al., 2015]. Recent studies have indicated that alkaline phosphatase is a superfamily of metalloenzymes known to catalyze the hydrolytic removal of phosphate from a variety of molecules [Millan J.L., 2006]. Intestinal alkaline phosphatase (IAP) is an important apical brush border enzyme expressed throughout the gastrointestinal tract and secreted into both, the intestinal lumen and the bloodstream. IAP exerts its protective effects against intestinal and systemic inflammation through

dephosphorylating of bacterial lipopolysaccharide (LPS) released from cells during stressful events [Fawley J and Gourlay D.M., 2016]. The mWAT in obesity may predispose the development of colitis [Malo M.S., 2015]. In the human trial, the administration of IAP daily over a 7-day course to patients with was associated with a short-term improvement in disease activity scores, and clinical beneficial effects were observed within 21 days and associated with reductions in C-reactive protein and stool calprotectin [Lukas M et al., 2010]. Our studies have demonstrated that voluntary exercise can improve the healing of experimental colitis due to release of protective myokines irisin from working skeletal muscles and normalization of leptin/adiponectin ratio in obese mice. The combined treatment of IAP with wheel running significantly reduced disease activity index (DAI) in high fat diet mice (HFD) and potentiated the beneficial ameliorating effect of exercise on experimental colitis. In genetically predisposed subjects, dietary patterns associated with a western lifestyle promote a pathological imbalance in the gut microbiota (dysbiosis). As the development of a “dysbiotic” microbiome is thought to be involved in the pathogenesis of IBD, the type of diet and meal portion size are now being considered as important etiological factors. Furthermore, we have found that exogenous IAP administration ameliorated gut inflammation favoring healing of colitis due to improvement in colonic blood flow and modification of intestinal microflora. Thus, our study documented that IAP may represent a novel therapeutic strategy to enhance the exercise benefit in human IBD. The pathomechanism of IBD is discussed in order to overview the potential “non-pharmacological” alternative and supplementary therapeutic approaches taking into account epidemiological and pathophysiological links between obesity and IBD, including changes in the disease activity, the functional parameters of the intestinal mucosa and alterations in the intestinal microbiome in both experimental animal models and human subjects.

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PART III – ABSTRACTS

ORAL PRESENTATIONS

SESSION OF YOUNG SCIENTISTS

OTHER ABSTRACTS

ORAL PRESENTATIONS

EFFECT OF SIMVASTATIN ON HUMAN DENTAL STEM CELLS

**Janka Pálešová¹, Ján Strnádel², Dana Dvorská², Slavomíra Nováková²,
Romana Záhumenská¹, Dagmar Statelová³, Henrieta Škovierová²,
Erika Halašová²**

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Adult dental stem cells have an excellent ability to proliferate and differentiate into other tissue types within their germ layer. Currently, successful stem cell therapy requires regulated stem cell proliferation and differentiation. Statins represent one group of these regulators. They decrease the level cholesterol by inhibiting of 3-hydroxy-3methylglutaryl coenzyme A reductase, a key regulatory enzyme in the cholesterol biosynthesis. Moreover, statins have a pleiotropic effect on proliferation as well as on differentiation of stem cells. When applied at lower concentrations, statins are able to improve stem cell survival.

In our study, we used a de novo prepared primary cell line obtained from human dental follicle tissue. For our experiments, cells were divided into three groups: (i) a control group, cells cultured in the absence of simvastatin; (ii) cells cultivated with simvastatin at higher concentration (5 $\mu\text{mol/L}$) and (iii) cells cultivated with simvastatin at lower concentration (0.05 $\mu\text{mol/L}$). Cells were grown in 3D conditions for 21 days to form spheroids. Subsequently, the expression levels of various genes included in pluripotency (NANOG), apoptosis (CASP3) and osteogenesis (RUNX2, BSP and ALP) were analyzed in spheroids.

We prove the pleiotropic effect of statins on 3D dental stem cells on different biological level.

The ALP and BSP gene products were not present in any sample, which indicating that spontaneous osteodifferentiation during prolonged cultivation did not occur. The expression level of NANOG and CASP3 increased in group with higher concentration of simvastatin comparing to other two groups. In contrast, the expression level of RUNX2 decreased with increased concentration of simvastatin.

We did not confirm the hypothesis, that simvastatin can stimulate stem cells towards osteodifferentiation.

This work was supported by the Slovak Research and Development Agency under the contract APVV-15-0217.

IN VITRO ANTIMETASTATIC PROPERTIES OF ESTRANE AND ANDROSTANE ANALOGS ON HUMAN CANCER CELLS

**István Zupkó¹, Zsuzsanna Schelz¹, Éva Frank², Erzsébet Mernyák²,
Ágnes Kulmány¹, Noémi Bózsity¹, Renáta Minorics¹**

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Cancer is the second major cause of mortality worldwide and in the high-income countries the number of cancer-related deaths seems to be higher than that from cardiovascular disorders. According to a generally accepted estimation up to 90% of cancer-related mortality is due to consequences of metastasis indicating that pharmacological interventions targeting metastasis formation may have a substantial impact on the overall mortality. Metastasis formation is a complex and well-organized procedure including the infiltrating cancer growth through the extracellular matrix, migration and initiation of distant colonies. Identification of innovative antimetastatic compounds requires *in vitro* methods of relatively high throughput.

The aim of the presentation is to give a summary of the currently available antimetastatic methods including different types of cell-based migration and invasion assays (wound healing and Boyden chamber assay, respectively).

The antiproliferative properties of modified steroidal compounds including both estrane and androstane analogs are widely investigated and documented [1,2]. On the other hand, experimental data concerning the antimetastatic potency of those agents are limited. Based on this discrepancy a project was initiated at our Department to investigate the action of such molecules on metastasis formation. Some preliminary results obtained so far will be additionally presented.

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PATHOPHYSIOLOGICAL ROLE OF ZINC (DEFICIENCY) – FOCUS ON CORONAVIRUS DISEASE 2019

**Urszula Doboszewska¹, Piotr Właź², Gabriel Nowak^{1,3},
Katarzyna Młyniec¹**

¹Department of Pharmacobiology, Jagiellonian University Medical College, Medyczna 9, PL 30-688 Kraków, Poland

²Department of Animal Physiology and Pharmacology, Institute of Biological Sciences, Maria Curie-Skłodowska University, Akademicka 19, PL 20-033 Lublin, Poland

³Laboratory of Trace Elements Neurobiology, Department of Neurobiology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Zinc is an essential element which is present in proteins of humans, other forms of life and of viruses. Zinc deficiency as well its excess in certain compartments has been implicated in the pathophysiology of several diseases. Recently, zinc has been suggested to play a role in the coronavirus disease 2019 (COVID-19), which remains a major threat worldwide. On one hand, zinc deficiency is common in populations at risk for COVID-19 such as the elderly. Administration of zinc has been suggested to limit the cytokine storm which may occur in those with severe COVID-19. Another aspect associated with the role of zinc in COVID-19 is the possibility of manipulation of viral proteins containing zinc sites which are easily disrupted, thus liberating zinc ions. As intracellular zinc was demonstrated to inhibit the RNA-dependent-RNA-polymerase of SARS-CoV, which is highly conserved among coronaviruses, targeting such zinc sites represents an exciting strategy for the design of novel antiviral drugs. Furthermore, the zinc metalloenzyme, angiotensin converting enzyme 2 (ACE2), is used by SARS-CoV-2 as entry point to cells in the alveoli.

MICELLAR THIN LAYER CHROMATOGRAPHIC APPROACH TO STUDIES OF THE SOIL-WATER PARTITION COEFFICIENT OF PHARMACEUTICALS

Anna W. Sobańska, Agata Wawszczak

Department of Analytical Chemistry, Medical University of Łódź, Poland 90-151 Łódź, ul. Muszyńskiego 1

The objective of this research was to evaluate the micellar thin layer chromatography (MTLC) as a tool to predict the soil-water partition of environmental pollutants emitted by pharmaceutical and cosmetic industry.

Soil-water partition coefficients $\log K_{oc}^{(1)} \div \log K_{oc}^{(4)}$ were calculated for 20 structurally-unrelated compounds (drugs, preservatives and sunscreens) according to 4 methods, as described in Ref. [1].

The R_M retention parameter was obtained according to Ref. [2]: $R_M = \log(1/R_f - 1)$.

MTLC was performed on the RP-18 stationary phase, with 2 series of micellar mobile phases containing THF at two concentrations (20 or 40% v/v), with the addition of Brij-35 at different concentrations (0.02 mol L⁻¹ to 0.06 mol L⁻¹). R_M^0 was obtained by extrapolation of R_M values to zero concentration of Brij-35, for each series separately.

It was established that the soil-water partition coefficients calculated as described above are linearly dependent on R_M^0 (MTLC) obtained for THF 20% (v/v), but no correlation was found for THF 40% (v/v). The correlations were better for $\log K_{oc}^{(1)}$ and $\log K_{oc}^{(2)}$ than for $\log K_{oc}^{(3)}$ and $\log K_{oc}^{(4)}$ ($R^2 = 0.83, 0.84, 0.56$ and 0.54 , respectively).

To conclude, MTLC may be used to study soil-water partition of pharmaceuticals, although it is possible that the results of the predictions would be better if the MTLC R_M^0 values were combined with other, easily calculated physico-chemical descriptors.

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**DETERMINATION OF ILLICIT DRUGS IN URBAN
WASTEWATER IN POLAND BY LIQUID
CHROMATOGRAPHY – TANDEM MASS
SPECTROMETRY METHOD**

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In recent years, an increased interest in measuring pharmaceuticals, including illicit drugs, in urban wastewater has been observed [1]. This approach, called wastewater-based epidemiology (WBE), is based on the assumption that the amounts of illicit drugs and their metabolites present in raw sewage reflect the use of drugs by the population of a given area. In contrast to the conventional population surveys, the wastewater analysis provides objective data that covers the entire communities and enables monitoring short- and long-term consumption trends over time. The aim of the research was to apply the WBE approach for the determination of illicit drugs in wastewater samples collected in Poznań, Poland [2]. Raw wastewater samples (influent) were collected using a time-proportional autosampler over a 24-h period and then subjected to a multi-step preparation procedure with solid-phase extraction as the main step [2,3]. The applied method employs high-performance liquid chromatography coupled to tandem mass spectrometry and enables quantitation of classic illicit drugs and their metabolites (amphetamine and their derivatives, benzoylecgonine, 6-acetylmorphine, 11-nor-9-carboxy- Δ^9 -tetrahydrocannabinol (THC-COOH)) as well as new psychoactive substances

(ketamine and mephedrone). Amphetamine and the main metabolites of both cocaine (benzoylecgonine) and marijuana (THC-COOH) were found the most abundant illicit drug residues in wastewater during the entire sampling period. The obtained results were in line with the EMCDDA and EUROPOL reports, which indicates that amphetamine is the most commonly used synthetic drug in Poland and the retail market for cocaine is expanding in Eastern Europe. Despite that the conducted research shows only a snapshot of illicit drug use in Poland, it provided a valuable insight into this problem. To conclude, the wastewater analysis (WBE approach) has great potential as an additional data source for monitoring the trends in drug abuse in communities.

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8-ALKYLAMINE DERIVATIVES OF PURINE-2,6-DIONE AS PDE INHIBITORS AND TRPA1 ANTAGONISTS: A NOVEL APPROACH FOR THE TREATMENT OF CHRONIC RESPIRATORY DISEASES

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Chronic respiratory diseases, such as asthma and chronic obstructive pulmonary disease (COPD), which are associated with pollution and smoking history, have a high prevalence around the world and constitute an important public health problem. These diseases are characterized by airway obstruction, chronic inflammation, and airway remodeling. Unfortunately, the current therapeutic management of chronic respiratory diseases does not affect airway remodeling, therefore the need for the specific therapy aimed at counteracting or preventing processes associated with lung fibrosis is very urgent. Phosphodiesterase (PDE) inhibitors and transient receptor potential ankyrin 1 (TRPA1) ion channel antagonists are considered one of the most relevant strategies in the treatment of chronic airway inflammatory diseases because of their anti-inflammatory and anti-fibrotic effects. Our recent studies revealed that 7,8-disubstituted purine-2,6-dione derivatives may effectively diminish airway smooth muscle cells remodeling and lung fibroblast to myofibroblast transition [1, 2]. Based on these findings, we have designed and synthesized a new series of 8-alkylamine derivatives of purine-2,6-dione with PDEs inhibitory activity and TRPA1 ion channel antagonistic properties. In this study we used the most active compounds and evaluated their anti-inflammatory activity and anti-fibrotic properties in the LPS-

induced mouse macrophages RAW264.7 cell line and in the TGF- β 1-induced human airway smooth muscle cells, respectively. Our research showed that selected 8-alkylamine derivatives of purine-2,6-dione significantly reduced LPS-induced IL-6, TNF- α , and nitrate level in RAW264.7 cells. Anti-inflammatory activity of these compounds was accompanied by additional anti-fibrotic properties as induced by TGF- β (a known pro-fibrotic factor) transcript level of ACTA2, FN1, and COL1 was reduced in the presence of their selected compounds. The observed effects were more pronounced for the tested derivatives than for the reference compounds (IBMX – a PDEs inhibitor and HC-030031 – a TRPA1 antagonist). These results indicate that a concomitant inhibition of PDEs and TRPA1 ion channel may represent a promising approach in the search for dually acting anti-inflammatory and anti-fibrotic compounds for the treatment of chronic respiratory diseases.

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DOUBLE-HIT AND TRIPLE-HIT DLBCLs: POSSIBILITIES OF THEIR IDENTIFICATION USING SIGNIFICANT PROGNOSTIC AND PREDICTIVE MARKERS

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Introduction: Bioptic analysis of diffuse large B-cell lymphomas (DLBCLs) includes identification of their prognostic and predictive markers such as morphologic variants, immunohistochemical subtypes GCB or non-GCB and double or triple expressions of proteins myc, bcl-2 and/or bcl-6. Of particular significance is verification of so called double-hit and triple-hit lymphomas with MYC, BCL2 and/or BCL6 gene rearrangements (DH, TH) by FISH analysis. They represent a highly aggressive group of DLBCLs with inadequate response to therapy and the worst outcome. However, in practice, a rational approach to FISH testing is required to lower the financial and workload burden for standard laboratories.

Objective: We attempted to find a suitable IHC selection method (GCB, double/triple expressors) for identification of those DLBCLs which should subsequently undergo genetic FISH analysis for examination of double and triple rearrangements.

Materials and methods: By retrospective analysis we have collected the data of all DH, TH patients from the database of the National Consultation Center for Haematopathology in Slovakia during 2008 and 2019. Immunohistochemical double/triple expressors and GCB/non-GCB subtypes were evaluated according to the latest WHO classification guidelines with application of IHC Hans algorithm.

Results: Total of 2,463 DLBCLs were diagnosed from which 70 cases were verified as DH, TH by FISH assays. Most of DH, TH (n=61) were concurrently double or triple expressors by IHC. Of the total 70 DH, TH, 58 were classified into GCB and 10 into non-GCB subtype.

Therefore by preferential testing of double/triple expressor DLBCLs for genetic rearrangements we would be able to detect just 87% of DH, TH and by preferential testing of just GCB DLBCLs the number would be even smaller preserving roughly the similar number of DLBCLs required to undergo FISH examination.

Conclusion: Despite some overlapping between FISH and IHC results, none of the selection method copies sufficiently changes happening at the genetic level in terms of double/triple gene rearrangements. That is why testing all DLBCLs seems to be the most reliable method to capture this group of highly aggressive DH, TH lymphomas which require using different therapeutic approaches.

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**DIFFERENCES IN ROS-SCAVENGERS AND
OSMOPROTECTANTS PATTERNS IN *ARABIDOPSIS
THALIANA* PLANTS WITH VARIABLE TOCOPHEROLS
COMPOSITION GROWING UNDER SALT STRESS**

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Tocopherols (TCs, α - and γ -forms of vitamin E) are important elements of plant protection against plethora of environmental stresses. TCs are linked by mutual dependencies and metabolic loops with different biosynthetic/metabolic pathways e.g. shikimate/chorismate, 2-C-methyl-D-erythritol 4-phosphate (MEP) and benzenoid-phenylpropanoid pathways. In presented study, TCs role is examined in *Arabidopsis thaliana* Columbia ecotype (WT), as well as in mutant *vte1* deficient in α - and γ -TCs, mutant *vte4* over-accumulating γ -TC and transgenic line *tmt* over-accumulating α -TC; exposed to salinity stress (200 mM NaCl) and low light condition.

The results indicate that α - and γ -TCs differentially affect accumulation patterns of non-enzymatic antioxidants (carotenoids like β -carotene, violaxanthin (Vx), antheraxanthin (Ax) and zeaxanthin or phenolic acids) in control and salt stress conditions. Under salinity β -carotene amount is lower in all plants with the altered α -/ γ -TCs content when compared to the WT plants. Parallely, the rate of Vx de-epoxidation is higher in mutants with lack or low level of γ -TC (*vte1* and *tmt*). On the contrary, mutants over-accumulating γ -TC (*vte4*) show lower Vx de-epoxidation. Additionally, the highest level of Ax accumulation, is observed in salt-treated *vte1*. The lowest level of zeaxanthin (Vx de-epoxidation product) is observed in *vte4* and in *vte1* under salt stress, which is caused respectively by the lowest rate of Vx de-epoxidation and

higher Ax accumulation. These results clearly show the relation between TCs and kinetics of Vx and Ax de-epoxidation under salt stress. Moreover, chemical modification of C=C, C-C bonds in selected molecules is confirmed by Raman spectroscopy. Further, proline content in plants with altered TCs composition is higher than in the WT plants, and its highest amount is detected in mutants with lack of both TC forms (*vte1*). At the same time, plants with α -TC deficiency show at least tendency to the reduced proline betaine and glycine betaine amount when compared to α -Tcaccumulating plants. Summarizing, α - and γ -TCs differentially impact on the accumulation of antioxidative and osmoprotective compounds what probably modifies ROS- and osmotic-dependent signal networks. TCs composition influences also on carbon and nitrogen metabolism.

UTILIZATION OF MEDICINES AND ENVIRONMENTAL PROTECTION

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Drug intended for use in humans and animals has a specific expiry date, therefore their disposal is a constant problem. As part of the legal regulations on the disposal of medical waste, which include drugs, it happens that not only expired, badly stored or transported drugs need to be disposed of, as well as drugs with the correct expiry date, which cannot be used in subsequent patients. This group includes, among others cytostatic drugs used in oncology that are not fully used for a given patient in chemotherapy and therefore, despite the appropriate expiration date, they should be disposed of simultaneously with the equipment used for their administration. Drugs that are questioned due to the finding of quality defects and withdrawn by the Pharmaceutical Inspection can also be disposed of. Unfortunately, there are no regulations in the Pharmaceutical Law regarding the supervision of companies storing drugs intended for destruction, as well as those carrying out the disposal process itself.

Another problem that has been identified for many years is the presence of active pharmaceutical substances (APIs), drugs and new psychoactive substances (NPS) and their metabolites in municipal wastewater. Some of them are recalcitrant and remain in the environment for many years. Such studies, coordinated by the EMCDDA (European Monitoring Center for Drugs and Drug Addiction), have been systematically

performed in Europe since 2011 for four selected substances [1]. In Poland, such tests are performed only in Kraków - in 2019, the following concentrations were found in the Vistula River: Cocaine 46.6 mg/1000p/day; amphetamines 65.9 mg/1000p/day; methamphetamine 2.7 mg/ 1000p/ day; MDMA 26.8 mg/ 1000p/ day.

