BALTPHARM FORUM 2019

PHARMACISTS AS DRUG EXPERTS: THEIR ROLE IN HEALTH CARE SYSTEM

The 22nd annual international scientific-practical conference

Book of abstracts

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The 22nd annual international scientific-practical conference „BALTPHARM forum 2019“ Pharmacists as drug experts: their role in health care system“ is organized by Lithuanian Pharmaceutical Association in collaboration with Pharmacists‘ Society of Latvia and Pharmaceutical Society of Estonia.

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Language of abstracts was not corrected.

CONFERENCE PROGRAMME

Park Inn by Radisson Cenference Center
K. Donelaičio street 27, LT-44240 Kaunas, Lithuania

APRIL 13, 2019

8:00-9:30 Registration	
9.30-13.00 PLENARY SESSION (I) **Chairs:** President of the Lithuanian Pharmaceutical Association Prof. Eduardas Tarasevičius President of the Latvian Pharmacists' Society Dace Kikute President of the Estonian Pharmaceutical Society Jaak Koppel	
9:30	**Conference opening remarks** Prof. Eduardas Tarasevičius
10:00	**The role of pharmacist in fight against antimicrobial resistance – perspective of the Commission** Vytenis Povilas Andriukaitis, European Commissioner for Health and Food Safety, Belgium
10:30	**The research-based pharmacy education: challenges in the 21st century** Prof. Ramunė Morkūnienė, Dean, Faculty of Pharmacy, Lithuanian University of Health Sciences, Lithuania
11:00	**Coffee break**
11:30	**New role of pharmacist in the health care system in the world, Europe and Lithuania** Gonçalo Soussa Pinto, Professional Development and Advocacy Manager of International Pharmaceutical Federation, The Netherlands
12:00	**Role of pharmacist in the UK healthcare system** Prof. Ashok Soni, President of the Royal Pharmaceutical Society, United Kingdom
12:30	**Present and future of hospital pharmacy** Assoc. prof. Juraj Sýkora, European Association of Hospital Pharmacists, Slovakia
13.00-14.00 Lunch	

14:00-16:00 PARALEL SESSIONS	
Community Pharmacy Session (IA) **Chairs:** Assoc. Prof. Asta Kubilienė, Lithuanian University of Health Sciences, Lithuania Assoc. Prof. Daisy Volmer, University of Tartu, Estonia	**Hospital Pharmacy Session (IB)** **Chairs:** Birutė Varanavičienė, Lithuanian University of Health Sciences, Lithuania Dr. Inese Sviestina, Rīga Stradiņš University, Latvia
14:00-14:20 **The actualities of pharmaceutical sector in Lithuania: what has been done and what awaits?** Justas Mačinskas, Simona Stankevičiūtė, The Departmnet of Pharmacy, Ministry of Health of the Republic of Lithuania 14.20-14:40 **Full-time-distance format of education within the curriculum "Pharmacy" in the National University of Pharmacy** Assoc. Prof. Fedosov Andrii, First Vice-Rector, National University of Pharmacy, Ukraine 14.40-15:00 **Assesing the effects of organizational factors on the pharmacy service at Lithuanian community pharmacies** Prof. Loreta Kubiliene, Prof. Gvidas Urbonas, Akvile Zieniūtė, Lithuanian University of Health Sciences, Lithuania 15:00-15:20 **New pharmaceutical legislation in Estonia** Representative of Estonia Delegation 15:20-15:40 **New pharmaceutical legislation in Latvia** Ieva Rutkovska, Rīga Stradiņš University, Latvia 15:40-16:00 **Economic evaluation of Pharmacy services** Indrė Trečiokienė, PhD Candidate, Vilnius University, Lithuania	14:00-14:30 **Importance of pharmacokinetic studies today in pharmacotherapy** Prof. Romaldas Mačiulaitis, Lithuanian University of Health Sciences, Lithuania 14.30-15:00 **Using contrast agents in clinical practice. The guidelines and competencies of the pharmacist** Prof. Algidas Basevičius, Lithuanian University of Health Sciences, Lithuania 15:00-15:20 **Hospital pharmacy achievements, goals and issues in Estonia** Kersti Teder, Tartu University, Estonia 15:20-15:40 **Hospital pharmacy achievements, goals and issues in Latvia** Dr. Inese Sviestina, University of Latvia, Latvia 15:40-16:00 **Hospital pharmacy achievements, goals and issues in Lithuania** Jolanta Jakaitė, Renalda Germanienė, Lithuanian University of Health Sciences, "Kauno klinikos", Lithuania
16.00-16.05 Coffee break	

16:05-17:00 POSTER SESSION **Chairs:** Prof. Ona Ragažinskienė, Vytautas Magnus University, Lithuania Prof. Vilma Petrikaitė, Lithuanian University of Health Sciences, Lithuania	
17:00	Pharmacy students surprise
17:10	Poster awards, Panel discussion / Cosing remarks
17.30 Closing	
19.00-22:00 Gala dinner	

APRIL 14, 2019

8:00-9:30 Breakfast
9.45-12:00 WELCOME CITY TOUR (What you have to see in Kaunas)

Kaunas castle (photo from Pixabay.com)

CONTENT

WELLCOME SPEECH

Dear Colleagues,

It is our pleasure to invite you to join us for the 22nd Baltic pharmaceutical conference **BaltPharm Forum 2019 on 13–14 April in Kaunas, Lithuania.** Baltpharm Forum is annual international pharmaceutical conference among the Baltic countries (Lithuania, Latvia, Estonia) organised to learn and update your skills. Every year since 1998, pharmacists across the Baltic States gather at this Forum to exchange ideas and experiences, meet Colleagues and participate in discussions, and most of all, develop professionally.

This year theme "**Pharmacists as drug experts: their role in health care system"** explores the evolution of pharmacists and helps understand transformation from that of apothecary with a drug product focus to that of patient centered care.

On this occasion, Baltpharm Forum will bring pharmacy experts, educators from different European countries, some of which already have stories of success to share. Conference will host well-known regional speakers, public institutions representatives, pharmaceutical industry representatives and much more.

On behalf of the organising and scientific committee
President of Lithuanian Pharmaceutical Association
Prof. Eduardas Tarasevičius

The research-based pharmacy education: challenges in the 21st century

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The goal of pharmacy education is to provide students the knowledge and skills they will need to succeed in the preparation, testing, storage and safe dispensing of medicinal products, professional advice and information to patients. The pharmacists occupied a significant place in the health care system: patient education and counseling, drug development, quality control, clinical research and services, fundamental research, therefore pharmacists need skills and abilities enabling them to assume many different functions. To reflect these changes, competence standards for the optimization of the pharmacist's qualifications and accessibility in the community have been developed. The key drivers and factors for the progress in pharmacy education include regulatory standards and guidelines for quality assurance, the curriculum and its intended outcomes, pharmacological, therapeutic, and technological innovation coupled with changing societal needs, national and international policy drivers and evidence- generated high-quality research. Pharmacists need to respond to the challenges of 21st century, therefore the novel scientific information on the improved drug delivery system, personalized therapy and genetic characteristics of patients, prevention and control of the chronic non-communicable diseases, rational phytotherapy, application of antioxidants, drug-nutrient and drug-food interactions is of great importance. The challenge for Pharmacy education is the steady progress in curicullum within the dynamic and complex health care environment. This results in pharmacy graduates as a specialists of medicines, capable making critical evaluations on therapy and advising the patient about the best use of medicines. Future directions - organizational change and better external relationships, eHealth technology, interprofessional and intraprofessional teamwork.

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2. Kennedy MJ. (2018) Personalized medicines – are pharmacists ready for the challenge? *Integr Pharm Res Pract*., 7, 113–123.
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Role of pharmacist in the UK healthcare system

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This presentation will review the traditional role of pharmacists.
It will explore what has caused this to change. This will be in the areas of training and education, professional development and resources to support the development.
I will then demonstrate the changes that have occurred in traditional practice and how this has helped to develop new roles for pharmacists in new settings.
Finally, I will try and demonstrate what the future opportunities could be and how pharmacists will work within a health care system.

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Full-time-distance format of education within the framework of the curriculum "Pharmacy" in the National University of Pharmacy

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Introduction. Full-time-distance education is a format of organizing the process of education to combine the traditional way of teaching in university classrooms and novel information technologies. The implementation of the above format of education into academic activities of educational establishments of Ukraine is regulated according to the legal documents "Provision on distance learning" (decree of the Ministry of Education and Science of Ukraine, 25 April, 2013, # 466) and decree of the Ministry of Education and Science of Ukraine, 30 October, 2013, # 1518.

Materials and Methods. Concerning specific stages of meeting the legal requirements of the Ministry of Education and Science of Ukraine as for implementing the full-time-distance education format by the National University of Pharmacy, they can be presented in the following way:

Stage 1: 2007-2012 – setting up the Center of distance learning technologies, becoming a university member of the network URAN (Ukrainian Research and Academic Network), developing electronic teaching resources and incorporating them into Moodle http://pharmel.kharkiv.edu/moodle/;
Stage 2: 2012-2015 – skills development further training of the teaching staff, providing our university departments with computer and periphery equipment to enhance distance learning, developing distance courses on a number of subjects of the major "Pharmacy", preparing audio resources to conduct lectures, seminars and workshops.
Stage 3: since the academic year 2015/2016 – involving students to take part in pedagogical experiments on implementing full-time-distance education pattern. So far 236 students have participated in the experiment, 83 of whom have already got the corresponding diplomas.

Results and discussion. The peculiarities of organizing the educational process according to the full-time-distance education pattern in the National University of Pharmacy are the following:

- students are taught during 40-42 weeks under their teachers' constant supervision. A concentrated educational technology is used; one subject is studied during 2-5 weeks;
- lectures, seminars and part of practice classes are in the distance format. Students get a certain number of points for working within the framework of a certain distance-leaning course, which constitutes a prerequisite for taking full-time format exams; lab assignments, the rest of practice and all module tests are to be done during full-time format final sessions only.

On the basis of analyzing the results of the pedagogical experiment as for implementing full-time-distance education it can be concluded that it is students who pursue their **second higher education.**

Conclusions

1. The implementation of full-time-distance education is a complex highly-professional long-term process that requires both considerable material resources on the part of higher educational establishments and the appropriate academic staff potential.
2. Full-time-distance education format enables providing the same quality level of students' progress as full-time education, it also gives an opportunity to plan time management.

References:

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The current and preferred organisational culture of Lithuanian community pharmacies and its impact on pharmacists' job satisfaction

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Introduction

Organisational culture is a pattern of shared beliefs in an organisation. There is emerging evidence that culture has an impact on how satisfied employees are with their work, which in turn influences their performance. The aim of this study was to design and implement a questionnaire to measure organisational culture in pharmacies in Lithuania for the first time and to see whether culture impacts pharmacists' job satisfaction.

Materials and Methods

The adapted Competing Values Questionnaire was chosen as an instrument to measure organisational culture (four types are distinguished: clan, adhocracy, market and hierarchy), and the short Brayfield-Rothe questionnaire to measure job satisfaction. 452 questionnaires were handed out and 384 were completed correctly (84% responsiveness). SPSS was used to perform data analysis.

Results and discussion

The obtained results revealed that the most prevalent culture among Lithuanian pharmacists was hierarchy. The analysed questionnaires also showed that Lithuanian pharmacists would prefer a clan culture in their workplace compared to the current situation in Lithuanian pharmacies. In addition, these working in a clan culture had significantly higher job satisfaction scores ($r=0,426$; $p=0.001$), which is in line with other authors' findings[1].

Conclusions

The prevailing organisational culture of Lithuanian pharmacies is hierarchical. However, employees in Lithuanian pharmacies reported that the culture of a clan would be preferred, and would result in more job satisfaction among pharmacists. There is emerging evidence that the culture of a pharmacy could have an impact on other outcomes such as performance[2]. The objective of future research is to find out whether a favourable culture would result in more effort to ensure patient safety among pharmacists.

References:

1. Park JS, Kim TH. (2009) Do types of organizational culture matter in nurse job satisfaction and turnover intention? *Leadership in Health Services*; 22:20–38.
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Economic evaluation of Pharmacy services

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Pharmacy is transforming from a role primary focused on dispensing to involving pharmacists into health care teams and providing professional pharmaceutical care. There is evidence, that pharmacy services have the clinical benefits for patients in hospital and community pharmacy. Professional pharmacy services (PPS) in community pharmacy are seen as divided into pharmaceutical services and other healthcare services. In 2012 Pharmaceutical Group of the European Union (PGEU) introduced a defined the community pharmacy's services into four dimensions: enhancing medicine safety and access to medicines, improving treatment outcomes of individual patients, improving public health, and contributing to the efficiency and quality of the health system. Pharmacy interventions introduced in practice were based on research evidence and economic evaluation performed alongside randomized controlled trial or observational studies of literature.

Economic evaluations of hospital and community pharmacy services enable to understand which health care services provide value for money. Economic evaluations of pharmacy services most commonly involve 4 types of pharmacoeconomic analyses: cost-minimization analysis (CMA), cost-effectiveness analysis (CEA), cost-benefit analysis (CBA), and cost-utility analysis (CUA). CEA and CUA are mostly used and reported recently. Few systematic reviews show there is evidence for cost-effectiveness of pharmacy services. Despite that findings of some interventions did not lead to strong conclusion or were in favor of their cost-effectiveness in the country context only, most of the services were cost-effective. Screening programs performed at community pharmacy and smoking cessation services showed solid positive results. PPS to improve treatment outcomes of individual patients did not lead to strong conclusion, yet there is evidence that medication review services and monitoring of inhalation technique and medication adherence in COPD patients showed to be cost-effective and cost-saving.

Pharmacy services that are cost-effective need to be implemented into the practice. Further full economic evaluations are needed to evaluate other services.

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Importance of pharmacokinetic studies today in pharmacotherapy

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Introduction

There are many Pharmacotherapeutic (PT) situations (PTS), when we do need discuss about more rational drug use (RDU) in Lithuania (LT). Although since 2003 we do have national plan to promote RDU in LT but we do need to explore the portfolio of possibilities implementing the plan, considering also observations based on WHO initiated country review in 2013. One of the tolls to be employed promoting RDU is Pharmacokinetic (PK) and Pharmacodynamic (PD) studies that should be elaborated more in Lithuania.

Materials and Methods

Overview of several cases and studies reflecting situation about the RDU and antimicrobial PT (AMT) and resistance (AMR) in Lithuania.

Results and discussion

There are obvious unmet needs in clinical practice in general: interpretation of drug inefficiency e.g., differential diagnosis (DDx); extrapolation of indications, dosages e.g., out of approved information (SmPC); interpretation of diverging Drug-Drug Interactions and Drug-Device Interactions; interpretation of PK analysis; interpretation of provisions in SmPC and off-label use; diagnosis, DDx, and management of adverse drug reactions; and the experimental therapy. For AMT we do need to cope with patient specific (PK/PD) and organizational problems. Real life cases highlight the option that PK analysis helps coping with a number of life-threatening PTS, especially in case of AMT. In particular, we need to prevent one of the main risk factors of AMR, i.e., underdosage, especially in cases of individualized PD targets attainment (PTA) when we do treat healthcare associated infection (HAI). Pharmacy specialists are well prepared contribute introducing drug concentration measurements. Pharmacy specialists advanced in clinical pharmacy could be beneficial to PK analyses and services, as it is the case in Czech Republic or Sweden. We have already elaborated a program in Lithuania (Prof. Liudas Ivanauskas, Kaunas, LUHS) applying HPLC methodology.