An additional threat to the environment is the growing presence of counterfeit drugs and dietary supplements in the illegal and legal distribution network, the composition of which never complies with the declaration, and which also contain unknown organic and inorganic pollutants as well as residues of toxic solvents and catalysts.

The most reliable method of assessing the size of the legal and illegal market for selected drugs, or rather active substances, is the study of wastewater (API metabolites) from a selected city / administrative area and the comparison of the obtained quantities with legal sales that took place at a specific time. The Dutch National Institute for Public Health and the Environment (RIVM) conducted such studies for sildenafil in 2013 and showed that up to three times more sildenafil came from illegal sources than was sold in the legal distribution network as Viagra [2].

Regardless of the way drugs and their metabolites get into the environment, there is a need for their disposal, for which biotechnological methods are increasingly used, thanks to which even such durable drugs as, for example, sulfonamides can be broken down. [3]

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CHEMICAL DERIVATIZATION WITH POLYCYCLIC AROMATICS: DEVELOPMENT OF REAGENTS AND METHODS

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The modern analytical methods are able to detect and quantify various organic substances at very low concentrations. However, chemical derivatization can push these limits even further, by altering the physicochemical and spectral properties of the analyte. Polycyclic aromatic scaffolds, like fluorene, naphthalene and others, increase the lipophilicity of the analyte and are both chromo- and fluorophores. These virtues make them great derivatizing agents for reversed-phase HPLC analyses. Introduction of these moieties in the derivatization process makes polar non-absorbing molecules easy to analyze even with simplest UV-VIS detection.

Within this contribution, we wish to present several examples of chemical derivatization with polycyclic aromatics like fluorenyl or naphthyl, where the physicochemical and spectral properties of the derivatizing agent are used to enable efficient RP-HPLC analysis as well as sensitive UV-VIS detection. The applications include stereochemical studies [1], optical purity determination and RP-HPLC analysis of drugs and biomolecules. In terms of the chemistry employed to derivatize the analytes, we used both the „traditional” amide and ester derivatization and the *Click chemistry* approach developed in the 21st century, including the copper-catalyzed azide-alkyne cycloaddition and maleimide chemistry. We show an easy way to obtain an alkyne fluorescent probe based on the fmoc-chloride [2] and demonstrate its applications.

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EDIBLE MEDICINAL MUSHROOMS SOURCE OF ORGANIC COMPOUNDS AND BIOELEMENTS WITH ANTIOXIDANT ACTIVITY

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There are about 3000 species of edible mushrooms, and many of them have a high amount of substances that are beneficial to human health, such as antioxidants. It is well known that oxidative stress plays an important role in the etiopathogenesis of many diseases, including cancer, cardiovascular disorders, and diseases of the central nervous system. One way to prevent homeostasis disorders that occur as a result of excessive production of pro-oxidative substances is to include the ingredients having antioxidant properties in the diet. Several compounds, such as those with phenolic and indole derivatives as well as carotenoids and some vitamins, exhibit antioxidant activity. These substances are present in many foods, including edible mushrooms. Mushrooms are a better source of antioxidants compared to the foods of animal or plant origin. In addition, they have certain unique compounds that are not found in other sources (e.g. norbadiene A).

Mushrooms are a rich, but not very popular, source of antioxidants, yet. Due to their high content of antioxidant organic compounds and bioelements, many mushroom species can be considered as functional foods. Moreover, they are cholesterol-free and contain organic substances such as tocopherols and carotenoids which help in protecting against the development of atherosclerotic lesions. Therefore, mushrooms can be treated as superfoods, which refer to unprocessed or low-processed foods having beneficial effects on human health and reduce the risk of a num-

ber of diseases. It is also worth noting that enrichment of medium and substrates used for mushroom cultivation–enhances the level of the substances possessing antioxidant activity.

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UNINTENDED DOPING AND ANTI-DOPING OVER-THE-COUNTER (OTC) MEDICATIONS

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Some over-the-counter (OTC) medications can be used as a doping substance because they improve the psychomotor condition of a human. It should also be noted that some OTC drugs are included in the Prohibited List of agents recognized by the World Anti-Doping Agency (WADA) as doping in sport.

An example is dehydroepiandrosterone (DHEA), which is classified in the S1 category (Anabolic Agents) and pseudoephedrine and n-kethamide, which is included in the S6 category (Stimulants). Phenylephrine and nicotine are covered a Monitoring Program. The uncontrolled use of OTC medications containing codeine predisposes to the detection of morphine (S7 category Narcotics) during doping control. Some OTC drugs have been included on the list of doping agents in the past, e.g. sildenafil or glycerol, and currently they are not the register of prohibited substances. OTC medications in the form of inhalation (nasal spray) are available for sale online, which contain glucocorticoids (GCs) used in the relief of subsequent allergies or inflammatory sinuses, and include, for example, budesonide or fluticasone. Selected OTC drugs may have an effect on physical performance, but are not considered doping agents due to insufficient scientific evidence.

Certain OTC drugs can also have the opposite effect on the human body, as anti-doping, i.e. having a negative effect on the human psychomotor performance, making physical activity difficult or impossible. Examples include I generation antihistamines drugs or herbal medicines that exhibit a sedative effect. The excessive or long-term application of non-steroidal anti-inflammatory drugs (NSAIDs), predisposes to the development of acute paroxysmal abdominal pain, which may be spastic (colic). Stomach pain sounds trivially, but it can disrupt or prevent train-

ing or competing in tournament. OTC laxatives drugs can be used not only for weight control, also as sphincter-fake anti-doping agents. Scopolamine (hyoscine) can be used for the same purpose.

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PRESENTATION OF THE EXHIBITION IN THE MUSEUM OF PHARMACY JAGIELLONIAN UNIVERSITY MEDICAL COLLEGE IN KRAKÓW

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Museum of Pharmacy at Jagiellonian University Medical College in Kraków was created in 1946 at the District Chamber of Apothecaries. Since 1961 museum is a part of the Pharmaceutical Faculty of University, which means that apart from typical museum's activity it is a scientific and didactic unit of Faculty for the History of Pharmacy. The museum's collections are on display throughout all premises of historical, 14th century building located at Floriańska 25 street, in 12 exhibition rooms, taking up all Gothic cellars, then spread out through the rooms on all three floors, and ending up right at the attic (stylized as the apothecary attic used for drying herbs). Those comprise a variety of pharmacy furnishing in different styles (Baroque, Empire, Biedermeier), pharmaceutical vessels from different eras, including a vast collection of majolica from various European manufactures, old medicinal ingredients of vegetable, mineral and animal origin (including human ones, e.g. fat), original emblems (coats of arms) of various pharmacies, pharmacy-themed stained glass windows, old pharmacy utensils (mortars, presses, filters, pill-making machines, dragee-making drums, herb slicers and grinders), old prints (e.g. Greek-Latin edition of the complete works of Hippocrates, medieval and early modern pharmacy textbooks, official and unofficial listings of medications, herbal books, handwritten manuals). The Museum also accommodates a thematically-oriented exhibition entitled: „What is left of ancient Kraków's pharmacies”. And finally, a collection of modern paintings clearly inspired by the Museum's prized artefacts.

One of the unusual museum's exhibits are antimonial laxative pills of multiple use, wellknown in 18th and 19th century medicine, which can be considered as an environmentally friendly medication.



TUBERCULOSIS AMONG THE HOMELESS IN CRACOW

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Homelessness is a widely perceived social problem, both in the sociological and psychological aspect. The multispectivity of this phenomenon stems from a number of causes, leading to homelessness. This is a relatively permanent situation of a human being without his/her own dwelling or altogether deprived of it. Among this group, the persons can be distinguished being compellingly or voluntarily homeless. The social problem of unemployment arises, which bars them from benefitting from medical services to which normally the insured persons are entitled. The Doctors of Hope Association, running the Clinic for the Poor and Homeless at Smoleńsk Street in Cracow, strives to fulfill its idea of decent treatment of the excluded people within the framework of medical assistance. The homeless people are much more exposed to civilization diseases, including chronic and infectious ones. The Association is doing its best, undertaking not only medical, but also preventive actions. Last year they resulted in conducting prophylactic examinations in the homeless population, aiming at the detection of tuberculosis infection and prevention of this disease spreading. These examinations were financed by the Centre of Social Assistance in Cracow in two series (July-September and October-December 2019). Several difficulties were encountered in the course of examinations, ensuing from the specificity of this group of the examined patients (their negligence of the fixed test dates, avoidance of medical visits, lack of contact, fake phone numbers, probability of falsified sputum samples, which made the annexing of the first series of tests necessary in order to convert the sputum examination into IGRA tests). Further obstacles included the lack of reliable anamne-

sis, lack of former medical and radiological documentation and lack of national medical benefits in cases requiring further examinations or treatment different from tuberculosis. In view of these difficulties the programme procedures were modified in order to achieve the greatest reliability possible. Within the programme, 60 patients were examined with the respiratory system symptoms or stating their contact with the infected individuals. Not only the patients frequenting the Clinic were examined, but also the nurses conducted the examinations in the St Albert Refuge Centre. All patients with the symptoms or at risk had their chests radiologically examined at the Smoleńsk Street Clinic, while the tests of interferon gamma emission T-SPOT TB were conducted in the Laboratory of Bacillus Bacteriology in Ulanów Street. All the related procedures (samples collection, their transportation for analysis, medical documentation) were supervised by the medical personnel (K. Komnata, A. Kopta, M. Kopciuch) and nursing team (Z. Zachara, K. Sarek). This cooperation resulted in the detection of two tuberculosis cases and one relapse. One case of emphysema and several cases of inflammatory and post-specific changes were ascertained. According to diagnoses, the patients were subjected to hospital treatment or to specific pharmacological procedures. Altogether, more than one hundred medical consultations were effected. It appears that all prophylactic actions aiming at the reduction of incidences within this social group should still enjoy the support of the self-governance organs.

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SESSION OF YOUNG SCIENTISTS

LEVELS OF SELECTED HEAVY METALS IN POLISH BEE POLLEN, ROYAL JELLY, AND PROPOLIS

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Bee products, including bee pollen, royal jelly, and propolis, due to their health-promoting properties, have been used in natural medicine since ancient times. These products contain large amounts of nutrients and biologically active compounds, like proteins, amino acids, carbohydrates, vitamins, phenolics and minerals (1,2). Therefore, they are willingly used as dietary supplements. However, given the natural origins of bee products, they could contain significant heavy metals contaminants (3).

The goal of this study was a qualitative and quantitative analysis of a broad spectrum of heavy metals in bee pollen, royal jelly and propolis, to assess bee exposure to various chemicals and to evaluate the quality of their products. All samples were collected in Poland (Greater Poland Voivodeship). The qualitative analyses were performed with inductively coupled plasma - mass spectrometry (ICP-MS) technique.

In total, levels of 16 trace elements and heavy metals have been determined in the samples. The obtained results revealed significant differences in contaminants contents between selected bee products. In general, bee pollen and propolis were found to be more contaminated than royal jelly. The most visible differences involved levels of aluminium, manganese, cobalt, nickel, cadmium, barium, and lead.

The contaminants involved in bee products may arise both from beekeeping practice and from the environment. The differences in heavy metals content between royal jelly, bee pollen, and propolis may result from the origins of these products – royal jelly is a pure secretion of bees' glands, whereas bee pollen and propolis contain constituents derived directly from plants. The evaluation of the toxic elements' content

of bee products will contribute to improving the safety of their intake. It is particularly important regarding the increasing interest of apitherapy and natural foods.

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LSD-MICRODOSING AS A NEW POTENTIAL STRATEGY IN PSYCHIATRIC ILLNESSES

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Breakthrough approaches to neuroimaging and to nervous system remapping made it possible to discover new connections between neurons and understand their actions. Recent studies used these technologies in order to study LSD effects on cerebral activity.

Discovered accidentally by A. Hoffman in 1938, Lysergic acid diethylamide, commonly known as LSD, is one of the most potent hallucinogens. This psychedelic drug is a chemical derived from *Claviceps purpurea*, an ergot fungus used since ancient times by different cultures during religious ceremonies as a revealing consciousness instrument [1].

The purpose of this review is to highlight how lately the research interest has been focused on potential application of microdose-LSD in the treatment of various psychiatric and alcohol use disorders. As a matter of fact, in recent studies LSD has been shown to mimic the serotonin mechanism by exerting its agonist action on 5-TH_{2a} postsynaptic receptors and on 5-TH_{1a} presynaptic receptors in order to affect the release of such endogenous neurotransmitter. [2-3] For the first time, sophisticated new RX crystallography techniques have allowed to isolate and observe images of LSD molecular structure in the active state and its binding with the 5-TH receptor.

Over the last few years multiple placebo-controlled clinical trials have been carried out on healthy volunteers in order to test the effect of low dosages of LSD (microdosing) on the nervous system. Moreover,

one double-blind, randomized clinical trial has been conducted by proving LSD potential efficacy on anxiety and depression diseases among patients with terminal cancer.

According to the Functional magnetic resonance imaging (fMRI) investigation, the LSD administration promotes functional integrity and new connections between cerebral areas compared to the placebo group [4].

In the future new researches focused on LSD may achieve new pharmacological milestones by offering a valuable alternative to conventional treatments.

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BIOELEMENTS ACCUMULATION BY *AGARICUS BISPORUS* FRUITING BODIES DURING CULTIVATION PROCESS

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Agaricus bisporus, also known as a white button mushroom, is the most common eaten and cultivated edible mushroom in the world. Its popularity is due not only to its taste and aroma but also to rich content of nutrients such as dietary fiber, essential aminoacids or unsaturated fatty acids. *A. bisporus* is rich source of bioelements, in particular selenium, zinc, magnesium, copper, iron, calcium, sodium and potassium [1]. Controlled environment and composition of compost used in commercial cultivation of *A. bisporus* make obtaining fruiting bodies with enhanced nutritional value possible. The aim of the study was to investigate the content of following bioelements: zinc, magnesium, iron, copper, calcium, sodium and potassium in three crops of fruiting bodies of *A. bisporus* from three different times – after 2, 3 and 4 weeks of cultivation on compost and choose which elements is accumulated the most effectively by bioconcentration element factor (BCF) determination. The samples were taken from the surface layer of fruiting bodies. In order to make an output sample, six samples from area of 50 cm² and depth of up to 25 cm was taken. Element analysis was performed using F-ASA after microwave mineralization. The highest BCF was observed in case of zinc, the lowest in case of iron. Gradual increase of BCF value was ob-

served in case of every bioelement which indicates that natural overgrowth of mycelium in the solid medium enhance capability of bioelements accumulation and transportation to fruiting bodies. Fruiting bodies harvested in second crop were characterized by the highest content of bioelements. Results from this study shows that zinc accumulation efficacy is the highest and iron accumulation efficacy is the lowest in *A. bisporus*. The increasing BCF factor with time is related with growth of mycelium and reveal the possibility of bioelements fortification in certain moments of cultivation to obtain fruiting bodies with higher content of elements, thus higher nutritional value in every crop.

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EVALUATION OF LIPOPHILICITY AND ADMET PARAMETERS OF 10-TRIAZOLYLMETHYL-1,8- DIAZAPHENOTHIAZINES

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At the Department of Organic Chemistry of the Medical University of Silesia were synthesized modified diazaphenothiazines with the structure of dipyridthiazines, which showed significant anticancer, immunomodulating, and antioxidant effects [1-3]. The results of these studies inspired the synthesis and evaluation of biological activity the novel dipyridthiazine structures.

The aim of the presented project became evaluation of lipophilicity and ADMET parameters of six novel 10-triazolylmethyl-1,8 diazaphenothiazines. The influence of the structure on the physicochemical properties was determined by theoretical and experimental methods. Reverse phase thin layer chromatography RP TLC was the experimental method. For theoretical methods were used computer programs such as Swisadme, ALOGPS 2.1, Molinspiration and Percepta. The Swisstarget prediction predict the most probable molecular target of testing drugs and the pharmacokinetics parameters were determined in the Percepta.

Varied values of lipophilicity coefficient were obtained in theoretical methods. It was determined that the values parameters *in silico* moderately correlate with the experimental results. The tested compounds showed good pharmacokinetic parameters and their molecular target were also predicted. The potential of the tested compounds to become

good candidates for medicinal substances was found based on the obtained results.

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NEW AZIDOTHYIMIDINE DERIVATIVES OF DIPYRIDOTHIAZINES – SYNTHESIS AND PROPERTIES

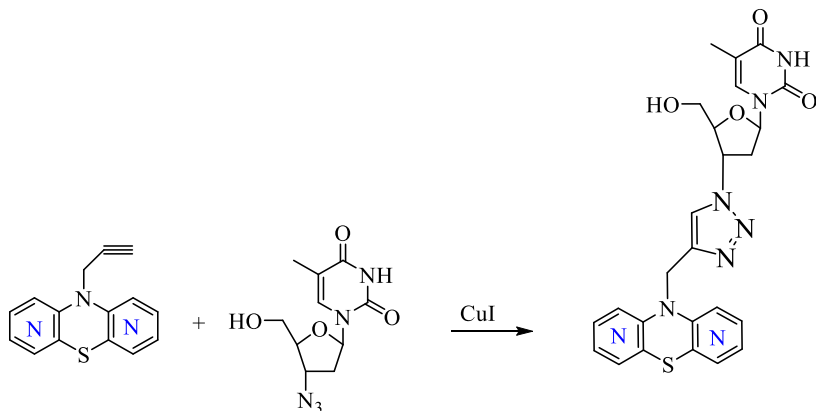
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Azidothymidine was primarily used only for the treatment of HIV. However, it also showed antibacterial and anticancer activity as it was described in the literature. The aromatic, heterocyclic 1,2,3-triazole system is present in the structure of some drugs used until now. It has wide range of biological properties such as: antibacterial, antiprotozoal, anticancer and antiviral proven in regularly updated scientific literature [1-3]. Tricyclic phenothiazines have been used for many years as neuroleptics for the treatment of schizophrenia. In recent years, significant publications have also proved their significant anticancer, antibacterial, immunomodulatory or reversing activity [4,5].

The aim of this scientific project was the synthesis of 1,6-, 1,8-, 2,7- and 3,6-diazaphenothiazines with a 1,2,3-triazole system and thymidine substituent, as well as an analysis of their structural and biological properties *in silico* using the PASS and PreADMET programs.



The structure of the newly obtained compounds was confirmed using ^1H NMR and HR MS spectroscopic and chromatographic methods. The *in silico* analysis of biological activities indicated a high pharmacological potential of the new derivatives obtained for anti-tumor and anti-proliferative activity.

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COMPARISON THE ACTION OF SELECTED AIR POLLUTION COMPONENTS ON ATHEROSCLEROSIS DEVELOPMENT IN APOE-KNOCKOUT MICE

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Several lines of evidence shows that air pollution is linked to numerous cardiovascular disorders, including atherosclerosis. Particulate matter (PM), the important component of air pollution, displays ability to induce inflammation and oxidative stress and thus can impair endothelial cells function and contribute to the atherosclerotic plaque development. Toxic action of PM particles strongly depends on their physicochemical properties, such as chemical composition, size and solubility. PM contains variety of organic and inorganic substances. Among inorganic components can be distinguished ions and metals, which among others are involved in reactive oxygen species generation [1,2]. The role of particulate matter, especially its inorganic parts, in the development of atherosclerosis is still under consideration. The aim of this study was to compare the effect of particulate matter and nanoparticles on atherosclerosis progression in apoE-knockout mice. Standard, commercially available particulate matter mixtures (NIST and LAP) and selected metal oxides in the form of nanoparticles (SiO_2 , Fe_2O_3), were used as the main components of inorganic parts of PMs. The animals were exposed to pollutants by inhalation in whole body exposition chamber. Histological, immune-histochemical and molecular methods were used to estimate the influence of treatment on the development of atherosclerosis. Our results show that only SiO_2 nanoparticles significantly increased the area of atherosclerotic plaque in mice. However, some PMs and nanoparticles exerted an influence on the plaque structure (e.g. content of macrophages, smooth muscle cells and necrotic cores size). Such changes could be at least partly attributed to the influence of PMs/nanoparticles on the bal-

ance of M1/M2 phenotype of macrophages within the plaques, however exact mechanisms explaining action of particulate pollutants on atherogenesis require further investigations.