Conclusions

We do have national plan to promote RDU in LT but we need to explore PK/PD and PTA portfolio of the possibilities stemming from more variable PK analyses into clinical practice. We should discuss the possible steps in benchmarking and implementation of progressive practices to LT. Advanced experiences from other centres involving pharmacy specialists into AMT and HAI issues encourage optimism.

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Using contrast agents in clinical practice. The guidelines and competencies of the pharmacist

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First ESUR guidelines booklet was published in 1994 (updated booklet every 2/3 years). Over the years, more than 200,000 copies of the booklet have been printed and it has been translated into many languages. Although the contrast agents in current use have been on the market for many years, minor changes occur in their adverse reaction pattern and new observations are reported. The 10th version (2018) of the Guidelines includes updated sections on acute adverse reactions, gadolinium contrast agents and other gadolinium issues, post contrast acute kidney injury (PC-AKI) and myeloma and contrast media. A contrast agent is a substance which alters the contrast in images produced by any method. It is a general term which can be used for X-ray, MR and ultrasound contrast compounds. A contrast medium is a substance which alters the contrast in X-ray images by altering transmission of the X-ray beam. This term should be reserved for X-ray contrast compounds, e.g. iodine-based, barium, air and carbon dioxide. **General adverse reactions** - Acute adverse reactions to iodine and gadolinium-based contrast agents; Management of acute adverse reactions to iodine and gadolinium-based and ultrasound contrast agents; Late adverse reactions; Thyrotoxicosis; Nephrogenic systemic fibrosis (NSF). **Risk factors for acute adverse reactions - Patient related.** Patients with a history of: previous moderate or severe acute reaction (see classification above) to an iodine- or gadolinium-based contrast agent; asthma requiring medical treatment; atopy requiring medical treatment. **Contrast medium related.** a) Iodine-based: high-osmolality ionic contrast media; there is no difference in the incidence of acute reactions between the non-ionic low-osmolar contrast agents and the non-ionic iso-osmolar contrast agents; there is no difference in the incidence of acute adverse events among the non-ionic low-osmolar agents. b) Gadolinium-based: the risk of a reaction is not related to the osmolality of the contrast agent: the low doses used make the osmolar load very small; there is no difference in the incidence of acute adverse reactions among the gadolinium-based extracellular agents. **Classification of contrast media:** Unmelting contrast media (barium) – for gastrointestinal radiology; Iodine-based contrast media (x-ray, angiography, CT): ionic; non-ionic; Gadolinium based contrast media (MRI); Ultrasond contrast media (US)

Iodine-based contrast media: The clinically important adverse effect of iodine-based contrast media on blood and endothelium is thrombosis. It is recognized that: all contrast media have anticoagulant properties, especially ionic agents, high-osmolar ionic contrast media may induce thrombosis due to endothelial damage, particularly in phlebographic procedures, drugs and interventional devices that decrease the risk of thromboembolic complications during interventional procedures minimize the importance of the effects of contrast media. **Examination with gadolinium.** Gadolinium-based contrast agents are not approved for radiographic examinations. Gadolinium-based contrast agents should not be used for radiographic examinations in patients with renal impairment (eGFR < 60 ml/min/1.73 m^2).

Gadolinium-based contrast agents are more nephrotoxic than iodine-based contrast media in equivalent X-ray attenuating doses. **Safety of ultrasound contrast media. Statements:** ultrasound contrast agents are generally safe; clinical evidence of ultrasound contrast agent related events in critically ill patients and patients with acute coronary disease is limited. **Contraindication:** avoid ultrasound contrast agents in the 24 hours before extracorporeal shock wave treatment. **Type and severity of reactions:** the majority of reactions are minor (e.g. headache, nausea, sensation of heat, altered taste) and self-resolving; more severe acute reactions are rare and are similar to those after iodine- and gadolinium-based agents. **Interaction with other drugs:** be aware of the patient's drug history, keep a proper record of the contrast agent injection (time, dose, name), do not mix contrast agents with other drugs in tubes and syringes. **Drugs needing special attention-** Metformin: renal adverse reactions; Nephrotoxic drugs (Cyclosporine, Cisplatin, Aminoglycosides, Non steroid anti-inflammatory drugs): stopping nephrotoxic drugs before administering contrast agents is not generally recommended; ß-blocker: ß-blockers may impair the management of bronchospasm and the response to adrenaline; Interleukin-2: late adverse reactions.

References

1. ESUR Guidelines. Contrast Media Safety Guidelines 10.0. (2019).

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Chemical stability estimation of the deflagilic ointment

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Introduction. The tendency of compounding medicines preparation in Ukraine is changing today. Preparation often held in the main compounding pharmacy of the network, and then medicines transmitted to other pharmacies. A big problem for such compounding pharmacies is the rather limited shelf life (only 10 days according to the State Pharmacopoeia of Ukraine (SPhU) article «Non-sterile compounding medicines» requirements). Often, medicines that are prepared for stock may remain stable over a longer period, but the shelf life increasing is possible only if there is scientifically confirmed information on studying the stability of the dosage form over a certain period.

One of the compounding ointments that is prepared for stock in the pharmacies of Ukraine is deflagilic ointment (sodium thiosulfate 5.0; urea 5.0; water purified 3.0 ml; paraffin liquid 10.0; wool fat 10.0). It is applied externally for the treatment of sinusitis. The aim of our work was to assess the ointment chemical stability during certain storage period to determine the possibility of its shelf life extending.

Materials and Methods. Three ointment samples from three different series prepared in the same pharmacy were selected for studies. The samples were stored at a temperature of 5±3 0C and relative humidity 60-65 %. Measured glassware of class A, reagents and volumetric solutions which correspond to the SPhU requirements, analytical balance AXIS ANG 200 (Poland), spectrophotometer Evolution 60S (USA) were used for the work. Ointment active ingredients quantitative content determination was carried out in a freshly prepared ointment, and then every next 30 days of storage (on the 60th, 90th, 120th and 105th day).

Results and discussion. Sodii thiosulfate quantitative determination was done by the iodometric titration method (by the SPhU article recommendations). For the urea quantitative determination spectrophotometric method was developed. Since there is no description of the developed method using for urea quantitative determination, its full validation was done. The robustness of the technique ($\Delta_{t\ test.}=0.463$; $\Delta_{t\ comp.}=0.195\leq1.024$) and its specificity ($\delta_{noise}$, $\%=0.95\leq1.02$) are correspond to the requirements as well as linearity ($b=0.98$; $a=1.38\leq5.12$; $S_0=0.39\leq1.81$; $r=0.9997\geq0.9925$); accuracy (δ, %=0.97≤1.02) and precision ($\Delta z,\%=0.78\leq3.20$) parameters. The determination of sodium thiosulfate and urea quantitative content in the ointment was carried out using both methods. In accordance with the article «Semisolid compounding dosage forms» requirements the content of active ingredients should be within ± 10 % throughout the entire storage period. The average value of both components quantitative content after 3.5 months of storage in each ointment sample does not exceed the permissible deviation (table 1).

Table 1

	Ointment 1	Ointment 2	Ointment 3
sodium thiosulfate	4.75 g (95.0 %)	4.74 g (94.8 %)	4.72 g (94.4 %)
urea	4.66 g (93.2 %)	4.59 g (91.8 %)	4.55 g (91.0 %)

Conclusions. The obtained results testify to the preservation of the deflagilic ointment chemical stability during 3.5 months of storage at a temperature of 5 ± 3 ^{0}C and it compliance with the SPhU requirements.