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ANALGESIC, ANTIDEPRESSANT-LIKE AND ANXIOLYTIC-LIKE PROPERTIES OF ROPINIROLE IN THE RESERPINE-INDUCED FIBROMYALGIA-LIKE MODEL IN MICE

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Introduction: Fibromyalgia (FM) affects around 2-4% of the total population of people worldwide, with the prevalence 10 times more common among women. The etiology of this condition is not known until now, with factors such as stress, other illnesses, dysfunction of central regulation of pain and co-occurring pain in some patients. These symptoms are also accompanied by neuroendocrine disorders and fluctuations in the concentration of neurotransmitters. These include: reduced biogenic amine levels, increased levels of neurotransmitter stimulants including substance P, and hypothalamic-pituitary-adrenal axis [1,2]. In general, the definition of "central sensitization" associated with the development of FM is also common [3]. FM is the second most common diagnosis in clinics of rheumatology [4].

Materials: In order to develop FM-like symptoms in mice, the animals were injected with reserpine at a dose 0.25 mg/kg subcutaneously during three consecutive days. Ropinirole was administered intraperitoneally on the 4th day at two doses: 5 and 10 mg/kg. Behavioral tests were performed 60 min after drug injection. To assess the influence of the test drug on tactile allodynia and heat hyperalgesia, the pain sensitivity threshold of experimental animals was assessed using the von Frey test and the hot plate test, respectively. Additionally, to assess the effect of ropinirole on comorbid symptoms in the reserpine-induced FM-like model, additional tests were performed to assess potential antidepressant activity – forced-swim test, and potential anxiolytic effect – four-plate test. The effect of ropinirole on locomotor activity and muscle strength was also assessed.

Results: Ropinirole showed an interesting and potent analgesic activity in von Frey test at a dose of 10 mg/kg. In addition, this drug showed a statistically significant antidepressant-like effect at two tested doses (5 and 10 mg/kg) in forced-swim test without without a negative effect on muscle strength and locomotor activity of mice.

Conclusions: Ropinirole seems to be an interesting option to attenuate FM-like symptoms.

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REGULATION OF ESTROGEN RECEPTORS AND NF- κ B IN METHYLPARABEN-STIMULATED HUMAN NEUTROPHILS

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Since parabens began used as preservatives of personal care products, foodstuff, pharmaceuticals, and cosmetics, these compounds become a ubiquitous element of the human environment. Parabens, including the most commonly used – methylparaben (MeP) – was regarded as xenoestrogens – compounds which mimic the action of estrogens. One of the ways of its action is modulation of estrogen receptor α/β (ER α/β)-signaling. Moreover, literature data suggested that activated ERs may modulate the expression of p65 NF- κ B.

This study aimed to assess MeP impact on ERs and NF- κ B, as well as verify the hypothesis about modulation of p65 NF- κ B via ERs in MeP-exposed human neutrophils.

Neutrophils were isolated from heparinized blood donated by 15 healthy male volunteer donors. To assess the involvement of ERs and NF- κ B in MeP-induced signaling, neutrophils were 1-hour pre-incubated with ERs inhibitor – ICI 182.780 (1 μ M) or NF- κ B inhibitor – PDTC (100 μ M); next, the MeP (0.06 μ M) was added for 24 hours. After isolation of cytoplasmic and nuclear fraction the expression of ER α , ER β , p65 NF- κ B, β -Actin and PARP-1 was measured by Western blot. The intensity of bands was measured; the results were analysed in Statistica 13.3.

Expression of ER α was decreased in cytoplasmic and nuclear fraction of neutrophils incubated with MeP or MeP and inhibitors. Addition of ERs inhibitor into MeP-stimulated cells decreases cytoplasmatic expression of ER β in compare with non-stimulated or MeP-treated neutrophils. ER β expression increases in nucleus of cells simultaneously incubated with MeP and inhibitors in compare with non-stimulated or only

MeP-stimulated neutrophils. 24-hour incubation with MeP or MeP with PDTC leads to increased expression of p65 NF- κ B in cytoplasmic fraction. MeP-induced expression of p65 NF- κ B was decreased in cytoplasm of cells incubated with ERs inhibitor. Expression of p65 NF- κ B in nuclear fraction of neutrophils incubated with MeP and PDTC was lower than in cells incubated only with MeP.

Based on our results, we suggest that MeP induce signal transduction via ER α , which may downregulate p65 NF- κ B in human neutrophils.

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SYNTHESIS AND INVESTIGATION OF PINANE-BASED CHIRAL TRIDENTATE LIGANDS IN THE ASYMMETRIC CATALYTIC REACTIONS

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The synthesis of chiral aminoalcohols and aminodiols has widely been exploited due to their variety of applications. Their chelating ability made them an important role in asymmetric catalysis. In the recent decade monoterpenes have been proven a very interesting and cheap source for the stereoselective synthesis of chiral aminoalcohols [1,2].

Key starting material 3-methylenopinone was prepared in 2 steps from commercially available (-)- β -pinene applying literature method.[3] The stereoselective reduction of the ketone function in presence of CeCl_3 as chelating agent afforded the key intermediate pinane-based allylic alcohol. Stereoselective epoxidation of allylic function followed by aminolysis of the resulting oxirane ring served aminodiol library.

O-benzylation of the allylic alcohol and similar transformation as above provided the bifunctional aminoalcohol library. While the ring closure of aminodiol towards 1,3-heterocycles was also studied.

The stereochemistry of the amino alcohols/diols was determined by 2D-NMR techniques (COSY, NOESY, HSQC and HMBC), and all compounds were tested as ligands for chiral addition of diethylzinc to aldehydes.

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OTHER ABSTRACTS

**PHOTOCATALYTIC DEGRADATION OF
SULFAMETHOXAZOLE USING A FLOATING TiO₂
– EXPANDED PERLITE PHOTOCATALYST**

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Sulfonamides are the oldest group of antibiotics used in human and veterinary medicine. They are polar, amphoteric, photo- and thermally stable substances that are readily soluble in water. For this reason, they possess high migration ability in the environment. The most commonly employed of this group of antibiotics is sulfamethoxazole (SMX). It is extensively used in human and veterinary medicine. It is particularly often used in bronchitis and in the treatment of urinary tract infections as well as is used as an antibacterial growth promoter. However, one of the most important negative consequences of the antibiotic pollution is an increased microbial resistance.

The aim of the presented studies was the photocatalytic degradation of SMX employing a novel floating photocatalyst composed of TiO₂ immobilized on expanded perlite (EP). We have checked that the EP-TiO₂-773 photocatalyst can be potentially used for SMX abatement as it markedly enhances SMX photodegradation in the aqueous medium in the wide range of pH values on irradiation with light of low energy (3.2 eV) from near UV spectral region. Kinetics of the process at different pH values were determined and photodegradation products at pH 5.1 were identified based on UPLC-MS/MS analyses. The mechanism of the photocatalyzed degradation indicates that the hydroxyl radicals are the main players in that process. The products of SMX hydroxylation and hydrolysis are the primary compounds formed during that process. We have found the formation of different photoproducts for SMX degrada-

tion using this photocatalyst than those described previously [1]. The results indicate that the process has the potential to be applied for facile removal of antibiotic pollutants from shallow water reservoirs. Taking into account the ability of the studied photocatalyst to float and consequently the possibility of its multiple use, its application is expected to be more economical than that of TiO₂ in suspension.

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PHOTOTOXICITY EVALUATION OF CEFUROXIME AXETIL PHOTODEGRADATION PRODUCTS USING THAMNOTOXKIT F

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Numerous pharmaceuticals have been detected in natural water reservoirs (rivers, lakes, regional discharges). The proliferation of β -lactam antibiotics, including cephalosporins, has had a huge impact on human health, allowing for the rapid treatment of patients with bacterial infections. The presence of antibiotics in aqueous environments is becoming a major worldwide concern, as they increase bacterial resistance to drugs. In environmental waters drugs can undergo various degradation paths, including biotic (biodegradation, bioaccumulation) and abiotic (hydrolysis, photolysis, sorption, oxidation) degradation.

Toxicity tests compare the response of an organism exposed to a specific chemical at various concentrations to the response of the same organisms unexposed to the chemical. The main goal of the presented work was the analysis of phototoxicity of cefuroxime axetil photodegradation products in solutions with the use of Thamnotoxkit F.

Thamnotoxkit F is a survival test for crustacean *Thamnocephalus platyurus*; the test reaction is his death (Creasel, Deinze, Belgium). A 24-hour LC50 bioassay was performed in a multivell test plate using larvae that had newly hatched from the cysts. The major advantage of the used microbiotest is that the test organisms are delivered as dormant or immobilized organisms that can be hatched or activated prior to the performance of the toxicity tests. This fact eliminates the need for the continual cultivation of test organisms. The used *Thamnocephalus platyurus* test was approved by the International Standardization Organization (ISO) as an acute toxicity test (published as ISO/14380 "Water quality-

Determination of the acute toxicity of *Thamnocephalus platyurus*” (Crustacea, Anostraca)).

Irradiation of samples containing cefuroxime axetil was performed in the sunlight simulator: SUNTEST CPS+(Atlas) with 1500 W xenon lamp emitted light of the UV–Vis (300–800 nm), radiation intensity was 750 W/cm². The temperature was about 37°C. Samples were irradiated from 1 to 5 h. To choose the optimal exposure time the kinetics of cefuroxime axetil photodegradation in solution was estimated. Concentration before and after light exposure was measured with the UPLC-MS/MS system.

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QUANTITATIVE ANALYSIS OF 2-HYDROXYGLUTARATE IN MALIGNANT GLIOMAS

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Genetic mutation analyses were performed from tumor tissue samples of glioma patients WHO grade I–IV (astrocytoma, oligodendroglioma, secondary glioblastoma and glioblastoma multiforme) with possibility of genetic mutation on isocitrate dehydrogenase (IDH) 1 or 2. They were performed by isolation of DNA directly from cancer tissue, PCR amplification and subsequent Sanger sequencing. Concurrently concentrations of both isomers D- and L- of 2-hydroxyglutarate (2-HG), as resultant oncometabolite of IDH mutations, of all (mutated and unmutated) samples were quantified *in vitro*. The method was implemented on and the concentrations were determined by HPLC-HRMS instrumentation using Agilent HPLC and Bruker Q-TOF high resolution mass spectrometer setup. This was to confirm the hypothesis of the correlation of the results of mutation analyses and the 2-HG *in vitro* concentrations in tumor tissues, plasma and urine.

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UPLC AS A MODERN ANALYTICAL TECHNIQUE IN PHARMACY

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UPLC (ultra-performance liquid chromatography) is a relatively young analytical method. It is a variant of liquid chromatography that uses columns with particles smaller than 2 μm . It is a modern modification of HPLC (high pressure liquid chromatography). The evolution and improvement of the method was possible thanks to the analysis of the Van Deemter equation. According to this equation, efficiency and resolution increase as the packing particle size decreases. For particles of this size, the performance does not change as the line velocity or flow rate is increased. Smaller particle sizes increase back pressure. Therefore, this method requires a higher eluent pressure. The main advantages of the UPLC technique are: reduction of analysis time, increased sensitivity and resolution. Due to the better separation of the peaks, the accuracy of the determinations was significantly improved. It should be noted that by reducing the time, the costs of the analysis are substantially smaller, through more efficient use of equipment and reduced solvent consumption. At the same time, the increased efficiency of the system allows for obtaining more information (the appearance of new peaks that were not found under the conditions of HPLC analyzes).

The main fields of UPLC applications are: chemistry, pharmacy, foodstuffs, biochemistry and chemistry of compounds used in heavy industry. These systems are also important research and production tools. Without UPLC, it would still be difficult, for example, to check the purity of manufactured drugs, to detect the presence of performance-enhancing drugs in samples supplied by athletes, or in the food industry to determine the concentration of vitamins in juices. They are also used to analyze the components of compounds, to separate and identify amino

acids, nucleic acids, proteins, hydrocarbons, pesticides, carbohydrates, antibiotics, steroids, and many other organic and inorganic substances.

The UPLC method is the most frequently developed analytical method for drug identification. A large group of researchers made an attempt to modify and switch from the well-used HPLC method to the new, much faster UPLC. Our goal was to collect published studies using the UPLC analytical method to identify drug substances. We focused on drugs commonly used in medicine, represented various pharmacological groups. Different methods of sample detection were used (most often spectrophotometry and mass spectrometry). Often the same drug has been tested under different conditions by other researchers as well as from various source material.

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ASSESSMENT OF ZINC CONTENT IN SELECTED PREPARATIONS TOPICALLY APPLIED TO SKIN

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Compounds of zinc are commonly used in dermatological and cosmetic products. Their popularity is related to the multifunctional profile of Zn²⁺: anti-inflammatory, anti-pigmentation, antioxidant, anti-ageing, anti-acne, antimicrobial, anti-odour, cleansing or stabilizing activity. The main inorganic and organometallic zinc salts as used in cosmetic formulations are: zinc glycinate, zinc sulphate, zinc gluconate, zinc chloride, zinc lactate. Furthermore, zinc oxide, a wide-band gap semiconductor, is also widely available in the skin care formulation. Most sunscreens contain inorganic UV filter - zinc oxide, that effectively absorbs in UVA-UVB range. The aim of the research was to determine the content of Zn(II) in selected preparations declared its presence. Flame Atomic Absorption Spectrometry was used to evaluate the content of investigated element. According to the manufacturers' declarations, zinc was detected in each of the eight preparations. The obtained values are in line with the standards applied by the European Commission. All of preparations conform European Union standards.

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BIOINDICATING TOXICITY TESTS

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Bioindication is a way of using a living organisms, the reaction of which can be the basis for the valuation of the total biological activity of the tested system, and allows to summarize the toxicity of all detrimental substances. The used organisms vary in their sensibility to miscellaneous toxic matter. Therefore, bioindication batteries belonging to different biological groups are used in the bioindicator investigation. A living organism is a type of environment inside which various processes take place, resulting in certain morphological changes or death. International organizations recommend the use of 4 types of bioindicators: crustaceans (*Daphnia magna* or *Daphnia pulex*), various species of fish, algae (*Scenedesmus algae subspicatus*, *Selenastrum capricornutum*), bacteria (*Pseudomonas putida* and *Vibrio fischeri*). Polish Standards recommending similar bioindicators as in international standards like algae (*Chlorella* sp.), crustaceans (*Daphnia magna* and *Gammarus varsoviensis*) and fish (*Lebistes reticulatus*).

Here, we presented tests used in our studies about potential toxicity analysis of cephalosporins photodegradation products.

Daphtoxkit FTM magna, using the *Daphnia magna* crustacean, is the standarddized toxicity test, according to OECD recommendations. This test is carried out on freshly hatched daphnia that survived the contact with the analyzed environment. The procedure makes it possible to eliminate the continuous cultivation of test organisms. The test reaction is the inhibition of crustacean movement (immobilization) observed after 24 and 48 hours of incubation.

Thamnotoxkit FTM uses the crustacean *Thamnocephalus platyurus*. The test reaction is the death of the crustacean, which in many cases is

more sensitive to the presence of toxic substances than the standard *Daphnia* test. The test complies with ISO 14380.

LumiMARA is a multi-species test using 11 bioluminescent strains (9 saltwater and 2 freshwater bacteria). Toxicity is determined by measuring the degree of decrease in light intensity. When the tested bacteria are exposed to a toxic environment, the luminescence decreases. LumiMARA is a "one test battery" and can be used to evaluate the toxicity of both water and soil samples.

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CHARACTERISTICS AND APPLICATIONS OF CYCLODEXTRIN COMPLEXES

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Cyclodextrins are cyclic oligosaccharides linked by α -1,4-glycosidic bonds. Among the natural cyclodextrins we can distinguish α -, β - and γ -cyclodextrin. However, derivatives are created by introducing various functional groups into natural cyclodextrin ring in order to improve their physicochemical properties. Due to this property, it is possible to create inclusion complexes in various stoichiometric ratios. Thanks to their hydrophilic outer surface, they are well soluble in water, while the interior space is hydrophobic and can contain another hydrophobic substance.

In the presented work we discussed some common method of complexation with cyclodextrins and their potential impact on physicochemical properties of tested compounds.

Depending on the type of cyclodextrin and the complexed substance, we can differentiate several methods of complex formation, such as: kneading method, co-precipitation method, lyophilization etc. The obtained complexes are tested using various techniques. In this way, for example inclusion complexes can be detected or molecular interactions between guest and host can be studied.

Cyclodextrins are widely used in the pharmaceutical, cosmetic and even food industries. By using these cyclic oligosaccharides in complexes with antibiotics, it is possible to increase their antibacterial activity and improve the production of antibiotics. Cyclodextrins increase the stability and solubility of poorly water soluble substances. They reduce the unpleasant, bitter taste of substances such as diltiazem or ranitidine. In addition, they can increase the thermal stability of drugs. Therefore they can be used as auxiliary substances in various drugs to ensure their optimal performance. In cosmetic products, cyclodextrins are often used

to eliminate unpleasant odors, minimize skin irritation as well as to improve the solubility and stability of the active ingredient. Cyclodextrins have the ability to form complexes with cholesterol and therefore they can be used in the food industry for the production of products with a reduced cholesterol content. By creating complexes with pesticides, we can significantly reduce their harmful impact on the environment.

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DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF VALSARTAN AND PRAVASTATIN IN MIXTURES AND PHARMACEUTICAL DOSAGE FORMS.

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Fast, accurate and precise method for the determination of valsartan and pravastatin was developed using derivative spectrophotometry. After analysis of derivative curves registered for the standard solutions it was found that quantitative analysis can be carried out by using first order (D1) and third order (D3) derivatives. For second order derivative (D2) and the "zero-crossing" technique in the case of valsartan it was not possible to determine the wavelength, which would have provided good validation parameters and hence the appropriate precision and accuracy of determination.

It was shown, that the developed method allows for the determination of the tested components in a direct manner, despite the apparent interference of the absorption spectra in the UV range. For quantitative determinations appropriate wavelengths for valsartan were: D1 $\lambda=235.85$ nm and D3 $\lambda=246.28$ nm. Pravastatin was determined at wavelengths: D1 $\lambda=249.29$ nm and D3 $\lambda=239.90$ nm.

The developed method was characterized by high sensitivity and accuracy, for valsartan LOD was $0.42 \mu\text{g}\times\text{mL}^{-1}$ and $0.90 \mu\text{g}\times\text{mL}^{-1}$ for D1 and D3 derivatives, respectively and for pravastatin 2.34 and $1.20 \mu\text{g}\times\text{mL}^{-1}$, respectively.

LOQ values determined for valsartan were 1.28 and $2.70 \mu\text{g}\times\text{mL}^{-1}$ (for D1 and D3 derivatives) and for pravastatin 7.09 and $3.65 \mu\text{g}\times\text{mL}^{-1}$, respectively. The recovery of individual components was within the range of $100\% \pm 5\%$. The linearity range was wide and estimated between $9.40 \mu\text{g}\times\text{mL}^{-1}$ and $28.20 \mu\text{g}\times\text{mL}^{-1}$ for valsartan and between $9.60 \mu\text{g}\times\text{mL}^{-1}$ and $48.00 \mu\text{g}\times\text{mL}^{-1}$ for pravastatin.

IS MICROEMULSION THIN LAYER CHROMATOGRAPHY (METLC) A SUITABLE TOOL TO STUDY THE BIOCONCENTRATION FACTOR OF PHARMACEUTICALS?

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The bioconcentration factor (BCF) is defined as the uptake of contaminants from the dissolved phase; it can be calculated using the equation (1):

$$\log \text{BCF} = \log (C/C_w) \quad (1)$$

where C - the equilibrium contaminant concentration in the organisms ($\mu\text{g}/\text{kg}$); C_w - the equilibrium contaminant concentration in the surrounding water ($\mu\text{g}/\text{L}$).

In the course of this study the bioconcentration factor was obtained for the thiepino[3,2-c:6,7-c']dipyridine and 16 benzothiepino [3,2-c]pyridine derivatives *in silico* using three different models:

- $\log \text{BCF}^{(1)}$ - regression-based model – using EpiSuite software [1]
- $\log \text{BCF}^{(2)}$ - Arnot-Gobas model - using EpiSuite software [1]
- $\log \text{BCF}_E$ - using the molecular weight and energy-based equation (2) [2]:

$$\text{BCF}_E = 0.00250227 M_w - 0.0723952 E_t - 0.21352 E_{\text{HOMO}} - 0.892481 E_{\text{LUMO}} - 2.58291 \quad (2)$$

Energies (E_t [Hartree] E_{HOMO} [kcal/mol] and E_{LUMO} [kcal/mol]) were calculated using HyperChem v. 8.0.

The METLC descriptor was R_M , calculated according to the well-known equation (3) [3]:

$$R_M = \log (1/R_f - 1) \quad (3)$$

R_f values were taken from Ref. [4]. METLC was performed on RP-18W stationary phase, with microemulsion mobile phases A and B:

A: *n*-heptane (2.8 mL) + butan-1-ol (19 mL) + water (75 mL) + sodium lauryl sulfate (7.7 g)

B: *n*-heptane (2.8 mL) + butan-1-ol (24 mL) + water (70 mL) + sodium lauryl sulfate (7.7 g).

The relationships between log BCF and R_M were linear, of moderate quality ($R^2 = 0.45 \div 0.69$; mobile phase B gave better results than A).

To conclude, METLC may be used to study the bioconcentration factor of pharmaceuticals, but the R_M parameter obtained in the above-described conditions is not suitable as a sole predictor of BCF – it is likely to be useful in combination with other, easily calculated physico-chemical descriptors.

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**INVESTIGATIONS ON PHENOLIC ACIDS ACCUMULATION
IN RED ARONIA (*ARONIA ARBUTIFOLIA*) IN VITRO
CULTURE DURING THE GROWTH CYCLES AND
ANTIOXIDANT ACTIVITY OF BIOMASS EXTRACTS**

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Red aronia is the plant species with North-American origin, experimentally cultured in Poland. Our phytochemical studies documented the presence of high amounts of different-subgroups of antioxidants (phenolic acids, anthocyanins and flavonoids) in fruits and leaves of the plant.

Stationary agar and agitated shoot cultures of red aronia established earlier in our laboratory produced high amounts of phenolic acids [1].

The aim of present study was establishment of agitated shoot cultures and investigations on dynamics of phenolic acids accumulation and antioxidant activity of biomass extracts during growth cycles.

The shoot cultures were maintained on the Murashige and Skoog medium [2] with BAP (1mg/l) and NAA (1mg/l) – 3 series. During 8-week growth cycles, biomass and culture media were collected at 1 week intervals. Methanolic extracts of biomass and lyophilized media were used for determination of phenolic acids (26 compounds) with DAD-HPLC method [3]. Additionally total content of polyphenols (Folin-Ciocalteu method) and antioxidant activity of the extracts was assayed using 3 different methods (CUPRAC, and with DPPH and FRAP reagents).

It was found that extracts of lyophilized culture media did not contain phenolic acids while all studies extracts of biomass contained the same 10 compounds. 3-Phenylacetic acid, 3,4-dihydroxyphenylacetic

acid, cryptochlorogenic acid and isochlorogenic acid accumulated in the highest quantities.

The total contents of phenolic acids, after an initial marked decrease (2nd week: 399.59 mg/100g d.w.), rapidly rose to the maximum value of 471.34 mg/100g d.w. (3rd week), and was maintained at a high level until the 5th week (440.46 mg/100g d.w.), to gradually decrease in the following weeks to 81.69 and 70.05 mg/100g d.w. (7th and 8th week, respectively).

The total content of polyphenols and antioxidant activity of biomass extracts distinctly correlated with phenolic acid contents in consecutive weeks of culture growth cycles.

This is the first report about the dynamics in the accumulation of phenolic acids in agitated shoot cultures of *Aronia arbutifolia* during the growth cycles and determination of antioxidant activity of extracts from the biomass cultured *in vitro*. We propose the agitated culture of red aronia as a rich potential source of phenolic acids, independent of environmental conditions and environmental pollution.

**ACCUMULATION OF PHENOLIC ACIDS IN *IN VITRO*
CULTURES OF BLACK ARONIA (*ARONIA MELANOCARPA*)
CULTIVATED IN PLANTFORM BIOREACTORS**

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Black aronia is North-American plant species successfully cultivated in Poland. Fruits of this plant are rich in antioxidants – phenolic acids and anthocyanins. Our earlier phytochemical studies documented that the leaves of plant are also rich source of phenolic acids and flavonoids [1].

Established in our biotechnological laboratory shoot cultures of black aronia cultivated as stationary agar and agitated cultures produced high amounts of phenolic acids [1].

The aim of the current study was the establishment of shoot cultures in temporary immersion system – in PlantForm bioreactors (Sweden) and evaluation of phenolic acids accumulation in such system.

Shoot cultures were cultivated on Murashige and Skoog medium [2] supplemented with plant growth regulators (BAP – 1 mg/l and NAA – 1 mg/l) for 4 and 6 weeks (3 series). In methanolic extracts of shoots and culture media samples the HPLC-DAD analysis of 26 phenolic acids was performed [3].

It was found that the extracts of lyophilized culture media did not contain phenolic acids.

In shoots extracts the presence of 11 phenolic acids was confirmed. The main compounds were: 3,4-dihydroxyphenylacetic acid, 3 phenylacetic acid and isochlorogenic acid (max. 58.64, 374.87 and 77.84 mg/100g DW). Total amounts of phenolic acids reached 693.29 and 365.11 mg/100g DW, after 4-week and 6 week growth cycles, respectively.

The obtained results documented high accumulation of phenolic acids in shoot cultures of black aronia cultivated in PlantForm bioreactors, especially after 4 week growth cycles. We propose this type of cultures

as a potential rich source of phenolic acids, independent of environmental conditions and environmental pollution.

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EFFECT OF ATORVASTATIN ON BRANCHED-CHAIN AMINO ACID AMINOTRANSFERASE IN RAT SKELETAL MUSCLES

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Skeletal muscles are the initial site of the branched-chain amino acids (BCAA) metabolism due to high activity of branched-chain amino acid aminotransferase (BCAT) - the first enzyme in BCAA catabolism. The activity and level of BCAT affects the availability of substrates for branched-chain α -keto acid dehydrogenase (BCKDH) complex which is the rate limiting enzyme of BCAA oxidation [1]. Statins are commonly used and well tolerated drugs with relatively few side effects, primarily affecting skeletal muscles. Statin-related myotoxicity includes mild changes such as asymptomatic increase of serum creatine kinase activity or tolerable myalgia and severe changes such as rhabdomyolysis or statin-induced necrotizing autoimmune myopathy [2]. Atorvastatin has been shown to influence the metabolism of skeletal muscles [3]. The precise mechanism of this action is unknown. It cannot be excluded that atorvastatin interferes with the catabolic pathway of BCAA and thus, taking into consideration the crucial role of BCAA in skeletal muscles, affects muscles function. The aim of this study was to evaluate the effect of atorvastatin on the relative level of mRNA for BCAT in rat's gastrocnemius muscle.

Male Wistar rats were divided into two groups: drug-treated group (ST+A, n=8), administered orally 50 mg/kg b.wt. of atorvastatin and control group (ST, n=8) which received 0,2% methylcellulose (vehicle for drug). The drug and vehicle were administered for 14 days. The level of mRNA for BCAT in gastrocnemius muscle was measured using semi-quantitative RT-PCR. Results: The level of mRNA for BCAT in drug-treated group was significantly lower when compared to control group ($1,18 \pm 0,11$ and $1,08 \pm 0,09$ respectively, $p=0,04$).

Atorvastatin leads to decrease of mRNA level for BCAT in rat's gastrocnemius muscle. It can disturb BCAA metabolism and muscles function.

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EVALUATION OF THE EFFECT OF SIMVASTATIN ON KEY ENZYMES OF MONOUNSATURATED FATTY ACID SYNTHESIS IN THE CARDIAC MUSCLE TISSUE OF RATS

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Fatty acid synthase (FAS), elongase 6 (ELOVL6) and stearyl-CoA desaturase (SCD) catalyze the synthesis of monounsaturated fatty acids (MUFA). The expression of these enzymes is the highest in the liver, but it also occurs in the myocardium. It is regulated mainly at the transcriptional level by transcription factors such as PPAR, SREBP and LXR. Studies conducted on various cultured cell lines and on animal models indicate, that the expression of genes encoding FAS, ELOVL6 and SCD is modified by HMG-CoA reductase inhibitors (statins). To date, no studies have been conducted on the effect of simvastatin on Fas, Elov16 and Scd mRNA levels in the myocardium. The aim of the study was to determine the effect of simvastatin on Fas, Elov16 and Scd mRNA levels in the cardiac muscle tissue of male Wistar rats. The study group (n=6, ST1-6) and the control group (n=5, C1-5) have received simvastatin (80mg/kg bw/day) and 0.3% methylcellulose (the vehicle), respectively. Relative levels of Fas, Elov16 and Scd mRNA were assessed by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). It has been found that simvastatin significantly decreases the Fas mRNA level in rat's cardiac muscle tissue. It can be assumed that simvastatin affects the activity of transcription factors that regulate Fas gene expression. Simvastatin does not influence the mRNA relative level for Elov16 and Scd, suggesting that, at dosage used in this study, it does not affect MUFA synthesis in rats' myocardium. Further studies are necessary to explain the mechanism by which simvastatin affects transcription factors regulating gene expression for these enzymes.

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NEW DERIVATIVES OF DIPYRIDOTHIAZINES – SYNTHESIS AND PROPERTIES

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Phenothiazines are a well-known and valued group of chemical compounds. Classic derivatives exhibit antipsychotic activity, which has been used in psychiatry of the years, in the treatment of various types of psychoses, manic states and depression. Some of these substances have gained new indications for use, such as: allergy, vomit, cough, neuralgias and even anorexia and Parkinson's disease. Today numerous researches are still being carried out on the actions of classic and modified phenothiazine derivatives. It has been proven, that they exhibit a number of new activities among others antitumor, antibacterial, antifungal, antiviral, neuroprotective, analgesic and suppressing multi-drug resistance[1]. Smiles rearrangement is an aromatic, nucleophilic substitution, that allows to obtain many different chemical compounds among others phenothiazines. The idea of the project was to research the possibility of the occurrence of a double Smiles rearrangement of the type S→N using 3-amino-3'-nitro-2,4'-dipyridyl sulfide. The study began with the reaction of sulfide with 4-chloro-3-nitropyridine in DMF. The reaction was carried out in neutral conditions, using equimolar amounts of reagents. The reaction mixture was heated for 1h (variant A) and 2h (variant B). The final product of the reaction was 10-(3'-nitro-4'-pyridyl)-3,6-diazaphenothiazine obtained with a yield of 59% (variant A) and 76% (variant B). Then the reaction of sulfide with 2-chloro-3-nitropyridine was carried out in an analogous condition. 10-(3'-nitro-4'-pyridyl)-1,6-

diazaphenothiazine was obtained with a yield of 49% (variant A) and 52% (variant B). The course of chemical reactions was monitored by thin layer chromatography (TLC). ¹HNMR, ²DNMR and HRMS spectroscopic analysis was used for identify of the chemical structure of the products. The obtained dipyridthiazines testify to a double Smiles rearrangement of the type S→N occurrence.

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NEW PERSPECTIVES OF AEROSOL THERAPY

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Aerosol therapy is one of the safest, effective and the most rapidly developing methods of drug delivery. Since the 50s of the twentieth century when Dry Powder Inhaler (DPI) and Metered Dose Inhaler (MDI) apparatuses were first presented, fast improvement of such kind of devices have been observed. Newly launched III-generation DPI-inhalers do not require patient's inspiratory effort. Drug can be delivered either by the use of electric equipment (microprocessor to control inspiratory flow, Inspiomatic[®]), or by air pump connected with cyclone separator (Spiromax[®]). In comparison to conventional DPI inhalers, both of these devices produce smaller size of aerosol and have higher lung deposition [1]. In addition, the technology of DPI's drug carrier is still improving. The new spray-dried powder has better lungs targeting and dose consistency than traditional sucrose-carrier powders. It is able to deliver higher doses of antibiotics such as tobramycin or ciprofloxacin (Pulmosphere[®]) [2]. Furthermore, nebulizers' parameters are improving as well. Recently, nebulizers with The Vibrating Mesh Technology (VMT) have been commercially available. We can distinguish VMT nebulizers which work passively (Omron[®] and Respironics[®]) and actively (Airon[®] and PARI[®]). Using VMT is possible to administer γ -interferon (Dual-membrane technology, Photo-defined aperture plate, PDAP[®]), antibiotics, mucolytics or prostaglandins even to mechanically ventilated patients and infants (Aerogen Pro[®] and Aerogen Solo[®]) [3]. All above-mentioned modifications in inhalers and nebulizers lead to improvement of aerosol parameters and better results in patients' compliance with treatment.

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THE MINI-GRADUATION TOWER – THE PROTOTYPE DEVICE

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One the biggest problems of the XXI-th century is the increase in air pollution contaminated with emitted gases such as (CO₂, SO₂, NO) and metals including Cd, Cr, Cu, Fe, Mn, Ni, As, and Pb. Air impurity is caused by the civilizing processes and the rapid development of industrialization. Numerous studies revealed that exposure to pollution present in smog leads to the heart, lung, skin, eye and diseases as well as cancer [1]. Therefore, it is very important to conduct studies which aim at monitoring the exposure to environmental contamination. One of the observation methods is to conduct *in vivo* tests with rodents and the prototype device specially designed for this purpose - the minigraduation tower. This device allows to assess changes in the concentration of metals in the air. Conducted pilot experiments characterized the physicochemical conditions and concentration of metal ions applied in the form of an aerosol. Aerosol was obtained from a zinc chloride in the concentration of 5000 ppm. Then, the vapor, condensed on membrane filters, was subjected to solution extraction with 1M HCl. Extracts obtained in this way were analyzed by the use of atomic absorption spectrometry (flame technique) for the content of zinc. The experiment shows that the mini-graduation tower allows testing of aerosols which can be useful not only in measuring the environmental pollutions but also drug contamination.

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REMEDIATIVE PROPERTIES OF MYCELIUM VS SYNTHESISED MATERIALS

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The specific ability of mushrooms to bioaccumulate metals is the subject of numerous studies and scientific publications, especially in recent years. The first reports on the bioaccumulation of some metals by mushrooms appeared nearly 100 years ago. Initially, the focus was on the evaluation of mushrooms toxicity, resulting for example from the accumulation of heavy metals (Cd and Pb). A little later, practical possibilities of using fungi as natural bioindicators of the environment began to be noticed [1, 2]. The uptake of specific elements by mushrooms is closely related to the species and place of occurrence (including their availability and soil reaction). Bioaccumulation of metals in mushrooms results, among others, from the presence of the low-molecular-weight proteins – metallothionein. This proteins has a strong affinity for metal cations. The presented study compares the remediation properties of selected species of mycelial cultures of mushrooms, including *Agaricus bisporus*, *Boletus Badius* and *Pleurotus ostreatus* with commercial TiO₂ powder and YsZ powder with neodymium modification, which was obtained by precipitation reaction after calcination and hydrothermal conditions. For this purpose, *in vitro* cultures of the aforementioned species of mushrooms were carried out on a liquid medium with the addition of heavy metal salts Pb and Cd at a concentration of 10 mg/L of the medium and adsorption of the same salts on the above-mentioned synthetic

nanopowders. On the basis of the conducted research, it was shown that all of the analyzed mycelium of mushrooms show the ability to accumulate metals, in particular *P. ostreatus* mycelium, for which the calculated adsorption efficiency is comparable to the hydrothermal nanopowder used.

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APPLICATION OF POWDERS FOR HUMIC ACIDS REMOVAL FROM AQUEOUS SOLUTION

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Humic substances constitute a group of natural organic matter present in soil, water reservoirs, peloids. They are formed by decomposition of plants and animal remains. Humic substances can be divided into three components depending on their solubility: humic acids, fulvic acids and humin. Humic acids (HA) are not soluble in water below pH 2 but become soluble at higher pH. They belong to high molecular weight fraction and they are aromatic and aliphatic compounds [1]. The presence of humic acids in water, especially in drinking water affects many quality parameters such as color, taste and odor. Humic acids (HA) contaminate membranes and ion-exchange resins in filtration systems. During the water treatment processes (disinfection) some carcinogenic products such as CHCl_3 can be formed. Humic acids form complexes with heavy metals and associations with organic pollutants. They may develop interactions with viruses and nanoparticles and affect transport of contaminants into the natural environment. Hence, there is a need to eliminate HA from water despite of their natural origin.

The titanium oxide, yttria-stabilized zirconia (also doped with neodymium), kaolin and montmorillonite were tested as humic acids adsorbents. The adsorption efficiency was tested in the series of experiments, considering various initial parameters, such as, pH of the solutions (pH 3,5) and mass of the adsorbent. The initial concentration of humic acid

was 25 mg/L, and time of sorption was 30 min. The quantitative measurements applied for determination of organic compounds removal effectiveness were made using the Ultraviolet-visible (UV-VIS) spectrophotometer.

The sorption effectiveness analysis of the used nanopowders was performed basing on humic substances measurements in solutions after sorbents centrifugation. All nanopowders were helpful in elimination of humic acid from water. The highest sorption effectiveness was found solutions with zirconium and yttrium nanopowders doped with neodymium.