References:
1. State Pharmacopoeia of Ukraine: at 3 volumes (2014) Kharkiv: State enterprise "Ukrainian scientific pharmacopoeial center of medicines quality", 2-nd ed., Vol. 3. 732 p.
2. State Pharmacopoeia of Ukraine: at 3 volumes (2014) Kharkiv: State enterprise "Ukrainian scientific pharmacopoeial center of medicines quality", 2-nd ed., Vol. 2. 724 p.

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Pharmaceutical poisonings in Lithuania: dynamics of epidemiological situation 2013 to 2017

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Introduction

Medicament poisonings remain significant cause of patients' hospitalization and reason of death. The aim of this retrospective epidemiological study was to investigate the epidemiological situation of pharmaceutical poisonings in Lithuania: to identify medicines mostly associated with poisonings and define changes in rates of poisoning with most common medicament groups and differences among age and gender groups of people in 5-years period.

Materials and Methods

Hospital and death records for pacients admitted with a diagnosis of medicament poisoning were analyzed. Data on pharmaceutical poisoning cases presented to medical institutions in Lithuania during period from 2013 to 2017 were obtained from National Health Insurance Fund under the Ministry of Health.

Results and discussion

The results showed, that total number of poisoning accidents present to medical institutions in Lithuania reduces every year, it decreased 1.75-fold from 1741 in 2013 to 992 in 2017. During 5-year period the main group of medicines causing one third of all pharmaceutical poisonings linked to hospitals are benzodiazepines (*code T42.4 on ICD-10*) and other groups of psychotropic medications remain among other leading groups. The second largest number of poisonings (approx. 10% every year) is occupied by anticoagulants (*code T45.5 on ICD-10*). The rate of poisoning with benzodiazepines has decreased by 10% and only by 1% of anticoagulants over 5 years. Since 2013 the frequency of poisoning with unspecified antipsychotics and neuroleptics (*code T43.5 on ICD-10*) has increased by 4,6%. Also, slight 1% increase is notable in the groups of butyrophenone and thioxanthene neuroleptics (*T.43.4*), unspecified psychotropic drugs (*T43.9*), other and unspecified antidepressants (*T43.2*) and 4-aminophenol derivatives (*T39.1*). Hospital admission rates by gender show that 61,64% of total number of hospital admissions every year are made by female and 38,36% by male. Approximately 40% of poisoning accidents happen for young and middle adults (age 19-55). Analysis of poisoning frequency among age groups revealed that since 2013 hospital admission rate of medicament poisoning has increased by 2,3% of adolescents, by 2,1% of young adults (*age 19-35*) and by 2,0% of seniors (*age 66+*).

Conclusions

Study revealed that people in the period from 2013 to 2017 suffered from poisonings with medicaments affecting mental state the most. Benzodiazepines were the most common, but the rate tend to get lower. Otherwise, no significant effort had been made to reduce the frequency of anticoagulant poisonings. Worsening problem is seen among adolescents and seniors and further investigation is needed to find out why. Also, more depth analysis should be done to identify reasons why total number of hospital admission rates of pharmaceutical poisonings in Lithuania has been decreased in the 5-years period. Currently increased availability of non-prescription drugs from nonpharmacy outlets will possibly change the situation of pharmaceutical poisonings in the future.

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Identification of anthocyanins in Cranberry extract

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Introduction

In botanical terms, cranberry is a wild, evergreen dwarf shrub of the family Ericaceae which grows in marshy coniferous forests and bogs. Common cranberry (Vaccinium oxycoccus) and the similar looking small cranberry (Oxycoccos microcarpus) are evergreen dwarf shrubs with small, narrow leaves and red edible fruits. Cranberry is commonly consumed as juice cocktail, juice, and other product forms to treat and prevent urinary tract infection (1-6). Researches claim that proanthocyanidins, phytochemical constituents of cranberry, act to inhibit a variety of Escherichia coli strains from adhering to uroepithelial cells in the urinary tract. Cranberry fruits contain the anthocyanins peonidin-3-O-galactoside, cyanidin-3-O-galactoside, cyanidin-3-O-arabinoside, peonidin-3-O-arabinoside, and smaller amounts of cyanidin-3-O-glucoside and petunidin-3-O-galactoside. The literature data present researches on purification, separation, identification, and quantitation of anthocyanins and anthocyanidins from different sources (fruits, vegetables, juices, etc.) using different analytical techniques: paper chromatography, thin-layer chromatography, UV–visible absorption spectroscopy, solid phase extraction, high-performance liquid chromatography, capillary electrophoresis and capillary electrochromatography, mass spectrometry, and nuclear magnetic resonance spectroscopy.

Materials and Methods

Thin layer chromatography (TLC) was used for identification of anthocyanins in Cranberry extract. TLC is still used as a routine technique in many laboratories due to their low cost and the constant development of better supports and mobile phases. The mixture of ethyl acetate-acetic acid-formic acid-water (100:11:11:25) was used as mobile phase. TLC chromatography was performed on cellulose glass plates.

Results and discussion

Cranberry extract contains four predominant anthocyanins: peonidin-3-O-galactoside, cyanidin-3-O-galactoside, cyanidin-3-O-arabinoside, cyanidin-3-O-glucoside. The result of investigation can be used for the identification of anthocyanins in different phythomedicines in order to establish their quality.

Conclusions

The result of study of anthocyanins in Cranberry extract by TLC method will be used for determination of authenticity of medicines as chromatographic fingerprints to show their quality.

The chromatographic analysis of anthocyanins in Cranberry extract also can be used for standardization.

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Continuous flow in situ phosgenation reactor

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Introduction

Phosgene gases are extremely toxic [1, 2, 3], however they are crucial for synthetic R&D chemistry. Our aim is a safer alternative for phosgenation reaction application in organic synthesis by developing closed continuous flow reactor.

Materials and Methods

Continuous flow reactor was developed by readily available HPLC parts, such as piston pumps, 1/16 tubings, 4.5mm × 250mm column with PID heater and controller. Triphosgene was converted to phosgene, catalytically using cooper phthalocyanine, which aids to reduce the triphosgene decomposition temperature. First pump is used to transfer solution, containing 1 molar triphosgene dissolved in inert organic solvent through heated column containing catalyst. The column acts as a phosgene generator, meanwhile, the converted phosgene solution is then cooled and transferred into the mixer (reactor). The second HPLC pump transferring nucleophile with base (1 molar), such as pyridine (0.2 molar) solution is connected to the reactor. Depending on reaction conditions (temperature and time) the resulting mixture is transferred to the collection flask. Initially for phosgene generation different solvents were tested in order to determine the optimal triphosgene conversion, where toluene showed most optimal results. By monitoring triphosgene cleavage with TLC staining method (p-dimethylaminobenzaldehyde/diphenylamine), ideal flow rate and temperature for full conversion was achieved at 120°C and 1ml/min. Reaction monitoring and purity of synthesized compounds were determined by means of LC – MS, GC – MS, FT - IR, TLC, proton / carbon NMR.

Results and discussion

In continuing the ongoing research generated phosgene was applied for phosgenation reactions. For example using different compounds containing amino, alcohol group different isocyanates, carbamates were synthesized. First attempts showed only 10% yield. However optimizing flow rates and temperatures the yield of isocyanates, ureas, carbamates substantually increased up to 90% according to LC - MS. Furthermore if the nucleophile is used in excess and reaction is carried out in elevated temperatures - high quantities of urea and carbamates are formed. Otherwise if electrophile excess is used in low temperatures - high quantities of isocyanates, carbamoyl chlorides are formed. Synthesized products were purified by distillation in high vacuum, the spectral data is in agreement with published literature results. Currently chloroformate synthesis is under development. Particularly for isocyanates FT – IR compared to NMR is much more informative showing strong absorption band at 2240-2270cm^{-1}, where C^{13} NMR shows only minor chemical shift at 129 ppm.