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FORTIFIED MYCELIA FROM *PLEUROTUS* SPP. A SOURCE OF Cl⁻ ANIONS

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Mushrooms of the *Pleurotus* spp. (incl. *Pleurotus citrinopileatus*, *Pleurotus djamor* and *Pleurotus pulmonarius*) have for years been valued all over the world not only for their taste, but above all because of the content of numerous compounds with health-promoting properties (including bioelements, phenolic and indole compounds, lovastatin) or ergothioneine [1]. The research undertaken in the study was aimed at determining the effect of the addition of inorganic magnesium salts ($MgSO_4 \cdot 7H_2O$, $MgCl_2 \cdot 6H_2O$) to the culture media and zinc salts ($ZnSO_4 \cdot 7H_2O$, $ZnCl_2$) on the increase of biomass growth as well as the degree of accumulation of subsidized anions. The actual bioavailability of the Cl⁻ anion was determined from the obtained enriched biomass by extracting the lyophilized mycelium in artificial digestive juices. Determination of the concentration of Cl⁻ ions in the biomass obtained from *in vitro* cultures was performed in quartz cuvettes using validated tests for chloride presence (Cat. No. 114897) using the spectrophotometric method (Spectroquant Nova 60 spectrophotometer). Artificial digestive juices samples were filtered through a 0.45 μm filter membrane. The conducted research has shown that the addition of inorganic salts to the culture media has a positive effect on the growth of biomass as well as on the efficiency of the accumulation of subsidized anions – Cl⁻. The following concentrations of the Cl⁻ anion were determined in natural fruiting bod-

ies: *P. citrinopileatus* – 1200, *P. djamor* – 910, *P. pulmonarius* – 1777 mg/100 g dry weight. However, in the case of *in vitro* cultures obtained on media enriched in both $ZnCl_2$ and $MgCl_2 \cdot 6H_2O$ salt, the determined concentrations of the Cl^- anion were respectively: *P. citrinopileatus* 2265, *P. djamor* –1020, *P. pulmonarius* – 1625 mg/100 g dry weight. The conducted research confirms that mushrooms of the *Pleurotus* spp. can be used as a natural component of the daily diet, helping to supplement deficiencies not only of bioelements in the human body but also of anions, including Cl^- .

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PHOSPHATE AND CHROMATE REMOVAL FROM AQUEOUS SOLUTION BY NANOPOWDERS OF YTTRIA-STABILIZED ZIRCONIA DOPED WITH RARE EARTH ELEMENT

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Contaminations by toxic metals such as Cr(VI) and eutrophication element (e.g. P) in soils and sediments may occur simultaneously, leading to an adverse effect on ecosystem. Presence of excess phosphate in water leads to significant eutrophication and water quality problems which results in the growth of harmful algal blooms, as well as depletion of dissolved oxygen which subsequently results in the decline of aquatic life. The origins of Cr(VI) in the environmental are predominantly anthropogenic, which are mainly discharged from industrial activities such as plating, texture dyeing, paper manufacturing, and wood preservation. In areas with poor irrigation/drainage and municipal sewers, Cr(VI)-containing wastewaters and PO₄-loaded domestic sewages may be discharged into the same ditch, including irrigation ditches. Therefore, the surrounding agricultural soils and waterbodies may be polluted simultaneously by these two elements. Hence, there is a need to eliminate chromates and phosphates from water.

The aim of the research was to demonstrate, for the first time, usefulness of the yttria-stabilized zirconia nano powders (also doped with neodymium) in removal of chromate and phosphate from aqueous solutions. The adsorption efficiency was tested in the series of experiments, considering various initial parameters, that is, pH of the solutions and

mass of the adsorbent. The desorption process was carried in distilled water. The effectiveness of chromate adsorption was examined in double distilled water and in the presence of phosphate ions. The chromate and phosphate concentration was analyzed using the Ultraviolet-visible (UV-VIS) spectrophotometer.

Nano powders were helpful in elimination of chromate and phosphate from water and the highest sorption was found solutions with zirconium and yttrium nanopowders doped with neodymium. The above materials worked well for remove of both anions from water solutions.

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**EVALUATION OF ANTIMICROBIAL ACTIVITY OF
NASTURTIUM OFFICINALE (WATERCRESS) – RITA®
BIOREACTOR GROWN IN VITRO CULTURES AND HERB
EXTRACTS**

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Nasturtium officinale R. Br. is an aquatic, partially protected in Poland perennial plant species. Its herb is the valuable medicinal, cosmetic and culinary raw material. It possesses scientific proven activities e.g. antioxidant, hepatoprotective and anticancer, conditioned by a rich chemical composition, e.g. glucosinolates, phenolic acids and flavonoids [1].

The aim of this study was to investigate the antibacterial and antifungal activity of *N. officinale* microshoot cultures grown in temporary immersion systems - RITA® bioreactors (MS medium with 1 mg/L 6-benzyladenine and 1 mg/L 1-naphthaleneacetic acid) and its comparison with the parent plant extract.

The extracts of 10-days bioreactor microshoot cultures and herb of *N. officinale* were screened for antibacterial and antifungal activities by micro-dilution broth method according to broth the European Committee on Antimicrobial Susceptibility Testing [2]. The Mueller-Hinton broth and Mueller-Hinton broth with 5% lysed sheep blood for growth of non-fastidious and fastidious bacteria, respectively or RPMI with MOPS for growth of fungi were used [3,4]. Minimal inhibitory concentration (MIC) of the tested derivatives were evaluated for microorganisms from American Type Culture Collection (ATCC), including Gram-negative bacteria (e.g. *Escherichia coli*, *Helicobacter pylori*), Gram-positive bacteria (e.g.

Staphylococcus aureus, *S. epidermidis*) and fungi (e.g. *Candida* spp., *Aspergillus niger*, *Trichophyton mentagrophytes*).

The tested extracts inhibited growth of the reference microorganisms with MIC of 1.25-20 mg/mL, depending on the studied strains. The biological activity of *N. officinale* herb extract and in vitro culture extract were comparable. The most sensitive was microaerophilic Gram-negative bacteria *H. pylori* (MIC 1.25 mg/mL). Gram-positive bacteria represented by *staphylococci* showed lower susceptibility to tested extracts (MIC 5-10 mg/mL). The lowest biological activity of both extracts was observed against Gram-negative strains.

Fungi represented by yeasts - *Candida* spp., mould species - *Aspergillus* spp. and dermatophytes - *Trichophyton* spp., were inhibited in 10-20 mg/mL concentration, irrespective of the tested extract. Higher bioactivity of the extracts was found against *Trichophyton* and *Microsporium* (MIC 1.25-5 mg/mL) with very slight difference related to tested extracts.

Moreover, the low values of minimal bactericidal concentration - MBC/MIC and minimal fungicidal concentration -MFC/MIC ratios (1-4) for the extracts suggested their good antimicrobial power for both bacterial and fungal strains.

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CHANGES OF CHEMICAL COMPOSITION IN SEEDS OF LEGUME PLANTS UNDER SOIL DROUGHT

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In the last years drought has become in Poland one of the main cause of crop yield reduction. Water stress induces many biochemical and physiological changes in plants. Legumes play a significant role in crop production, animal feeding and human diet, because of high protein content and well balanced aminoacids. One of the factors which directly determines legumes' yield is water deficit in critical stages during the greatest growth of biomass, the flowering period and seed filling. In legume plants soil drought affects the quality of seeds by changing the protein and antinutritional substances, including phenolic compounds.

The aim of the study was to determine the impact of soil drought on the content of selected chemical compounds in legumes' seeds.

Seedlings of pea (*Pisum sativum* L.), lupine (*Lupinus luteus* L.) and faba bean (*Vicia faba* ssp. *minor* L.) were subjected to the soil drought (25% field water capacity, FWC) for 2 weeks. Control plants and plants after drought were watered to the 70 % FWC. In the dry seeds the content of soluble proteins, phenolics, proline, sugars, polyamines and antioxidant activity were measured.

In the seeds of all tested species soil drought reduced content of polyamines by 37%. The composition of identified polyamines consisted mainly of putrescine, spermidine, spermine and agmatine. Drought reduced also the content of antioxidants (ca. 8%). Besides that water deficit caused: the decrease of protein (17%) and sugars (14%) contents in yellow lupine, the increase of phenolics (8%) and proline (32%) in faba bean, and the increase of proline (21%) in pea.

Conducted experiments confirmed that water stress causes the oxidative stress and leads to the change in the chemical composition of the legumes seeds.

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EXOGENOUS MELATONIN CAN IMPROVE *IN VITRO* PLANT REGENERATION

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The presence of melatonin (MEL) in plants, known as a pineal hormone, was confirmed in the 90s and since then, its ability to enhance tolerance and plants survival under abiotic stress was postulated by many authors (Tan et al. 2007; Posmyk and Janas 2009; Szafrńska et al. 2013). Plant MEL might act as a dual agent: antioxidative cell protector and plant morphogenic regulator. In the present study, MEL was investigated as possible regulator of winter triticale (*×Triticosecale* Wittm.) microspore embryogenesis (ME). The process of ME – the most effective and widely used method for doubled haploids (DHs) production – requires a complete re-programming of the developmental plan, to embryo instead of pollen grain formation. It is accompanied by many physiological, metabolic, and molecular changes, among which changes in hormonal homeostasis are one of the most deeply studied aspects of ME. It can be induced by various stress factors, but the most successful and most often applied is tillers pre-treatment with low temperature (LT). In the study, the effectiveness of ME was examined in *in vitro* cultured microspores isolated from triticale tillers pre-treated with LT (4 °C for 21 days) alone or in combination with MEL (50 or 100 µM),

applied for 4 days before the scheduled date of microspore isolation. It was accompanied by simultaneous analyses of hormonal profile: auxins (Auxs) and cytokinins (CKs).

Despite MEL pre-treatment kept microspore viability at the control level, it increased the number of green regenerated plants and declined the percentage of albino regenerants, especially at its lower concentration. Such effect could be possibly the result of MEL ability to scavenge hazardous reactive oxygen species (ROS) (Arnao and Hernández-Ruiz 2015) generated under LT treatment and microspore isolation procedure. It was associated with decreased content of active and conjugated Auxs. Surprisingly, MEL did not significantly change the content of the most of identified CKs.

The obtained results confirm MEL potential to strengthen plants subjected to stressors and constitute a premise for further research on the possibility of increasing the efficiency of ME in winter triticale.

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INFLUENCE OF Cu^{2+} , Zn^{2+} AND Ag^+ IONS IN OAT (*AVENA SATIVA* L.) ANDROGENESIS

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Androgenesis is a process of plant development from microspores in anther or isolated microspore cultures, usually induced by the applica-
tion of the stress treatment e.g.: low/high temperature, nitrogen starva-
tion or osmotic stress which trigger re-programming of the developmen-
tal plan to embryo rather than pollen grain formation. Also the addition
of some metal ions to the medium may stimulate the conversion of mi-
crospores to the path of androgenesis. Ions such as Cu^{2+} and Zn^{2+} stimu-
late the division of plastids and the chloroplasts development and pre-
vents the formation of albino plants. Cu^{2+} together with Ag^+ act as an
inhibitor of ethylene biosynthesis in in vitro cultures, delaying the aging
process.

The aim of study was to determine the effectiveness of oat andro-
genesis induction in anther cultures depending on the concentration of
 Cu^{2+} , Zn^{2+} or Ag^+ ions added to the regeneration medium.

The plant material consisted of two oat (*Avena sativa* L.) cultivars:
‘Bingo’ and ‘Chwat’. Collected panicles were pre-treated in Hoagland’s
liquid medium (Hoagland and Arnon 1938) at 4°C for 2 weeks, and then
placed at 32°C for 24 h. The anthers were isolated on solid W14 medium
(Ouyang et al. 1989) with 2.0 mg/dm³ 2,4-dichlorophenoxyacetic acid
and 0.5 mg/dm³ kinetin. Salts were added to the media in two concentra-
tions:

$\text{CuSO}_4 \times 5 \text{H}_2\text{O}$ (10 or 20 μM), $\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$ (90 or 180 μM) or Ag -
 NO_3 (25 or 50 μM). Anther cultures were incubated in the dark at
28 ± 1°C.

Embryo like-structures (ELS) were observed after 6–8 weeks of cul-
ture. The highest number of ELS was obtained after panicles pre-

treatment in Hoagland's medium with the addition of CuSO_4 at a concentration of 10 or 20 μM (2.1% or 1.8%, respectively). Most ELS were founded on W14 medium with 25 μM AgNO_3 , where 'Chwat' produced 33 ELS, while 'Bingo' 5 ELS.

Panicle pre-treatment with 10 or 20 μM CuSO_4 increases the effectiveness of ELS formation. The obtained results can contribute to the development of an effective method for the oat plant regeneration by androgenesis.

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PARTICULATE MATTER OF AIR POLLUTION FROM THE KRAKÓW AREA PROMOTES PRO-INFLAMMATORY ACTIVITY OF TH LYMPHOCYTE SUBSETS OF PATIENTS WITH AUTOIMMUNE DISORDERS

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Introduction: In recent years, an increase of air pollution, especially particulate matter PM10 and PM2.5, seriously impacts human health. However, the knowledge on their specific effects on the cells of the immune system is still scarce. Here we asked whether PM from the Kraków area has an effect on the activity of human CD4⁺ T cell subsets (Th1, Th2, Th17, and Treg) of patients with autoimmune disorders, namely multiple sclerosis (SM) and rheumatoid arthritis (RA).

Materials and methods: Ten patients of each group (SM and RA) and ten healthy donors (age and sex matched) were included in the study. Peripheral blood mononuclear cells (PBMC) of healthy donors and patients were isolated by standard Ficoll/Isopaque density gradient centrifugation and cultured with or without PM of air pollution collected from the Kraków air, at the previously established concentration of 10 ug/ml. As a positive control, PBMC stimulated with phorbol myristate acetate (PMA; 50 ng/ml; InvivoGen) and ionomycin (1 µg/ml; Sigma) were used. After 3 hours of culture, the expression of intracellular IFN-γ, IL-4, IL-17A and Foxp3, characteristic for Th1, Th2, Th17 and Treg, respectively, was detected using Human Th1/Th2/Th17 Phenotyping Kit and Human Th17/Treg Phenotyping Kit (both BD Biosciences, Pharmingen) and analyzed by flow cytometry (FACSCantoX, BD Biosciences).

Results: The results showed that treatment of PBMC with PM samples from Kraków air increased the expression of IFN- γ and IL-17A, specific for Th1 and Th17 cells respectively, both in patients and healthy donors, while the frequency of cells positive for IL-4 (Th2) and Foxp3 (Treg) was unchanged. This effect was more pronounced in patients with RA than MS in comparison to the control.

Conclusions: The results, although preliminary, indicate that in vitro treatment of human PBMC with PM from the Kraków area skews the balance of Th1/Th2 and Treg/Th17 cells, promoting pro-inflammatory activity of the Th1 and Th17 subsets. This observation may confirm the hypothesis that air pollution plays a role in exacerbation of autoimmune disorders.

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BIOREACTOR CULTURES OF *HYPERICUM PERFORATUM* CULTIVARS – A POTENTIAL RICH SOURCE OF BIOACTIVE COMPOUNDS

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St. John's wort (*Hypericum perforatum* L.) is a widely used medicinal plant species. It is known for its antidepressant, antimicrobial, anti-inflammatory, antioxidant, anticancer, cholagogic, spasmolytic and astringent effects. The aim of the study was the quantitative analysis of phenolic acids, flavonoids and catechins in *in vitro* cultures of three cultivars of *Hypericum perforatum* – 'Elixir', 'Helos', 'Topas' – maintained in PlantForm bioreactors (Sweden).

Liquid medium by Murashige and Skoog [1] with the addition of growth regulators – 6-benzylaminopurine (BAP) and 1-naphthaleneacetic acid (NAA) in various concentrations – 0.1, 1.0, 2.0, 3.0 mg/l was used. The analysis was conducted using the HPLC method [2].

In all analyzed biomass extracts the presence of following phenolic acids were confirmed: protocatechuic acid, neochlorogenic acid, 3,4-dihydroxyphenylacetic acid, chlorogenic acid and cryptochlorogenic acid. The presence of vanillic acid was confirmed only in selected extracts. In all extracts the presence of hyperoside, rutoside, quercitrin, quercetin, luteolin, kemferol (flavonoids) and catechin and epicatechin (catechins) have also been documented. Among phenolic acids, the main metabolite was 3,4-dihydroxyphenylacetic acid (max. 209.10 mg/100 g DW). Quantitatively dominant metabolite from flavonoids group was quercetin (max. 753.59 mg/100 g DW). Epicatechin and catechin contents were comparable, 197.37 and 176.17 mg/100 g DW, respectively. Media variants supplemented with higher amounts of growth regulators (2.0 mg/l or 3.0 mg/l BAP and NAA) favoured the accumulation of all

analyzed groups of metabolites in *in vitro* cultured biomass. The maximal obtained total amounts of these metabolites were as follows: 353.30 mg/100 g DW (phenolic acids, cv 'Elixir'), 906.16 mg/100 g DW (flavonoids, cv 'Helos'), 373.54 mg/100 g DW (catechins, cv 'Helos'), respectively. The experiment proved that the bioreactor's cultures could be an effective and alternative for field crop herb (sensitive to environmental factors) source of phenolic acids, flavonoids and catechins, what we had confirmed before for another type of *in vitro* cultures [3,4].

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THE ANALYSIS OF THE CONCENTRATION Cl^- AND SO_4^{2-} ANIONS IN MYCELIUM OF *FOMITOPSIS OFFICINALIS*

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Fomitopsis officinalis is a popular polypore mushroom widely used in traditional European medicine. The medicinal properties of *F. officinalis* are related to the high content of biologically active metabolites in fruiting bodies, e.g. organic compounds such as indole and phenolic compounds, terpenoids, vitamins and macro- and microelements and anions such as Cl^- , SO_4^{2-} [1]. Both the Cl^- and SO_4^{2-} anion are necessary for the proper functioning of the human body because they are responsible for the acid-base balance. Hence, conducting research with the use of these anions and the species *F. officinalis* with documented pro-health properties is fully justified. In the conducted research, the composition of the culture medium enriched with the addition of inorganic sulphate(VI) salts and chloride metals of Mg and Zn was optimized. For the first time, the effect of anions addition (Cl^- and SO_4^{2-}) on the *F. officinalis* biomass growth was determined. The obtained biomass was extracted into artificial digestive juices in order to determine their bioavailability for the human body.

The concentration of selected anions in obtained biomass was determined using the spectrophotometric method (Spectroquant Nova 60 spectrophotometer). The sample after extraction to the digestive juices, were filtered using a 0.45 μm membrane to obtain a clear solution for analysis. The experiment results revealed that enriched culture medium

has a positive effect not only on the growth of biomass, but also on the efficiency of accumulation anions. In experiment was found that fruiting bodies of *F. officinalis* contain a higher concentration of Cl^- – 1904 mg/100 g of dry weight (d.w.) than SO_4^{2-} – 429 mg/100 g d.w. Moreover, it was shown that the addition of chloride salts did not increase the concentration of Cl^- anions in the obtained biomass.

Contrary to the addition of sulphate salts(VI), in this case both the addition of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ increased the sulphate ions. The best result was obtained in the in vitro cultures of *F. officinalis* carried out on a medium enriched with the addition of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (SO_4^{2-} : 1733 mg/100 g d.w.). Such content of ions in biomass allows used it as a natural component of the diet supporting the supplementation of Cl^- and SO_4^{2-} anions deficiencies.

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LIPOPHILICITY STUDY OF NOVEL BUTANEHYDRAZIDE DERIVATIVES OF PURINE-2,6-DIONE BY REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY

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Autoimmune diseases are a group at least 80 types of diseases, which might affect around 5%–10% of the total population and the prevalence is rising rapidly with no effective therapy against these disorders, basing on symptomatic treatment [1,2]. Increasing evidence has shown that the abnormal inflammatory response is closely associated with many chronic diseases, especially in autoimmune diseases [3]. Phosphodiesterase (PDE)-targeted therapies become an incentive for treating patients with a variety of inflammatory diseases [4]. Selective PDE4 or PDE7 inhibitors are presently regarded as potential agents for the treatment of many T-cell mediated autoimmune diseases [2]. The lipophilicity of drug substance is an important physicochemical parameter is binding with its biological activity and affects absorption, distribution, metabolism, excretion, and toxicity of a drug [5].

In this case, the lipophilicity of twelve novel butanehydrazide derivatives of purine-2,6-dione as dual PDE4/7 inhibitors² has been investigated. Reversed-phase thin-layer chromatography (RP-TLC) method with densitometric detection was applied to determine parameters of lipophilicity. The mobile phases were prepared by mixing phosphate buffer 0.01M pH=7.4 with organic modifier (methanol, acetone). The obtained experimental R_{M0} factors of examined substances were compared with the calculated results of the same butanehydrazide deriva-

tives. The log P values of examined substances were calculated using following algorithms: AlogPS, ChemAxon, AClogP, miLogP, ALogP, MLogP, KOWWIN, XLOGP2, XLOGP3 using stand-alone programs (ChemAxon) or Virtual Computational Chemistry Laboratory. The parameters RF, RM and RM0 were calculated using Excel 2007 (MS Office Excel 2007, Microsoft, Redmond, USA). Regression procedures were run using Statistica 13.3 (TIBCO Software, USA). Principal Component Analysis was done using built-in functions of GNU R 3.1.1 (www.r-project.org).

The obtained results show that RP-TLC method may be the reliable technique for butanehydrazide derivatives in describing their lipophilic nature as well as the activity.

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DEVELOPMENT AND VALIDATION OF THE THIN-LAYER CHROMATOGRAPHY DENSITOMETRIC METHOD FOR THE DETERMINATION OF SELECTED CANNABINOIDS

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Cannabinoids or phytocannabinoids are compounds found in different varieties of hemp plant species (*Cannabis sativa* L.). The most important of them are cannabidiol (CBD), delta-9-tetrahydrocannabinol (Δ^9 -THC), cannabigerol (CBG), cannabichromene (CBC), cannabinol (CBN). These compounds present pharmacological activity related to the endocannabinoid system in vertebrate organisms. Cannabis-based preparations relieve autoimmune disorders, such as multiplesclerosis, rheumatoid arthritis, and inflammatory bowel disease. Some studies indicate that they can be very useful in the future as a important medication in the treatment of Alzheimer's disease, amyotrophic lateral sclerosis, and reducing the spread of cancer cells [1-4].

The aim of this study was to develop and validate the TLC method with densitometric detection in the UV range for the determination of four cannabinoids: CBC, CBN, CBG and CBD. As the stationary phase HPTLC LiChrospher chromatography plates were used; the mobile phase was a mixture of n-hexane - acetone – diethylamine. UV detection was performed at $\lambda=300$ nm for CBC and CBN, and also at $\lambda=215$ nm for CBG and CBD.

The method was validated for specificity, linearity, detection/quantitation limit, precision and intermediate precision and accuracy in accordance with ICH guidelines.

Under the developed conditions, well-separated and symmetrical peaks of tested substances were obtained. The validated method meets the criterion of specificity. The method has a wide range of linearity and is characterized by high sensitivity, the obtained values of LOD and

LOQ are respectively from 0.0208 µg/band to 0.0625 µg/band and from 0.0416 µg/band to 0.2500 µg/band. The method is also characterized by good accuracy, the recovery percentage is from 99.20% to 102.00%. Precision and indirect precision, determined on the basis of RSD values from the obtained results (lower than 2.79%), for determined cannabinoids also meet the required criteria.

The validation of the method shows that it is specific, sensitive, precise, accurate, has a wide range of linearity and therefore can be used for the quantitative analysis of the tested cannabinoids in CBD oils used as dietary supplements.

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RESEARCH ON THE BIOSYNTHETIC POTENTIAL OF *KALANCHOE PINNATA IN VITRO* CULTURES

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The progressive pollution of the environment is the cause of many civilization diseases such as allergies, cardiovascular diseases, and cancer. The most common factors that can cause them are, among others, free radicals and heavy metals. The use of antioxidants is recommended in the prevention of civilization diseases. Many plant metabolites have antioxidant and free radical scavenging properties. Phenolic compounds such as phenolic acids and flavonoids are particularly known for this action. Plants are the best source of these types of compounds. An alternative to ground plants is the use of plant cultures *in vitro*. This technique allow to stimulate the production of selected secondary metabolites and independence from external conditions. *Kalanchoe pinnata* (Lam.) Pers. (family Crassulaceae) is a popular plant used in traditional medicine in many regions of the world. Extracts of this plant show multidirectional healing effects, including antioxidant, hepatoprotective, anti-inflammatory, antiallergic properties. Pharmacological activity is due to the varied chemical composition. *Kalanchoe pinnata* contains phenolic acids, flavonoids, triterpenoids, tannins and steroid glycosides. The aim of this study was to investigate *in vitro* cultures of *Kalanchoe pinnata* and to compare the metabolic profile of the parent plant and *in vitro* cultures. The cultures of *Kalanchoe pinnata* were maintained on solid Murashige - Skoog medium supplemented with plant growth regulators (auxine: NAA – 0.5 mg/L, cytokinine: BAP – 1 mg/L). Four groups of secondary metabolites were analysed by HPLC method in the

methanolic extract from dry biomass of *in vitro* cultures and the parent plant.: amino acids, catechins, phenolic acids, flavonoids. Quantitatively dominant group of metabolites in both studied extracts was flavonoids. The most dominant metabolites were quercetin and kaempferol glycosides. Differences in the chemical composition of the parent plant and the *in vitro* cultures were found.

EFFECT OF ATORVASTATIN ON BRANCHED-CHAIN KETOACID DEHYDROGENASE (BCKDH) IN RAT'S LIVER

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Branched-chain ketoacid dehydrogenase (BCKDH) complex is a regulatory enzyme of branched-chain amino acids (BCAA) metabolism. It is composed of multiple copies of 3 catalytic subunits (E1, E2 and E3) and two regulatory enzymes, specific kinase (BDK) and specific phosphatase (BDP). The most important regulatory mechanism of BCKDH is covalent modification achieved by a reversible phosphorylation of E1 subunit catalyzed by BDK and BDP [1]. Therefore, BCKDH is characterized by total activity (fully dephosphorylated), actual activity (partly phosphorylated) and activity state (percentage of BCKDH complex in active dephosphorylated state).

It has been found that simvastatin, one of the HMG-CoA reductase inhibitors used in hypercholesterolemia treatment modifies liver BCKDH complex. It upregulates liver BCKDH activity and stimulate BCAA catabolism, especially under the condition of dietary protein deprivation [2]. At present, atorvastatin is one of the most frequently prescribed drugs in the world. Effect of atorvastatin on liver BCKDH activity has not been studied yet.

The present study was aimed at investigating the effect of atorvastatin on liver BCKDH activity in rats fed with a standard diet (18% protein). For 14 days, atorvastatin (50 mg/kg b wt/day) or the vehicle (0.2% methylcellulose) were administrated orally by gavage to the treated (n=8) and control groups (n=7), respectively. The actual and total BCKDH activities were assayed spectrophotometrically prior to and following incubation with lambda phosphatase, respectively. Then BCKDH activity state was calculated. In rats treated with atorvastatin

actual and total BCKDH activities were lower (by 10% and 12%, respectively), while the activity state was 3% higher than in control group. The differences were not statistically significant ($p > 0.05$).

In conclusion, atorvastatin has no effect on BCKDH activity in rat's liver and thus it does not affect liver BCAA catabolism. These results confirm the safety profile of atorvastatin.

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***CORDYCEPS MILITARIS* AND *HERICIUM ERINACEUS* INFLUENCE ON COGNITIVE FUNCTIONS, MENTAL AND PHYSICAL FATIGUE**

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Mushrooms of the species *Cordyceps militaris* and *Hericium erinaceus* have a long tradition of use in Asian ethnomedicine as adaptogenic agents, strengthening the body and stimulating the immune system. *C. militaris* contains bioactive compounds, including cordycepin, polysaccharides (β -glucan derivatives) and γ -aminobutyric acid (GABA). Analytical studies on mycelium *H. erinaceus* showed the presence of: diterpene compounds (hericenone, erinacin), indole compounds (5-hydroxytryptophan, melatonin) and polysaccharides.

Reduction of physical and mental fatigue, affects on strengthens the body and improves exercise capacity. In vivo experiments on rodent showed anti-fatigue activity of *C. militaris* and *H. erinaceus* in the swimming test. In a study involving healthy men, it was proved that supplementing a mushroom mixture containing *C. militaris* for 1–4 weeks contributed to the improvement of physical endurance. *C. militaris* was one of the ingredients in a mushroom blend (trade name PeakO₂®) which also included *H. erinaceus*. A polypeptide isolated from *C. militaris* has been found to improve memory and learning in animal model in mice. The mechanism of biological activity has been associated with the inhibition of acetylcholinesterase (AChE) and the intensification of GABA neurotransmission. In vivo neuroprotective activity has been proven for cordycepin. Clinical trials are ongoing regarding the influence of *C. militaris* on the active of central nervous system in humans – the regulation of emotions and mood.

More experiments in the area of effects on the central nervous system have been carried out for *H. erinaceus*. Among young students, the use of *H. erinaceus* has been associated with an improvement in sleep

quality and overall well-being. In the elderly group and in patients with Alzheimer's disease, the procognitive effect of *H. erinaceus* has been demonstrated. In obese or overweight patients, it has been proven that 8-week use of *H. erinaceus* contributed to the reduction symptoms of depression, anxiety and improvement of sleep quality. Clinical trials are underway on the possibility of using *H. erinaceus* in the treatment of Parkinson's disease and depression.

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CHRONIC ADMINISTRATION OF ANTIDEPRESSANT DRUGS INDUCES ADAPTIVE CHANGES IN GLUA1 AMPA RECEPTOR SUBUNIT IN SELECTED STRUCTURES OF RAT BRAIN

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Depression is a mental disorder for which current treatment has many limitations. Despite thorough research in this field, the biological mechanisms underlying this psychiatric illness are still poorly understood. The factors influencing the development of depression may be biological, and environmental. There are reports connecting mood disorders with air pollution. Studies show relation between environment and mental health [1,2]. Small compounds of the atmosphere may affect the central nervous system by penetrating the brain's blood barrier.

Brain structures that have been linked with depressive disorder have been located in prefrontal cortex and hippocampus [3]. Some studies suggest the role of cerebellum in pathophysiology of depression. Neuroimaging techniques confirmed decreased volume and grey matter concentration [4,5].

The aim of this experiment was to evaluate the molecular adaptive changes in prefrontal cortex, hippocampus and cerebellum, after chronic administration of antidepressant drugs. Drugs with different mechanisms of action were selected: imipramine, reboxetine and S-citalopram. Saline was used for the control group. Drugs were administered to rats (n=8/group) intraperitoneally for 21 days. Brains were isolated and protein expression was determined using Western blot analysis.

GluA1 subunit of ionotropic glutamatergic AMPA receptor (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) has been chosen for the experiment as it plays role in brain signalling and

pathophysiology of depression. AMPA receptor has also been suggested to mediate the rapid antidepressant effect of ketamine (6,7).

Adaptive changes after chronic administration of tested drugs have been identified in all investigated structures. GluA1 subunit level was significantly increased in prefrontal cortex of rats treated with imipramine ($p<0.01$), reboxetine ($p<0.01$) and s-citalopram ($p<0.05$) when compared to NaCl treatment. In hippocampus the up-regulation of GluA1 was demonstrated only after imipramine ($p<0.01$) while in cerebellum after s-citalopram ($p<0.01$) administration.

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THE IMPACT OF THE ELICITATION ON THE ACCUMULATION OF PHENOLIC ACIDS IN *TRAMETES* SPP. MYCELIAL CULTURES

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Phenolic compounds occurring in mushroom fruiting bodies and accumulated in biomass from mycelial cultures possess a high health-promoting potential including antioxidant, anti-inflammatory, or antimicrobial activity.

The study aimed to investigate the effect of the elicitation with methyl jasmonate (MeJa) on increasing the production of phenolic acids in aerated mycelial cultures of three *Trametes* species: *Trametes gibbosa* (Pers.) Fr., *Trametes hirsuta* (Wulfen) Lloyd, and *Trametes versicolor* (L.) Lloyd. Two variants of elicitation were tested: the addition of MeJa on the first and ten of the 20-day growth cycle.

Based on quantitative determinations by the HPLC-DAD method, a significant increase in the accumulation of phenolic acids in the biomass obtained in mycelial cultures was demonstrated, depending on the day of elicitation in the growth cycle (as compared to control samples). Derivatives of hydroxybenzoic acid: gallic, p-hydroxybenzoic, and protocatechuic were determined qualitatively in the examined extracts. Additionally, L-phenylalanine and L-tryptophan were determined. The analysis of the antioxidant activity (DPPH method) and the determination of the total content of polyphenols in the extracts from the elicited biomass were carried out. A significant increase in antioxidant activity

and an increase in the total content of polyphenols in the biomass was found depending on the day of elicitation in the growth cycle.

The tested mycelial cultures of *Trametes* spp. species can be proposed as a model for research on the dynamics of the accumulation of phenolic compounds – structures with recognized antioxidant activity.

**IN VITRO CULTURES OF GANODERMA SPP.
– BIOTECHNOLOGICAL SOURCE OF ANTI-AGING
COMPOUNDS**

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The objects of research were species of medicinal mushrooms from the genus *Ganoderma*: *Ganoderma adspersum* (Schulzer) Donk, *Ganoderma applanatum* (Pers.) Pat., *Ganoderma carnosum* Pat., *Ganoderma lucidum* (Curtis) P. Karst., *Ganoderma pfeifferi* Bres., *Ganoderma resinaceum* Boud. These species are acknowledged the medicinal raw material in TCM.

The study aimed to determine the biosynthetic possibilities of *in vitro* cultures maintained in specially constructed biofermentors with an aeration system. As part of an experiment, the conditions of conducting aerated cultures were optimized according to culture duration (10- and 20-day growth cycles). The content of phenolic acids, hydroquinone, indole derivatives, and selected tyrosinase inhibitors were determined in biomass extracts using the HPLC-DAD method. The total content of polyphenols compounds was analyzed by the Folin-Ciocalteu method. The antioxidant activity of the tested extracts was determined using the DPPH method.

Depending on the length of the culture cycle, the content of individual compounds varied slightly. Extracts grown in 10-day cycles found higher contents than in extracts from biomass grown in 20-day cycles. Among phenolic acids, gallic, protocatechuic and *p*-hydroxybenzoic

acid predominated quantitatively in mostly studied species. Among indole compounds was predominated L-tryptophan, the presence of a trace of melatonin in most study species were detected. Among tyrosinase inhibitors – kojic acid was determined in *Ganoderma appalanatum* and *Ganoderma carnosum* and hydroquinone was confirmed in *Ganoderma applanatum*, *Ganoderma pferifferi* and *Ganoderma resinaceum*.

The model of conducting aerated *in vitro* cultures proposed in studies promotes the accumulation of secondary metabolites with multidirectional therapeutic effects including the anti-aging effect.

THE HISTAMINE H3 RECEPTOR AS A POTENTIAL TARGET FOR THE TREATMENT OF OBESITY

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Nowadays, the pharmaceutical industry is experiencing a crisis in terms of introducing new drugs for the treatment of obesity. The reason for this type of phenomena are frequent side effects resulting from the use of pharmacotherapy, but also the low effectiveness of individual drugs. Currently, the pharmacotherapy of obesity is limited to only a few drugs, such as: bupropion/naltrexone, orlistat, liraglutide. Often, however, these drugs cause only a small degree of weight loss and are discontinued by patients due to adverse reactions occurring during their use. The small number of effective and safe drugs, and the prevalence of obesity in the world is a challenge for scientists and the pharmaceutical industry to look for new potential drugs that may be used in the pharmacotherapy of obesity.

Since the stimulating effect of histamine via histamine H1 receptors located in Central Nervous System on food intake (e.g.: appetite reduction, white adipose tissue lipolysis increment and increment of motor activity) had been discovered, there is a strong need to search for an anti-obesity drug among compounds that may increase the synthesis and release of histamine.

One of the therapeutic goals in the treatment of obesity is the use of histamine H3 receptor ligands. H3 histamine receptors are located in the Central Nervous System and modulate histamine synthesis and release. Histamine has been proven to play a significant role in eating behavior because it causes a loss of appetite and is considered a satiety signal released during food intake. Taking into consideration the location of the histamine H3 receptor (H3R), as well as their physiological functions the use of histamine H3 receptor ligands in treatment of diseases such as obesity has been postulated.

In order to determine potential anti-obesity properties of selected H3R ligands, preliminary pharmacological *in vivo* tests have been performed. In these studies, the influence on body weight, food intake, selected biochemical parameters in plasma and spontaneous activity on rats in the model of excessive eating of preferential feed was tested.

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INDIVIDUAL THERAPY OF PATIENT WITH ACUTE CERVICAL C4/C5 DISCOPATHY

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A male, 54 years old, was referred to rehabilitation center with very strong pain in the cervical spine, with radiation of the pain to the right shoulder. Problems with cervical spine were from many years, but in the last 3 weeks pain increased, and now patient suffers constantly. Static positions without motion (for example: sitting) increase pain. This pain was described as very big. Cervical spine range of motion was decreased significantly. Physical examination: passive and active motion is painful, especially right rotation, extension and lateral right flexion. Repeated flexion, and lateral left flexion increase pain. Upper thoracic spine is hypokyphotic and painful during palpation. Neurologic examination revealed no deficits. Radiologic examination showed diminished cervical lordosis, decreased C3/C4 space, C4/C5 discopathy and small degenerative changes in C3/C4/C5 segments. Established diagnosis: C4/C5 discopathy.

The goal of treatment was to decrease pain and regain painless, full range of motion in cervical spine and cervico-thoracic junction. During therapy, retraction elements and lateral flexion up to pain were applied due to disc centralization. Manual therapy was applied in order to improve the global motion of cervical spine. At home patient had to perform exercises according to McKenzie, for maintaining the recovery. Some elements of deep massage was used for diminishing the muscle

tension in the area of neck and shoulder girdle. This treatment lasted a few weeks, first three times a week, then twice a week. After 10 meetings pain decreased to small; spine mobility improved significantly. Next, we tried to restore full mobility at the individual levels of cervical spine. Due to this, a manual therapy with manipulations on intervertebral joints was applied. Exercises were expanded in order to improve the movement stereotype and muscle strength of the shoulder girdle.

End result after 5 weeks therapy and 15 meetings: we achieved painless full of range of motion and complete pain relief.

INDIVIDUAL REHABILITATION THERAPY OF THE PATIENT WITH DEGENERATIVE CHANGES IN CERVICAL SPINE

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Female, age 51, was admitted to rehabilitation center due to severe pain in cervical spine and upper thoracic spine. Additionally she reported often headaches and shortness of breath. Physical examination: thoracolumbar scoliosis, increased muscle tone of back, significant rib stiffness and significant global restriction of neck motion in all directions. Passive motion was significantly also restricted with firm feeling at the end of motion. Neurologic examination revealed no deficits. Radiologic examination showed reversed cervical lordosis (due to insufficiency of ligamentous apparatus), multi-level discopathy with degenerative changes from C4 to C7, arthrotic changes in Luschka joints. Additionally functional x-rays show instability signs ant levels: C3-C4-C5. Diagnosis: multi-level discopathy with degenerative disease at the same levels and instability at the level C3-C5. The goal of therapy was to decrease muscle tone of the neck and shoulder girdle, improve the mobility of the cervical spine, shoulder girdle (if possible) and increasing thoracic chest (especially ribs) elasticity.