Conclusions

At the current moment we have developed the phosgene generator capable of performing phosgenation reactions safer by eliminating phosgene exposure and consuming it in situ, thus making it broader applicable in R&D laboratories, where previously such approaches were too hazardous. Furthermore, our developed continuous flow in situ phosgenation reactor allows to synthesize important intermediates, such as aromatic and aliphatic carbamoyl chlorides, isocyanates, carbamates, unsymmetrical ureas. Our ongoing research focuses on expanding the phosgenation reactions to the broader range of organic compounds including amino acids, heterocycles, etc.

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The effect of *Cannabis sativa* L. extract on malondialdehyde (MDA) level in mice brain

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Introduction

Oxidative stress is a biological process, caused by an imbalance between reactive oxygen species (ROS) and antioxidants. The excess of ROS may attack the unsaturated lipids of the biological membranes and induces the lipid oxidation process where malondialdehyde (MDA) is produced as an advanced oxidation product and it is recognized as an oxidative stress biomarker [3]. *Cannabis sativa* L. is a plant which contains specific phytochemicals in its leafy anatomical parts – phytocannabinoids or cannabinoids, which has antioxidant properties in vitro [1]. Antioxidants are chemical compounds that can donate one or more electrons to free radicals, so that these free radicals can be muted and protect the cells against oxidative stress [4]. The main aim of this study was to evaluate the MDA level as oxidative stress biomarker in brains obtained from mice fed with *Cannabis sativa* L. extract.

Materials and Methods

Experiments were done on 4-6 weeks old outbred mice weighing 20-25g. Mice were randomly assigned to five groups and weighed. *Cannabis sativa* L. herb extracts were administered intragastrically to mice via a stomach tube for 21 days and MDA concentrations in the brain of mice were determined. Oxidative stress was induced by $AlCl_3$ solution. Control mice received the same amount of saline. Hemp extracts were alcohol- based, so another control group received the same volume of 10% ethanol. The content of MDA was carried out spectrophotometrically at 535 and 520 nm by measuring thiobarbituric-acid-reactive substance [2].

Results and discussion

The concentration of MDA was evaluated in the brain homogenates of laboratory mice after 21 days of *Cannabis sativa* L. herb extract intragastrically administration. The concentration of MDA in mice brain was insignificantly ($p \geq 0.05$) increased by 49.60 % in case of ethanol, by 4.28 % in case of hemp extract and decreased by 2.47 % in case of hemp extract with $AlCl_3$ administration compared to a control group that received saline. The MDA levels were significantly ($p \leq 0.05$) increased by 416.64% under $AlCl_3$ effect compared to control group that received saline.

Conclusions

Our studies revealed that *Cannabis sativa* L. extract alone or in combination with $AlCl_3$ did not affect MDA levels in mice brain significantly, whereas $AlCl_3$ treatment resulted in highly increased MDA concentration. It means *Cannabis sativa* L. extract protects from lipid peroxidation that is caused by negative $AlCl_3$ effect.

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CYP2C19, CYP2D6 and CYP2C9 allelic variants and its possible effect on drug metabolism: a retrospective study

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Introduction

Cytochrome P450 enzyme superfamily is responsible for the phase I drug metabolism. In particular, enzymes CYP2C19, CYP2D6 and CYP2C9 are responsible for the metabolism of 39,6% of commonly prescribed drugs [3]. However, CYP2C19,CYP2D6 and CYP2C9 enzymes are encoded by genes where genetic polymorphisms are prelevent, thus causing altered or little to no enzyme activity.[2] Such polymorphisms are influental in drug metabolism, the occurance of adverse drug reactions and to the efficacy of the treatment[1]. These type of events can be prevented by pharmacogenetic testing and personalized medicine.

Materials and Methods

A total of 54 patients' pharmacogenetic tests were conducted by Autogenomics Infiniti system in Kaunas Clinics, Department of Genetics and Molecular medicine, Lithuania. Based on the genotype-metabolic phenotypes of CYP2C19, CYP2D6, CYP2C9 enzymes, patients were classified according to the terms, that were made valid by CPIC: Normal metabolisers (NMs), intermediate metabolisers (IMs), Rapid metabolisers (RMs), Ultrarapid metabolisers (UMs) and poor metabolisers (PMs).

Results and discussion

CYP2C19 enzyme genotype-phenotype distribution: 18pts (33,33%) with *1/*1 genotype were normal metabolizers (NMs); 14 pts (25,93%) with*1/*2; *2/*17 genotypes were intermediate metabolisers (IMs); 15 pts (27,78%) with *1/*17 genotype were rapid metabolizers (RMs); 4 pts (7,4%) with *17/*17 genotype were ultrarapid metabolizers (UMs); 3 pts (5,56%) with *2/*2 genotype were poor metabolizers(PMs). CYP2D6 results: 26 pts (48,15%) with*1/*1,*2/*2,*1/*2,*1/*41,*2/*41 genotypes were normal metabolizers (NMs); 21 pts (38,89%) with *1/*5,*2/*4,*10/*41,*1/*4,*1/*3,*2/*5,*2/*4,*2/*6 genotypes were intermediate metabolizers (IMs); 2 pts (3,7%) with*2XN genotype were ultrarapid metabolizers (UMs); 5 pts (9,26%) with *4/*5,*4/*10,*4/*9,*4/*41 genotypes were poor metabolizers (PMs); CYP2C9 enzyme results: 44 pts (81,48%) with*1/*1 genotype were normal metabolizers (NMs); 10 pts (18,52%) with *1/*2;*1/*3 genotypes were intermediate metabolizers (IMs). Our study indicates that when prescribing a particular agent, individual genetic background, in some cases, should as well be evaluated to prevent therapeutic failures.

Conclusions

Our study showed that patients with rapid, ultrarapid and poor metabolic phenotypes, combined for CYP2C19 are as follows 22pts (40,74%) and for CYP2D6 7pts (12,96%). Individuals containing these metabolic phenotypes are more susceptible to adverse drug reactions, therefore drug monitoring and pharmacogenetic testing is a must for developing personalized therapeutic regimen.

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Optimisation of pretreatment and derivatization method for analysis of organic acids by gas chromatography-mass spectrometry

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Introduction. Chromatography is the preferred method of analysis because it adequately addresses the simultaneous identification and quantification of targeted compounds. However, not all chromatographic protocols are suitable for the given task. Although the separation using this method generally targets volatile, non-polar species, the use of derivatization for polar low molecular weight species enables detection with a good resolution and sensitivity. Derivatization as and pretreatment can improve chromatographic results.

The aim of our work was to develop reliable and accurate method for quantitative and qualitative analysis of organic acid, such as levulic, heptanoic, malic, lactic, glycolic, oxalic, nonanoic, maleic, succinic , stearic, citric.

Materials and methods. The research was done using methodology on SHIMADZU GC-MS-QP2010 Ultra chromatography system with RXI-5ms (Restek Corporation) capillary column (30 m long, with 0.25 mm outer diameter and 0.25 µm liquid-stationary phase thickness) with a liquid stationary phase (5% diphenyl and 95% polysiloxane), as carrier gas of chromatography we used helium. Organics acids were identified by comparison with database mass spectra of compounds or analyzing ions characteristic of mass spectra. The oven temperature was programmed from 75 °C for 5 min, then 10 °C/min to 290 °C for 5 min, after 20 °C/min to 320 and held constant for 5 min. The injector temperature was 260 °C, injection volume 1 µL, injection mode split, split ratio 1:10, the ion source voltage 70eV. Mass spectra scan range of m/z 35-500 amu with mass scan time 0.2 seconds, interface temperature 280 °C. All solvents were HPLC grade.

Results and discussion. In presence work we examined an effects of different derivative agents, solvents for sample dissolution and reaction solvents. The weights of samples were placed into 5 ml volumetric flasks, 3 ml (of the following solvents: water, acetonitrile, methanol, 80% methanol and 80% acetonitrile) were added and ultrasonicated for 5 minutes, then the volume was filled up to mark. 1 mL of test solutions were evaporated to dryness with a gentle stream of nitrogen, after that 100 µL of acetonitrile (methanol) and 100 µL of N,O-Bis (trimethylsilyl)trifluoroacetamide (BSTFA) or N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) were added. The derivatization time 4 hours and temperature 70 °C were selected.

In suggested conditions the investigated organic acids have the following retentions times (as shown at the Figure 1): Levulic acid (12,39 min), Heptanoic acid (12,85 min), Malic acid (13,05 min), Lactic acid (14,28 min), Glycolic acid (14,51), Oxalic acid (15,06 min), Nonanoic acid (15,53), Maleic (2-Butenedioic) acid (17,49 min), Succinic (Butanedioic) acid (17,63), Stearic acid (24,72), Citric acid (25,81).

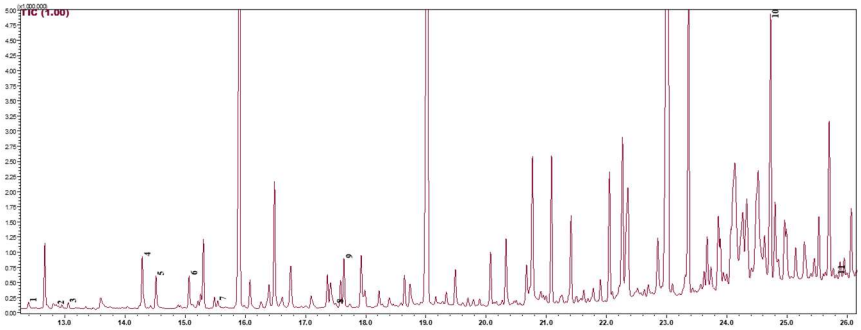

Fig. Typical chromatogram of mitochondria sample.

Conclusion. According to received data, the most optimal derivatization conditions were as the following: as derivatization agent was MTBSTFA, as the solutions for dissolution and reaction - water and acetonitrile, respectively. We detected the most effective chromatographic conditions for analysis of organic acids, the results suggest that the method could be usefully integrated in analysis of organic acid.

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The evaluation of the particle size changes of oil-in-water microemulsions containing quercetin

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Introduction

Microemulsion is thermodinamically stable dispersed system. Its quality is characterized by particle size (10–200 nm) and polydispersity index (PDI≤ 0.5). Microemulsions can enhance solubility of poor water soluble drugs as quercetin. Quercetin is a natural flavonoid, known for its antioxidant, anti-inflammatory, antiviral, antifungal and photo-protective properties. The aim of this study was to evaluate the changes of the particle size of three different o/w microemulsions containing quercetin within 4 weeks.

Materials and Methods

Three oil-in-water microemulsions (QME-EtOH, QME-Pg, QME-PEG-400) consisted of 3% of isopropyl myristate, 33% of purified water and 64% of mixture of labrasol and co-surfactant (5:1). Ethanol (EtOH), propylene glycol (Pg) and PEG-400 were used as co-surfactants. Quercetin was dissolved in microemulsion particles forming components. Average particle size and PDI of the investigated microemulsions (ME) were measured applying light scattering method. Measurements were carried out after formulation, after 2 and 4 weeks.

Results and discussion

After formulation, particle size of microemulsion was varying according co-surfactant type: ME containing ethanol was in the range of 1.5–5.6 nm, ME containing propylene glycol – 28.2–141.8 nm and ME formulated with PEG-400 as co-surfactant was in the range of 78.8–615.1 nm. Particle size changes were observed in all ME after 2 and 4 weeks measurements. It was determined, that after four weeks there were no 1.5–2.0 nm size particles in ME containing ethanol, but formation of 24.4–91.3 nm size new particles was observed. In the range of 2.3–2.7 nm were 1.8–9.7% less particles and in the range of 3.1–4.2 nm were 0.9–4.4% more particles than after formulation. Incresement of particle size was observed in ME containing propylene glycol: after four weeks 0.1–5.8% increase of particle number in the range of 50.8–164.2 nm was observed. It was obtained, that there were no particles in the range of 78.8–105.7 nm in ME containing PEG-400 after four weeks. In the range of 122.4–164.2 nm were 1.8–6.8% less particles than after preparation. The increase (0.1–4.5%) of particle number in the range of 190.1–712.4 nm was observed. PDI values of microemulsions were determined less than 0.4, although changes were observed within 4 weeks.

Conclusions

Changes of particle size were observed in all investigated microemulsions. Particle size of ME could be determined by nature of used co-surfactant: large particle size (>200 nm) of QME-PEG-400 could be attributed to polymers property to formulate micelles. According to observed PDI values, all formulated microemulsions were corresponding quality requirements.

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Optimization of assay method of trans-10-hydroxy-2-decenoic acid (10-hda) in royal jelly and food supplements by high performance liquid chromatography

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Introduction

Royal jelly (RJ) is a worker bee gelatinous secretion from the hypofaringeal and mandibular glands. It is composed by proteins, carbohydrates, lipids, amino acids, sugars, minerals and small amount of vitamins. Nowadays RJ is widely used in producing of food supplements, in pure state or lyophilized, also in cosmetic products. The major and the most important of RJ is (2E)-10-hydroxydec-2-enoic acid (10-HDA). 10-HDA is unsaturated fatty acid, no one other bee products contain such substance. Since it is specific, the substance can be used as biomarker of RJ. Thus an amount of 10-HDA shows the quality of those products.

Materials and Methods

HPLC analysis has been carried out using Waters 2695 chromatography system. Separation was done with usage of ACE 5 C18 column (250 ×4.6 mm, particle size 5µm. The two elution solvents were exchanged: the solvent A (0.1% TFA) and the solvent B (acetonitrile). The following linear gradient elution profile was used: 95% A/5% B–0 min, 25% A/75% B–20 min, 5% A/95% B–21 min, and 5% A/95% B–28 min. The flow rate was 1 mL/min and injection volume was 10μL. The effluent was determined at a wavelength of 240 nm.

Results and discussion

For extraction of 10-HDA from raw material and products, such as tablets and capsules, which contains RJ, we used 3 different solvents (methanol, chloroform and ethyl acetate). Standard was dissolved in each of these solvents, the best peak shape was obtained from methanol solvent. For preparation test solutions were used the same method, weights of samples were placed into measure flask added methanol and sonicated for 15 minutes. This method was suitable for tablets, but not for capsules and raw material, we could not receive separation of 10-HDA and other excipients. Because of that, the content of capsules was extracted with a portion of diethyl ether (15 ml), then non-soluble material was extracted with methanol (3x15ml), duration of each extraction was 15 min. Extracts were evaporated with liquid nitrogen and were diluted in 1 ml of the same solvent. The test solution of raw material was prepared in the same way. The average amount of 10-HDA in raw material was 0.93%, in capsules – 0.71 mg per average mass of capsule, in tablets – 1.50 mg. per average mass of tablet. The linear calibration curve was made and expressed by the following quadratic equation: 2=0.9996, (y = 3.36·104x-1.01·105), the linearity range: 4–260μg/ml. The precision of the method met all requirement of ICH, since all the obtained relative standard deviation (RSD) values were lower than 2.0%. The retention time of main substance is about 13.5 minutes.