First, the work with the patient included only muscle-fascia techniques. Due to the level of structural changes and intensified muscle tone, mobilization, as well as manipulation techniques were not used. After first 6-7 meetings, we did not achieve permanent improvement: just after therapy patient felt better, but complaints came back. ON the

7th meeting we added new techniques: manipulation acc. to Ackermann for thoracic spine and global, non-targeting manipulation for cervico-thoracic junction. It was done because we wanted the motion to be performer in the whole cervical spine and moved to the upper thoracic segments (not only in the middle cervical spine as previously). The area with the biggest degenerative changes was also the most overstretched region during normal daily activity of the patient. It was connected with the posture, motion stereotype and gradually worsening (over years) of the motion in the shoulder girdle and whole chest. Such therapy was repeated during 5 meetings with only small improvement. After 10 sessions, range of motion improvement slightly, medium movement

restriction remained. Pain decreased from big to medium one. Special set of exercises was prepared for patient, in order to improve slowly the length of neck and shoulder muscles. Exercises aimed to remain the best mobility of upper trunk. Patient returned after several months. During anamnesis we found out that a few weeks after completing therapy, she exercised very systematically and her state of being improved significantly. Later, she stopped exercises and after months pain and neck stiffness increased. It is a typical finding in patients with advanced structural changes: for stable pain control systematically done exercises plus rehabilitation therapy is necessary or complete change of lifestyle.

ENZYMATIC DEGRADATION OF POLYMERIC MATERIALS PLA, PCL AND PLA/PCL MIXTURES IN ENVIRONMENTS WITH DIFFERENT ENZYMATIC ACTIVITY

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The aim of the work was to produce biodegradable films from PLA, PCL and PLA/PCL (50/50 wt.%) with an alternative extrusion method and subjecting these materials to enzymatic degradation tests [1].

The paper presents the results of research on the susceptibility of PLA, PCL foil and PCL/PCL mixture to enzymatic degradation with the use of enzyme solutions of microbiological origin. The scope of topics included analyzes in enzymatic solutions containing proteinase K, protease, esterase and lipase. The loss of foil mass was determined after the samples were incubated in enzymatic solutions.

It was shown that the enzymes proteinase K and protease had the greatest influence on the enzymatic degradation of the tested materials. Under the conditions of the enzymatic degradation tests performed, the greatest weight loss was found in the PLA film, and the smaller in PCL and PLA/PCL. We have shown that the foil has biocidal properties against human pathogens and phytopathogens. Moreover, the obtained films, especially the PLA/PCL mixture, have a potential application in the pharmaceutical industry due to changed and favorable physico-chemical properties.

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BIOFILM FORMATION DURING BIODEGRADATION PLA, PCL AND PLA/PCL MIXTURES IN ENVIRONMENTS WITH DIFFERENT MICROBIOLOGICAL ACTIVITY

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A key aspect in biodegradation processes is the ability to create bacterial biofilm on a given surface. Bacterial biofilm is a structure that protects cells from bactericidal substances. In addition to its protective function, it also plays a huge role in the initial biodegradation process [1-7].

The paper presents the results of research on the susceptibility of PLA, PCL foil and PCL/PCL mixture to biodegradation determined by the biochemical oxygen consumption by microorganisms (BOD) method. The thematic scope included analyzes of the biodegradation process in compost, activated sludge, river and sea water. The amount of oxygen consumed by the microorganisms was determined in the presence of the tested films. Images of the surfaces of individual samples were made by scanning electron microscopy (SEM), confirming the formation of a bacterial biofilm.

It was shown that the compost and sludge had the greatest impact on the biodegradation of the tested materials. The best degradation environments turned out to be compost and activated sludge, and the most susceptible materials to biodegradation were PCL and the PLA/ PCL mixture. Moreover, the obtained films, especially the PLA/PCL mixture, have potential applications in medicine and the pharmaceutical industry due to their changed and favorable physico-chemical properties.

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DETERMINATION OF CITICOLINE IN VARIOUS DOSAGE FORMS BY HPLC METHOD WITH UV DETECTION

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The analysis of the content of citicoline in various dosage forms was performed by high performance liquid chromatography (HPLC) with UV detection. The Merck-HITACHI D-7000 liquid chromatograph with the L-7400 UV detector and RP-18 column with a particle size of 5 μm was used. An isocratic elution was used, the mobile phase was methanol : water 75 : 25 (v / v). The detection wavelength was set at the wavelength $\lambda = 225 \text{ nm}$. The flow rate of the mobile phase through the column was 1 mL/min; dosing volume of 20 μl . The column temperature was 21°C.

The quantitative analysis was carried out using the calibration curve method, based on the assumption of linear dependence of the area under the curve and the concentration of citicoline. Standard curve was made on the basis of chromatographic analysis of dilutions of standard substance: 5-200 $\mu\text{g/mL}$. The content of citicoline, an active substance of selected dietary supplements in the form of eye drops, capsules and tablets, as well as the drug in the form of an oral liquid, was determined. The specified contents were consistent with the manufacturers' declarations and amounted to 100,61% for the drug and 97,20-104,46% of the tested dietary supplements.

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PRODUCTION OF IRIDOID GLYCOSIDES – VERBENALIN AND HASTATOSIDE IN SHOOT CULTURES OF VERVAIN (*VERBENA OFFICINALIS*) MAINTAINED IN VARIOUS *IN VITRO* SYSTEMS

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Verbena officinalis is a widely distributed medicinal plant species with valuable biological activities especially due to its antioxidant, antibacterial, and anti-inflammatory properties. Vervain is a rich source of iridoids, phenylpropanoid glycosides, flavonoids, terpenoids, and essential oil [1]. In our earlier studies we confirmed high production of verbascoside and isoverbascoside in undifferentiating callus, suspension and bioreactor cultures. cultures of this species [2,3].

In our recent research we focused on the production of hastatoside and verbenalin in *V. officinalis* shoot differentiating cultures cultivated under various conditions. Four types of cultures: agar cultures, agitated cultures, stationary liquid cultures maintained in Magenta vessel, and cultures grown in RITA bioreactors were tested. Two basal media – Murashige & Skoog (MS) medium and Schenk & Hildebrandt medium (SH) and three different compositions of plant growth regulators (6-benzylaminopurine - BAP, indole-3-butyric acid - IBA, tiazaurone - TDZ, 2-izopentyladenine – 2-iP and adenine) were tested. The content of iridoid glycosides was determined in biomass extracts using HPLC-DAD method [4].

The amounts of these compounds glycosides were varied depending on in vitro conditions. The accumulated amounts of verbenalin reached 64.2 - 181.0 mg/100g DW and hastatoside 60.1-173.9 mg/100g DW. The highest contents of both compounds were confirmed in cultures main-

tained in RITA bioreactor on SH medium supplemented with 2 mg/L 2-iP and 0.22 mg/L TDZ.

The obtained amounts of verbenalin and hastatoside, especially in biomass cultivated in Rita bioreactor are interesting from practical point of view. Bioreactor shoot cultures of vervain could be the potential reach source of iridoids independent of environmental conditions and environmental pollution.

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**ANTIMICROBIAL ACTIVITY OF *SCHISANDRA HENRYI*
EXTRACTS OF BIOMASS FROM MICROSHOOT CULTURES
AND LEAVES OF PARENT PLANT**

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The object of this research was the endemic for Yunnan province China plant species from *Schisandraceae* family – *Schisandra henryi* C.B. Clarke. Only few research has been done on this species. *S. henryi* has been shown to have similar biological effects to the pharmacopoeial species - *Schisandra chinensis* (Turcz.) Baill [1].

The aim of the study was an evaluation of the antimicrobial properties of *S. henryi* in vitro culture and leaf extracts of parent plant.

Under the experiment the *S. henryi* microshoot cultures were maintained on agar Murashige-Skoog medium [2] supplemented with 1 mg/l BAP and 1 mg/l IBA, over 30 days growth periods (3 series). The leaves were harvested from the parent plant in May 2016 (“Clematis”, Poland). Antibacterial and antifungal tests were performed using the micro-dilution method in accordance with the requirements of the European Committee on Antimicrobial Susceptibility Testing using Mueller-Hinton broth and Mueller-Hinton broth with 5% sheep blood for bacterial growth or RPMI from MOPS for fungal growth [3,4].

Under the study the following bacteria were tested: Gram positive strains - *Staphylococcus aureus* ATCC25923, *S. aureus* ATCC43300, *S. epidermidis* and Gram negative strains - *Escherichia coli*, *Helicobacter pylori* and *Pseudomonas aeruginosa*.

For studied bacteria, the MIC (minimum inhibitory concentration) values of biomass extracts ranged from 0.625 mg/ml (*H. pylori*) to 10 mg/ml (*E. coli* and *P. aeruginosa*). MBC (minimum bactericidal concen-

tration) values ranged from 0.625 to 10 mg/ml. The lowest MBC value was obtained for *H. pylori*.

The MIC values of *S. henryi* leaf extracts ranged also from 0.625 mg/ml to 10 mg/ml. For all strains of Gram positive bacteria tested, the MIC values were 1.25 mg/ml, and for Gram negative bacteria varied from 0.625 mg/ml for *H. pylori* to 10 mg/ml for *E. coli* and *P. aeruginosa*. MBC values for *S. henryi* leaf extracts ranged also from 0.625 mg/ml to 10 mg/ml. The lowest MBC value was shown for *H. pylori*.

For studies of antifungal activity the following strains were used: *Aspergillus niger*, *Candida albicans*, *C. glabrata*, *C. parapsilosis* and *Trichophyton menthagrophytes*. The MIC and MFC (minimum fungicidal concentration) for the biomass extracts were 10 and 20 mg/ml, respectively. The MIC values for leaf extracts ranged from 5 to 10 mg/ml, and MFC from 10 mg/ml to 20 mg/ml. The lowest MIC value was obtained for *C. albicans* and *C. glabrata* strains.

This is the first report documented the antimicrobial activity of extracts from *S. henryi* microshoots cultured in vitro.

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IN VITRO OSTEOGENIC DIFFERENTIATION OF HUMAN DENTAL PULP STEM CELLS.

A VIEW THROUGH SPECIFIC PROTEIN PROFILING

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One of the main challenges in regenerative medicine is identification of strategies for bone injuries repair and regeneration originated from various pathological problems. Bone regeneration is complex and well organized physiological process, in which mesenchymal stem cells (MSCs) have a important role. Their differentiation is regulated by specific signaling molecules (growth factors/cytokines and hormones) and activated intracellular pathways [1].

Human dental pulp stem cells (hDPSC) are multipotent adult mesenchymal dental-derived stem cells. Among other, they can differentiate into osteoblasts and odontoblasts and form reparative tissue. One from frequently used methods imitating osteogenesis microenvironment is differentiation using specific induction media [2]. The exact mechanism by which cells differentiate and form a mineralized extracellular matrix is not fully understood, yet. In this work, we focused on protein profiling of this specific process. Commercial human hDPSC cells was in vitro cultivated in specific osteogenic cultivation media for 24 days and differentiation kinetics was monitored by light microscopy. During all the time we detected presence of mineral deposits (calcificates) by Alizarin Red staining. Following that, calcificates were also quantified to amount of total proteins. On 24 day, recommended in the literature where maxi-

mal differentiation was observed, total proteins were isolated by optimized protocol. To understand molecular details of this process, in solubilized protein fraction, abundances of specific osteogenic proteins were detected. We found out, not only osteoblastic but also odontoblastic specific proteins were produced with various and unexpected abundances.

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BIOLOGICAL EFFECT OF WIRELESS SYSTEM ON HUMAN HEALTH

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The increasing presence of electromagnetic sources in our daily life and environment persuade us to investigate the mechanisms of electromagnetic field's (EMF) influence on biological structures. Several studies reported effects of EMF exposure, which can affect many cellular mechanisms [1]. However, EMF exposure could be without any detectable adverse effect to living organisms. There are only a few in vitro studies focused on human health risks at intermediate frequency range and almost no studies about wireless power transfer used for charging electric vehicles.

Human dermal fibroblasts are routinely used in cell laboratories for various types of experiments. Because skin cells are the cells which are most exposed to EMF in living organisms, we decided to use this cell type in our study to analyze different cellular processes.

To analyze the effect of EMF radiation of the wireless power systems on human cells, an experimental prototype of wireless charging system utilizing circular shaped coupling coils was constructed at University of Žilina. During the experiments, 86 kHz intermediate EMF was used to meet IEEE standards. We compared the metabolic activity (MTT test), genomic stability (Comet assay) and cell viability (Annexin assay) in exposed cells to control. After exposition (12 mT, 700W, 30 min, 37°C), cells were cultivated at standard conditions for another 44 hours

to regenerate and to see the possible cumulative effect on cellular processes.

We did not find any differences in the metabolic activity, cell viability as well as in DNA damages and did not prove the negative effect of intermediate EMF generated by wireless energy transfer system human fibroblasts. However, it will be very useful to analyze the prolonged exposition or pulse charged points together with different types of cells to know the safety of constructed prototype.

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THE ELICITATION EFFECTS OF SALICYLIC ACID ON *ISATIS TINCTORIA* L. SHOOT CULTURES GROWTH

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Isatis tinctoria L., belonging to the *Brassicaceae* family, is an herbaceous biennial, or short-lived perennial plant species also known as woad. It is spread widely across Europe, Eastern Asia and North-Africa and is mainly known for its properties as a dye, but the scientific literature reports a long history of its medical usage since ancient times [1,2]. Lately its potential anti-inflammatory, anti-tumor, antiviral, antimicrobial, antioxidant and analgesic effects, due to its bioactive compounds, have been discovered [1,3]. The aim of the study was to perform elicitation of *I. tinctoria* in vitro agitated shoot cultures with salicylic acid (SA), which is the model of environmental stress.

The elicitation was performed by inoculating 1 g of *I. tinctoria* developed biomass in 250 ml flasks containing 100 ml liquid Murashige and Skoog (MS) medium [4] supplemented with 1.0 mg/l 6-benzylaminopurine (BAP) and 0.5 mg/l α -naphthaleneacetic acid (NAA) as growth regulators. After three weeks of growth, SA was added in three different concentrations 100, 200 and 400 μ M. The cultures were harvested respectively, at three different times, after the addition of the elicitor: 24 h, 48 h and 120 h.

Fresh biomass increments varied from 3.88- to 7.69-fold 24 h after addition of SA, from 4.35- to 9.22-fold after 48 h, and from 5.01- to

8.75-fold after 120 h. The best results after elicitation with 100 μM SA (5.03-fold) were obtained after 120 h, with 200 μM SA (7.69-fold) after 24 h, with 400 μM SA (9,22-fold) after 48 h, respectively. The results showed that the addition of SA positively affects *I. tinctoria* cultures' growth and biomass morphology. The phytochemical analysis of biomass extracts is now in progress. In the future other biotic and abiotic elicitors will be also tested.

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THE EFFECT OF EICOSAPENTAENOIC ACID ON HUVEC ENDOTHELIAL CELLS ACTIVATED WITH BENZO(A)PYRENE

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Endothelial cells are characterized by intense metabolic activity. Exposure to environmental pollution plays an important role in the etiology of inflammation and atherosclerosis. Polycyclic aromatic hydrocarbons (PAHs) are widespread lipophilic impurities present in the air, soil, and also in grilled foods.

The aim of the study was to determine the effect of eicosapentaenoic acid (EPA, C20:5 n-3) on pro-inflammatory gene and protein levels in human umbilical vein endothelial cells exposed to benzo(α)pyrene (BaP).

The effects of EPA (50 μ mol) and/or BaP (1 μ mol) in HUVEC cells on cyclooxygenase-2 (COX-2, PTGS2), S-glutathione transferase (GSTM1) and aromatic hydrocarbon receptor (AHR) was analysed by RT-qPCR and Western blot.

In HUVEC cells incubated with EPA and BaP, significant repression PTGS2 and AHR gene was observed when compared to BaP activated cells. Overexpression of GSTM1 and a decrease in the level of 8-isoPGF2 α have also been shown which may be associated with the antioxidative properties of EPA in endothelial cells exposed to BaP.

The results suggest that EPA can significantly affect the function of HUVEC cells and reduce the harmful effects caused by benzo(α)pyrene.

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EICOSAPENTAENOIC ACID SUPPLEMENTATION IN MURINE 3T3-L1 FIBROBLASTS ACTIVATED WITH INFLAMMATORY FACTORS

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The cellular, biochemical and pharmacological mechanisms of action of lipid mediators are still not fully understood. Therefore reprogramming of fatty acid metabolism under inflammatory conditions in adipocyte *in vitro* models seems advisable.

The aim of this study was to determine the effect of eicosapentaenoic acid (EPA, C20:5 n-3) on murine 3T3-L1 fibroblasts (preadipocytes) activated with inflammatory factors, lipopolysaccharide (LPS), tumour necrosis factor (TNF- α) and benzo (a) pyrene (BaP).

3T3-L1 cells before and after differentiation into adipocytes were incubated with EPA (50 μ mol) for 24 h and activated by inflammatory factors (LPS, TNF- α or BaP). Cyclooxygenase-2 (COX-2), Toll likereceptor 4 (TLR4), peroxisome proliferator-activated receptor γ (PPAR γ), glucose transporter type 4 (GLUT4) and Fatty acid binding protein 4 proteins level were analysed by Western blot. Isoprostanes and prostaglandin F3 α level was determined using EIA technique.

Statistically the highest levels of COX-2 and TLR4 were found in cells activated with inflammatory factors (TNF- α and LPS). The activation of the FABP4 fatty acid receptor and the GLUT4 receptor suggests a significant role of EPA in the regulation of adipocyte metabolism. The addition of EPA to 3T3-L1 cells significantly decreased the amount of 8-iso PF2 α and 8-isoPGF3 α isoprostanes and increased the level of prostaglandin F3 α (PGF3 α) despite BaP activation. A high level of PGF3 α may suggests an anti-inflammatory effect, because it is a product of the transformation of eicosapentaenoic acid.

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ANTI-INFLAMMATORY ACTIVITY OF BIOMASS EXTRACTS FROM BIOREACTOR'S *IN VITRO* CULTURES AND LEAF AND FRUIT EXTRACTS OF *SCHISANDRA CHINENSIS*

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Extracts from plants rich in bioactive compounds are effective in the mitigation of inflammation processes which are an important risk factors in the pathogenesis of numerous chronic diseases. *Schisandra chinensis* is the valuable Far-East medicinal plant species; it has been used in official phytotherapy. *Schisandra* fruit extract shows, among others hepatoregenerative, adaptogenic, antioxidant, and antitumor properties. The main constituents of *S. chinensis* extracts are dibenzocyclooctadiene lignans [1,2].

The aim of this study was to investigate the anti-inflammatory potential of biomass extracts of *S. chinensis* microshoots maintained in Plantform (Sweden) bioreactor (on Murashige-Skoog [3] medium supplemented with 3 mg/l BA (6-benzyladenine) and 1 mg/l NAA (1-naphthaleneacetic acid), over 30 days growth periods), and in comparison in the extracts of parent plant material (leaves and fruits) (Clematis, Poland). The studies were involved also the most abundant lignans present in plant extracts: 6-O-benzoylgomisin O, schisandrin, gomisin D, gomisin N and schisantherin A.

For the evaluation of anti-inflammatory activity, tests based on the *in vitro* inhibition of 15-lipoxygenase (15-LOX), phospholipase A2 (sPLA2), cyclooxygenase-1 (COX-1), and cyclooxygenase-2 (COX-2) enzymes (Cayman Chem. Co.) were applied.

Evaluation of 15-LOX inhibition showed that fruit, leaf, and microshoot extracts moderately inhibited this enzyme: 25, 28, and 21% (at 17.5 µg/ml).

The sPLA2 enzyme inhibition assay showed that leaf and microshoot extracts inhibited its activity to 25 and 35%, respectively (at 175.0 µg/ml).

For COX-1 and COX-2 enzyme the percent of inhibition (at 175.0 µg/ml) was 69% and 77% for leaves, and 80% and 55% for in vitro cultures, respectively.