Conclusions

We found the most suitable conditions of 10-HDA analysis in tablets, capsules and raw material.The developed method proved to be a good instrument for measurinf of 10-HDA in raw material and different products.

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Determination of muscimol in *Amanita muscaria L.* by high–performance thin–layer chromatography (*HPTLC*) method after decarboxylation

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Introduction

The fly agaric well known as *Amanita muscaria L.* Ibotenic acid and muscimol are substances which mostly participate in psychotropic properties [1]. First symptoms of fly agaric intoxication apparent within 15 – 30 minutes after ingestion. Nausea, vomiting, diarrhea, vasodilation, sweating and salivation are the first signs of muscarinic poisoning [2]. A reliable analitycal method was developed for the identification of muscimol (MUS) and ibotenic acid (IBO).

Materials and Methods

Decarboxylation method was applied to convert ibotenic acid into muscimol [3]. The powder of lyophilizate herbal material (0.2g) were weighed onto aluminum plate and placed on the CAMAG TLC plate heater III for 1 hour. Temperature modes were selected 75°C, 95°C and 110°C. After decarboxylation herbal substance was replaced into a 10 ml volumetric flask and extracted with 70% methanol (5ml) in an ultrasonic bath at room temperature for 10 min, with periodic shaking of solution. After 24 hours extract was centrifugated (4000 rpm) for 5min. We used high-performance thin-layer chromatography method (HPTLC) for qualitative and quantitative analysis of muscimol. Injection of muscimol standard solution was 5 µl and 10 µl for test solution. Chromatographic plates (HPTLC Silica gel 60 F_{254}, 10x10 cm) coated with silica gel was used with 1-butanol:96% ethanol:acetic acid:water (75:25:5:7:5) as mobile phase, migration distance was over path 70 mm. The detection was performed after sprayed with a solution of ninhydrin, then plate was heated at 100°C and examined with using CAMAG TLC Visualizer 2 in daylight. The obtained chromatograms of samples by the main spot correspond to the typical reference solution chromatogram with R_F = 0.25. Calibration curve was done for quantitative determination. We counted of muscimol amount for determination of the most suitable temperature for decarboxylation.

Results and discussion

Three samples with different temperatures of heating were analysed. Muscimol standard was compared with test samples after decarboxylation. Muscimol was identified after reaction with ninhydrin. The biggest amount of muscimol (17,013±0.51 mkg/ml) was obtained with usage 75°C as temperature of decarboxylation. Less ammounts of muscimol were obtained by the temperatures 95°C (16,910±0.50 mkg/ml) and 110°C (11,403±0.57 mkg/ml).

Conclusions

Decarboxylation method by heat was suitable to convert ibotenic acid into muscimol. High-performance thin-layer chromatographic method was developed for determination of muscimol in extractions.

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The manipulation of nutraceuticals contents under fluctuating light intensity in vegetables

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Introduction

Vegetables are the essential part of balanced diet because of good source of phytonutrients and nutraceutical compounds. Leafy vegetables are rich source of vitamins such as ascorbic acid, folic acid as well as minerals like iron. Most phytochemicals demonstrate antioxidant activity and protect our cells against oxidative damage [1]. Lettuce and radish are model plants for lighting treatments because of attained commercial status and wide spread cultivation in world. Due to low production costs and high yield letucces and radishes are one of the cheapest leafy vegetables in the market and could be rightly described as 'poor man's vegetables [2]. In this study we present how different intensity of light-emitting diode (LED) lighting influence the metabolism of nutraceuticals in red lettuces and radish leaves under controlled environment conditions.

Materials and methods

Red leaf lettuce (*Lactuca sativa* L., 'Lobjoits Red Cos') and radish (*Raphanus sativus* L., 'Cherry Belle') were grown under combinations of red (660 nm) and blue (445 nm) LED lighting under different photosynthetic photon flux density (PPFD) at 150 and 250 μmol m^{-2} s^{-1} for three weeks. Plants were treated as follows: 1) 150 μmol m^{-2} s^{-1}; 2) 150 μmol m^{-2} s^{-1} irradiated with 250 μmol m^{-2} s^{-1}; 3) 250 μmol m^{-2} s^{-1}, and 4) 250 μmol m^{-2} s^{-1} irradiated with 150 μmol m^{-2} s^{-1} PPFD of LED lighting.

The contents of folic acid, ascorbic acid and xanthophylls' (lutein and zeaxanthin) in technical maturity plants were determined by high performance liquid chromatography (HPLC-DAD) methods. The content of iron was evaluated by microwave digestion technique combined with inductively coupled plasma optical emission spectrometry (ICP-OES).

Results

Results showed that light intensity leads to different accumulation of xanthophyll's, folic acid, ascorbic acid and iron in various growth strategy vegetables. In contrast to red leaf lettuces, leaves of radishes were less sensitive to changes of PPFD during vegetation period. Moreover, the accumulation of nutraceuticals in radish leaves is two time lower than in red leaf lettuces. The highest contents of nutraceuticals were found when vegetables were irradiated with PPFD at 250 μmol m^{-2} s^{-1} till maturity stage and subsequently irradiated with PPFD at 150 μmol m^{-2} s^{-1}. Lettuces grown under 250 μmol m^{-2} s^{-1} PPFD level and irradiated with 150 μmol m^{-2} s^{-1} PPFD at maturity stage accumulated about three times more determined nutraceuticals than radish leaves.

Conclusions

The accumulation of treated nutraceuticals of different life strategies vegetables varies depending on LED lighting intensity. The most favourable condition for red leaf lettuce and radish is cultivation only under 250 μmol m^{-2} s^{-1} PPFD and illumination by lower PPFD during maturity stage. Thus, selecting plants, which naturally accumulates important for human health metabolites and choosing light intensity during vegetation, enables manipulate the metabolic response.

Acknowledgements

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Anticancer activity of beta adrenoblockers in non-small cell lung cancer cell lines A549 and H1299

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Introduction

Beta adrenoblockers (also known as beta blockers) are a class of drugs used for the treatment of cardiovascular diseases and glaucoma. The first evidence of beta adrenergic receptor signalling pathway involvement in lung cancer development appeared in 1989 [1]. Moreover, beta adrenoblocker application is associated with the increased survival outcomes in patients with non-small cell lung cancer [2]. According to recent studies, beta blockers also possess anticancer activity in pancreatic, breast, colorectal, prostate and ovarian cancer [4-6].

Materials and methods

The aim of our research was to investigate the anticancer activity of seven beta adrenoblockers (β_1 selective: atenolol, metoprolol, esmolol, betaxolol, and non-selective compounds: propranolol, timolol, pindolol) in non-small cell lung cancer cell lines A549 and H1299. Compound effect on cell viability was established by MTT assay. The activity of beta adrenoblockers on clonogenicity of lung cancer cells was tested by evaluating the effect on the colony-forming ability. The type of cell death was evaluated by cell staining with Hoechst 33342 and Propidium iodide.

Results and discussion

Propranolol and betaxolol inhibited lung cancer cell growth. Among tested compounds propranolol had the greatest effect on cell viability (EC_{50} values after 72 h on A549 and H1299 cell lines were 119.3 ± 12.7 μM and 98.8 ± 10.3 μM, respectively). Betaxolol effect on cell viability was similar in both cell lines (EC_{50} was 251.33 ± 14.61 μM against A549 and 252.23 ± 7.62 μM against H1299). Other compounds did not reduce the cancer cell viability at concentrations less than 500 μM. Propranolol and betaxolol at concentrations equal to 90% of calculated EC_{50} values completely suppressed cell colony formation. Betaxolol possessed this effect on A549 cell clonogenicity at a concentration equal to 10% from EC_{50} value, while the rest of the compounds did not induce statistically significant effect.