Evaluation of anti-inflammatory properties of individual lignan solutions and the “average lignan composition” sample (MIX) showed that they were not active against the sPLA2 enzyme. All studied lignans showed from 49% to 57% 15-LOX inhibitory activity at 0.175 µg/ml.

The anti-inflammatory activity of biomass extracts from *S. chinensis* bioreactor cultures under this study was examined for the first time. Extracts from in vitro cultures showed higher and/or similar anti-inflammatory activity compared to plant material.

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**ANTIMICROBIAL ACTIVITY OF *SCHISANDRA CHINENSIS*
BIOMASS EXTRACTS FROM PLANTFORM BIOREACTOR
GROWN MICROSHOOTS AND OF PARENT PLANT
MATERIAL**

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Schisandra chinensis (Turcz.) Baill. (Chinese magnolia vine) it's pharmacopoeial plant species in East Asia and also in Europe with well-established e.g. adaptogenic, hepatoprotective, antioxidant and anti-cancer activity. Biological properties of the fruit extracts are attributed mostly to dibenzocyclooctadiene lignans, called the schisandra lignans (SL) [1]. The aim of the present study was to investigate the antimicrobial properties of biomass from *in vitro* cultures and parent plant leaf and fruit extracts.

Under the experiment the *S. chinensis* microshoots were maintained on Murashige-Skoog medium [2] supplemented with 3 mg/l BA (6-benzyladenine) and 1 mg/l NAA (1-naphthaleneacetic acid), over 30 days growth periods in Plantform (Sweden) bioreactors (3 series). The leaves and fruits were harvested from the parent plant in June 2018 (Clematis, Poland). Antibacterial and antifungal tests were performed using the micro-dilution method in accordance with the requirements of the European Committee on Antimicrobial Susceptibility Testing using Mueller-Hinton broth and Mueller-Hinton broth with 5% sheep blood for bacterial growth or RPMI from MOPS for fungal growth [3,4].

Under the study the following bacteria were tested; Gram positive strains: *Staphylococcus aureus* ATCC25923, *S. aureus* ATCC43300, *S. epidermidis*, and Gram negative strains: *Escherichia coli*, *Helicobacter pylori* and *Pseudomonas aeruginosa*. For Gram positive bacterial strains, the minimum inhibitory concentration (MIC) values for the *in*

in vitro biomass extracts ranged from 1.25 to 10 mg/ml. The lowest MIC was confirmed for *S. epidermidis*. For Gram negative bacteria strains the MIC values ranged from 0.625 to 10 mg/ml. The lowest MIC value was showed for *H. pylori*.

The MIC values of leaf and fruit extracts ranged from 0.625 to 10 mg/ml for all strains. The lowest MIC was obtained for Gram negative bacteria - *H. pylori*.

For tests of antifungal activity, the following strains were used: *Aspergillus niger*, *Candida albicans*, *C. glabrata*, *C. parapsilosis* and *Trichophyton menthagrophytes*. The lowest MIC values (2.5 mg/ml) were showed for *C. albicans* and *C. parapsilosis*.

This is the first report documented the antimicrobial activity of extracts from *S. chinensis* microshoots cultured *in vitro*.

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BEHAVIORAL FLEXIBILITY DEFICITS IN ZINC-SENSING GPR39 RECEPTOR KNOCKOUT MICE

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Background: Zinc modulates the function of ~10% of proteins in the human body. In Alzheimer's dementia (AD), a peripheral zinc deficiency is accompanied by increased zinc levels in AD patients' brains, where the amyloid beta protein (a biomarker of AD) binds zinc with high affinity. Not only does the amyloid beta become more toxic when bound to zinc, but it also depletes zinc from synapses, where the ion plays an important role in physiological mechanisms of learning and memory. We have recently shown that a metabotropic receptor specifically activated by zinc: the GPR39, is one of the factors behind episodic-like memory in mice. Here we extend these findings to behavioral flexibility, i.e. the ability to adapt responses to changes in the environment.

Methods: We measured behavioral flexibility of wild-type (WT) and GPR39 knockout (KO) mice with the Attentional Set-Shifting Task (ASST). In the ASST animals are presented with two bowls filled with two different digging materials (tactile dimension) and scented with two different odors (olfactory dimension). Mice have to locate a food reward, which is associated with one of the exemplars of a dimension, while ignoring the other dimension. After forming this „attentional set”, the reward becomes associated with one of the exemplars of the previously ignored dimension and the number of trials required to relearn the task (extradimensional shift, EDS) is measured.

Results: KO mice did not differ from their WT conspecifics in the number of trials and errors made during the stages leading to the EDS (including reversal learning), but required significantly more trials to perform the EDS (mean \pm SEM: 21.7 \pm 1.9 vs 16.6 \pm 1.3).

Conclusions: The lack of deficits in reversal learning combined with a slight but specific impairment in EDS suggest, that GPR39 KO affected the medial prefrontal cortex, which along hippocampus is highly prone to AD pathology. Since episodic memory and cognitive/behavioral flexibility deficits are the only psychological impairments correlated with amyloid beta levels in humans, these results suggest that GPR39 may be a promising target for adjunct pharmacotherapy of AD.

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THE ANTIOXIDANT ACTIVITY OF SELECTED SPECIES OF THE *PLEUROTUS* SPP.

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Mushrooms are a valuable source of proteins, lovastatin, mineral salts and vitamins. They are categorized as functional foods because they have a multidirectional and positive effect on the human body. To date, their anti-cancer activity has been demonstrated in studies on aqueous and methanol extracts. They have been found to lower blood cholesterol and triglyceride levels, as well as anti-diabetic, antibiotic, antiviral, immunomodulatory, anti-cancer, anti-inflammatory and antioxidant activity.

The *Pleurotus* spp. is a group of edible mushrooms, which covers about 70 species occurring on all continents except *Antarctica*. In Poland, parks, gardens and forests are places of their common occurrence. The main source of nutrients for *Pleurotus* spp. is wood, usually deciduous trees. Most often they grow on the lateral surface of the tree, hence their common name – the oyster mushrooms.

In the present study we used *in vitro* cultures of the following *Pleurotus* spp. species: *Pleurotus osteratus*, *P. pulmonarius*, *P. djamor*, *P. eryngii*, *P. citrinopileatus*. We established that all of these species are sources of health-promoting substances, which include polyphenols, phenolic compounds, phenylalanine, hydroquinone and azelaic acid. These raw materials can be used in care products directed primarily at people with mature skin that requires regeneration, as well as people struggling with the problem of hyperpigmentation. Oyster mushroom extracts are also useful in the treatment of skin diseases, among others in atopic dermatitis. The experiment confirmed the antioxidant activity of selected species of the *Pleurotus* spp., which also has a therapeutic effect and is important in cosmetology.

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ENRICHED *FOMITOPSIS OFFICINALIS* MYCELIUM AS A SOURCE OF INDOLE COMPOUNDS

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Fomitopsis officinalis is a polypore mushroom, which – due to its extensive use in folk medicine – is endangered with extinction. Therefore, the mycelia from *in vitro* cultures of *Fomitopsis officinalis* enable scientific studies of possible nutritional and medicinal properties of the mushroom to be conducted, without its further degradation. Moreover, the *in vitro* cultivation method also enables precise control of nutritional content of the medium upon which the mycelia grow.

In the present study we enriched the medium of *Fomitopsis officinalis*'s cultures with inorganic sulphate(VI) and chloride salts of magnesium (Mg) and zinc (Zn). Both biometals are important nutrients and accumulate in mushroom mycelia, which could be utilized as an organic means of supplementation. To this date, however, the impact of Mg and Zn enrichment of *Fomitopsis officinalis*'s growth and chemical content has not been studied. Thus, we also measured the amount of indole compounds in the enriched mycelia with RP-HPLC method with UV detection, and established their absorption via an in-house artificial digestive system.

We observed no impact of Mg and Zn enrichment on mycelium growth, which was optimal regardless of the medium content. The following indole compounds were detected in *Fomitopsis officinalis* mycelia: 5-hydroxytryptophan, L-tryptophan, tryptamine, melatonin, serotonin and 6-CH₃-D,L-tryptophan. Among these, 5-hydroxytryptophan was present in the highest amounts ranging from 118 to 518 mg/100 g of dry mushroom weight. After extraction to gastric and intestinal juices, 5-hydroxytryptophan was also detected in the highest amount.

In summary, the mycelium of *Fomitopsis officinalis* enriched with Mg and Zn is a good source of indole compounds (5-hydroxytryptophan, L-tryptophan). Since these compounds are metabolized into serotonin in the human body, the mycelium obtained from *in vitro* cultures could potentially be used as a supplement in disorders characterized by serotonin deficiency (ex. affective disorders).

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DISINFECTANTS USED IN STOMATOLOGY EFFECTIVE AGAINST SARS-COV-2 INFECTION

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Dentists are a group of healthcare professionals who are constantly exposed to contact with infected patients and infectious materials. It is therefore necessary to implement additional safety procedures related to the ongoing pandemic in order to prevent the infection of medical personnel and patients. Disinfection in the dental office involves disinfection concerns not only of hands but also of surfaces, medical equipment, and air. To reduce the amount of the virus in the oral cavity, it is recommended to rinse the oral cavity with an antiseptic. On the basis of chemicals effective against HCoV, it was concluded that 0.1% sodium hypochlorite, 0.5% hydrogen peroxide, 62–71% ethanol in 1 min and 78–95% ethanol in 30 s to 1 min have the disinfectant effect on SARS-CoV-2 contaminated surfaces. Similarly, WHO recommends 70% ethanol for disinfection of small surfaces. Moreover, to reduce the amount of SARS-CoV-2 in saliva, the authors recommend rinsing the oral cavity with 0.5–1% hydrogen peroxide or 0.2% povidone-iodine. The recommendations for dentists developed by the Polish Dental Society (PTS) together with those developed by the Center for Technology Transfer Ltd., Academic Dental Clinic of the Medical University of Wrocław, and external experts were published in March 2020, in which the authors recommend antiseptic agents that can effectively inactivate the SARS-CoV-2 virus, e.g. antiseptics containing 78–95% ethanol; 0.23–7.5% iodopovidone solution; 0.01–0.21% sodium hypochlorite; 0.5% hydrogen peroxide. It

should also be noted that it may be worthwhile to use mats saturated with disinfectants at the entrance to the dental surgery office to eliminate viruses from the soles of shoes of the patients.

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MONITORING OF Mg CONCENTRATION IN A LIQUID MEDIUM FOR *IN VITRO* CULTURES OF *BASIDIOMYCOTA* MUSHROOMS

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Due to the natural ability of mushrooms to bioaccumulate metals, mushrooms have been considered as bioindicators of environmental pollution since last years. Contrary to the popular opinion that mushrooms are all about taste, numerous scientific studies proved that mushrooms from the *Basidiomycota* (including *Agaricus bisporus* and *Imleria badia*) are rich in numerous ingredients which have a confirmed biological activity. These include phenolic, indole, terpenoids compounds, polysaccharides, vitamins, and bioelements. In the last decade, more and more experimental works have been carried out in which the ability of mushrooms to adsorption of metals are used in order to obtain biomass – mycelium enriched with selected bioelements. During the conducting research in *in vitro* conditions allows for the optimization of the breeding process – obtaining a repeatable amount of biomass (mycelium) with a specific quantitative and qualitative composition. Due to the fact that the cultures are provided in sterile conditions, it is justified to determined the changes in the composition of the medium during mycelium growth. In the presented experiment were carried out to obtain of the change in Mg concentration in the liquid medium according to Oddoux for *in vitro* cultures of *A. bisporus* and *I. badia*. The analysis was carried out over a period of 14 days with a special device. The collected medium was sub-

jected to the microwave mineralization process in a closed system with the use of 30% perhydrol and 65% nitric acid. The obtained solution was tested using the Atomic Absorption Spectrometry method. Based on the tests performed, it was found that during the growth of mushroom mycelia the Mg accumulation from the medium lead to accumulation of tested bioelement for *A. bisporus* was higher – 82% than for *I. badia* – 63%. The conducted research confirms the ability of the mushrooms to remediate.

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POSSIBILITIES OF APPLYING MODIFIED POLYMER MEMBRANES FOR WASTEWATER TREATMENT

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It is known that modern industries use a huge amount of water which need treating before the final discharge. Dairy industry is one of the most polluting industries with high pollution load of fats, proteins and other contaminants [1]. Membrane technology could be an efficient solution to purify wastewaters thanks to its advantages (easy operation, high purification efficiency, etc.), however the main limiting factor for economical application is the fouling. There are several studies proposed that the antifouling properties of polymer membranes can be enhanced by adding nanoparticles into the membrane [2]. Thus, the goal of this study was to understand filtration mechanism of bovine serum albumin (BSA) with different types of modified and neat membranes.

The aim of my study was the investigation of the efficiency of nanoparticle- modified PAN 50 kDa and PVDF 100 kDa polymer membranes on the ultrafiltration of BSA protein as a model dairy wastewater. The main topic of my work was the comparison of the two types of membranes by their flux, resistance, removal effectiveness and degradation by UV light, and the effect of the photocatalyst on these parameters.

UV light caused a structural change in the neat membranes. During the filtration of BSA, the decrease in flux was significant on both membrane types. This decrease was less for TiO₂- covered PAN membranes and PVDF without a photocatalyst. In terms of PVDF membranes, resistances were lower for those without photocatalyst, while regarding PAN membranes, for those that were covered with TiO₂. Removal effi-

ciencies were excellent both on the neat and modified PAN membranes, as well as on PVDF photocatalyst-free membranes, but it was lower for the TiO₂ modified PVDF.

My research revealed that the TiO₂ photocatalyst is may have been capable of modifying the structure of the protein, thus resulting in different filtration parameters.

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**JAN ANTONI MIKULICZ-RADECKI – AN OUTSTANDING
DOCTOR, INVENTOR OF THE PROTECTIVE MASK
(1850-1905)**

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On May 16, 2020, 170 years have passed since the birth of an outstanding Polish surgeon, Jan Antoni Mikulicz-Radecki. His contribution to humanity at the time when the SARS-CoV-2 virus has significantly affected lives of people around the world, the role of protective masks is emphasized. Their inventor was exactly Jan Mikulicz-Radecki. This outstanding doctor was not only one of the pioneers of antisepsis and asepsis, but also the inventor of surgical instruments (for example endoscope, Mikulicz's tamponade, scoliozometer). He dealt with general surgery, gastroenterological surgery, thoracic surgery, urology, as well as orthopedics (the problem of valgus knee) and an oncological surgery. In the initial period of his work, as an assistant of Prof. Billroth, discovered giant cells named after him (Mikulicz cells) and the pathogenesis of rhinoscleroma. Later in his life he described what we now call Mikulicz's disease (a noninflammatory disease of the salivary and lacrimal glands). He was the founder of the Kraków and the Breslau School of Surgery. He was also interested in wound healing. He spread the use of iodoform dressings for this purpose, replacing them with the carbolic acid (he used to clean tools and hands). For the treatment of wounds, he recommended an ointment (Mikulicz's ointment), which contained a balm of Peru. He modified general anaesthesia by replacing chloroform with ether. He was the first to describe gastric cancer in an endoscopic

examination. It is worth emphasizing that it was characterized by an interdisciplinary approach to diagnostics and treatment.

After moving to Wrocław, he created the most modern clinic in Europe with first aseptic operating room (1897). The Clinic used sterile cotton gloves and masks that covered mouth and nose. Hand and operating field disinfection was introduced at the Clinic in Wrocław and with time also rubber gloves. Prof. Mikulicz-Radecki introduced aseptic rules and required employees to follow them. He used a steam sterilizing apparatus and replaced the sponges with gauze during the operation. Masks were used for the first time in the world by Mikulicz-Radecki in 1897. They were used to prevent cross-infections in the course of scarlet fever and diphtheria. Their effectiveness was appreciated during the First World War. Gradually over the course of the 20th century, wearing masks in the operating room became a matter of course. Today we cannot imagine medicine without them.

Jan Mikulicz-Radecki worked as a professor in Kraków, Königsberg and in Breclau. He was an extremely hardworking, demanding, multi-talented man, characterized by perseverance and consistency. He was not only a doctor for the patients, but also a friend. As a student he was forced to earn a living because he chose medicine against his father's will. At that time, he earned his living by tutoring German and giving piano lessons. Before he became an assistant to Professor Theodor Billroth he worked as a volunteer in his Clinic for 3,5 years (1877). He was a comprehensively gifted man, a musician, he played not only the piano but also the organ. Mikulicz-Radecki loved travelling and hiking. He has undoubtedly rendered great services to the whole Europe and the world, and now 170 years after his birth, it is hard to believe that without him humanity would have had the protection of a mask.

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ASSESSMENT OF ZINC AND MAGNESIUM LEVELS IN TEETH OF RATS FED WITH DIFFERENT DIETS

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It is known that different diets may affect the level of certain substances necessary for proper functioning of the body. Eventually, following a certain diet may influence the level of particular compounds deposited in hard tissues, for instance teeth and bones.

In the present study we aimed at assessing the levels of zinc and magnesium in teeth collected from male and female Wistar rats that were fed with different diets: standard diet (SD), high-fat diet (HFD), high-carbohydrate diet (HCD) or mixed diet rich in fat and carbohydrate (HMD). Teeth were collected either from 28-days-old or 70-days-old rats. Assessment of zinc and magnesium levels was done by the means of atomic absorption spectrometry (AAS). Before the analysis, the samples were subjected to wet mineralization.

It was found that zinc levels were not significantly affected by the type of diet, regardless of the sex or age of the animals. Significantly higher magnesium level was found in teeth from 70-days-old male rats fed with HFD in comparison to the ones fed with SD. Similarly, in teeth from 70-days-old female rats fed with HFD, magnesium level was significantly higher than in female rats fed with SD. Significant differences in magnesium levels in teeth from 28-days-old rats were only found for males: for animals fed with HCD or HFD it was significantly higher than for animals fed with SD.

The obtained results show that different type of diet may affect the level of magnesium but not zinc in Wistar rats' teeth.

COVID-19 AS A PERFECT MURDERER...

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What is the (supposedly) unattainable perfection in murder? Of course to murder leaving no, even the tiniest trace. Even better, to leave the false scent, a “red herring” which misleads the police pursuit! Translating this Agata Christie – inspired background into biomedical reality we think that it is rightful to denounce COVID-19 as very probable murderer that deserves the highest appreciation (of being a “perfect” one). This conjectures are the result of the autopsy findings in the deceased patient strongly suspected of COVID-19 infection but who turned out to be twice COVID-19 negative in PCR tests and also negative in the test based on nasopharyngeal and direct lung section smears performed during autopsy. Of most intriguing autopsy findings in this prior to the very short disease apparently healthy and well fit middle aged man the truly puzzling was diffuse and conspicuous presence of numerous myeloperoxidase (MPO) positive blast cells in many organs like lungs, liver, spleen, lymph nodes. Moreover there were some morphological features of hemophagocytic syndrome (hemophagocytic lymphohistiocytosis). It seems to be noteworthy that hemophagocytic syndrome (well-known of being one of the deadliest acute syndromes) is typically attributed to previous viral infection (including maybe COVID-19?). In turn the diffuse blastic “seed” in our opinion may represent the most initial phase of hematogenic systemic outgrowth (esp. myeloid leukemia or else so called “blastic plasmacytoid dendritic neoplasm”). But is this really a newly-born leukemia?

Could it be that the true culprit was the COVID-19 which yet has left no “traces” and what is more, tried to “frame in” the hypothetical “pre-leukemia” as a sole or main culprit of death? Is it an example of the “perfect” murder?

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