The majority of tested compounds induced cell death through apoptosis and necrosis. In A549 cell lines apoptosis was mainly induced while in H1299 cell line compounds induced both apoptosis and necrosis.

We did not find any difference between the activity of β_1 selective and non-selective compounds. However, the most active compounds possess the membrane stabilizing activity, which is possibly associated with their anti-inflammatory effect [7].

Conclusion

Beta adrenoblockers, especially betaxolol and propranolol, possess anticancer activity in non-small cell lung cancer cell lines A549 and H1299.

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Determination of solvent system for qualitive analysis lime flores of *Tilia cordata* and *tilia platyphyllos* using tlc metodology

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Introduction

Lime flowers have a prominent importance in a folk medicine. It is stated to possess expectorant, diuretic, diaphoretic, antispasmodic activities. Tilia is used in traditional medicine of many European countries primarily as a non-narcotic sedative for sleep disorders or anxiety. Essential oils, vitamins, mucilage and flavonoids components are known as the active ingredients. *Tilia cordata* and *Tilia platyphyllos* are the most popular and numerous species. The officinal raw material is flowers (inflorescences) with flower bunds (*Tiliae flos*), which are introduced into many pharmacopoeis of the world. Nowadays there are only packed raw material and collections as medicines on the pharmaceutical market of Ukraine. The development of gallenic and newgallenic drugs is advised as Ukraine has a good recourse base of lime flowers.

Aim

Development a method of TLC analysis is necessary for quality control of raw materials and drugs on its base in future. Therefore the major aim of study was selection a mobile phase for TLC analysis, which gives visible difference and resolution of derivatives of kaempferol, quercetrin and acacetin in extracts from the flowers of *Tilia cordata* and *Tilia platyphyllos.*

Materials and methods

Tilia flos was used as a plant material for analysis, collected in Warsaw, Poland, in 2014, 2015, 2016. For determining the mobile phase of TLC analysis of extracts from *Tiliae flos* were used: tetrahydrofuran (POCH basis), isopropanol (POCH basis), dichloromethane (POCH basis), formic acid (Merck), acetic acid (Merck) and distilled water in different ratios.

The analysis was carried out at the Department of Pharmacognosy and Molecular Basis of Phytotheraphy of Medical University of Warsaw. For analysis used: CAMAG Linomat 5, CAMAG ADC 2 Automatic Developing Chamber 2, CAMAG TLC plate heater III, CAMAG derivatizer, CAMAG TLC Visualizer 2; HPTLC plates, silica gel 60 F254, Merck. Standard samples of flavonoids which were used for comparison: quercetin derivatives (isoquercitin, routine, avicularin), keampferol derivatives (keampferol 3-O-glucosid-7-rhamnozide, trans-tyrolizide, astragalin) and acacetin derivatives (linarin).

Results and discussion

The analysis showed that the best resolution of standard substances was provided by a mobile phase which contains tetrahydrofuran - dichloromethane - formic acid - acetic acid – water P in a ratio of 16:20:4:2:4, accordingly. This solvent system ensures more visible difference between species.

Conclusions

The identified solvent system will be used in the development of normative documentation and quality control for raw materials and extracts on its bases.

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Influence of proton pump inhibitors on doxorubicin delivery into 2D and 3D cancer cell cultures

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Introduction

In order to improve the efficacy of chemotherapy an increasing attention is given to the drug transport to tumors. Various methods, such as tumor pH modulation by proton pump inhibitors (PPIs), can be used to improve drug delivery in cancer cell cultures. PPIs are a group of drugs that are used to reduce gastric acid production (Shin et al, 2008). Recent studies show that they can reduce extracellular acidity, thus preventing basic drugs from ionisation and increasing their delivery to cancer cells (Raghunand et al, 2000).

The aim of our study was to evaluate the influence of two PPIs (omeprazole and lansoprazole) on doxorubicin (DOX) delivery to monolayer-cultured 4T1 murine breast cancer cells and three-dimensional cultures (spheroids).

Materials and Methods

The effect of PPIs on cell viability was evaluated by MTT assay. 3D cell cultures were formed using *3D Bioprinting* method (Tseng et al, 2015). DOX penetration cancer cells and spheroids at pH 6.0 and 7.4 was assessed using fluorescence microscopy. All the experiments were done in at least triplicate independent measurements. Student's *t*-test was used, and *p*-values were calculated. A value of $p<0.05$ was considered as the level of significance.

Results and discussion

Among tested PPIs, omeprazole had no effect on the 4T1 cell viability at 250 μM concentrations. Lansoprazole reduced cell viability (EC_{50} was 158.3 ± 11 μM). PPIs concentrations (100 μM), used in further experiments were below the toxicity level. At pH 7.4 both, omeprazole and lansoprazole, did not enhance DOX (5 μM) delivery in 2D cell cultures but in acidic conditions both compounds increased the amount of drug in cancer cells and their nucleus. Lansoprazole also increased the penetration of DOX (20 μM) in cancer cell spheroids but the effect was temporary and after 8 h there were no difference compared with the control group.

Conclusions

Tested PPIs, especially lansoprazole, increased DOX penetration in monolayer-cultured cancer cells and three-dimensional cultures and are worth further studies as transport modulators of basic drugs.

Acknowledgement

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Terpenoids of *Veronica teucrium* L. leaves and flowers

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Introduction

Veronica L. is a genius of family *Plantaginaceae* Juss. and including around 500 species in the world's flora [1]. An unofficial species – *Veronica teucrium* L. is distributed worldwide and is grown as an ornamental plant. Though a plant has been used in folk medicine of many countries a long while, a critical analysis of scientific primary sources has shown that it's chemical composition is not completely studied for today [1, 2]. Terpenoids of *V. teucrium* L. leaves and flowers haven't been studied [1, 2], and it is of scientific interest. The aim of the research was to identify and quantify different groups of terpenoids of *V. teucrium* L. leaves and flowers.

Materials and Methods

Objects of a study were *V. teucrium* L. leaves and flowers, harvested in 2018 in a Kharkiv region, Ukraine. Chromatographer Evolution 60S was used for quantification of iridoids (λ=515±5 nm), triterpene saponins (440±5 nm) and carotenoids (440±5 nm). We used a modified procedure of iridoids and saponins quantification based on the reaction with Stahl reagent and with vanillin reagent, respectively.

The essential oil of *V. teucrium* L. leaves and flowers had been obtained by the microsteam distillation method by means «Agilent» 22 ml vials, then an essential oil had been washed from the condenser to vial by pure pentane and concentrated by blowing with nitrogen. Chromatography-mass spectrometer Agilent Technology HP6890 GC with mass spectrometric detector 5973N had been used for an analysis of the essential oil content. The analysis conditions: quartz and capillary chromatographic column HP-5MS (a length – 30 m, an internal diameter – 0.25 mm), carrier gas – helium with a speed 1 ml/min, the injection sample – 2 μl, a mode splitless, the temperature of detector and evaporators – 250 °C. A content of essential oil in total content had been calculated in relation to internal standard – tridecane. The components of essential oil had been identified by comparing mass spectra of chemical substances obtained in process of chromatographic study with data from the mass spectrum library NIST02.

Results and discussion

In the result have been found, that the content of iridoids was 0.93% in *V. teucrium* L. leaves and was 0.80% in flowers in terms of harpagide; a content of triterpene saponins was 0.04% in *V. teucrium* L. leaves and was 0.02% in flowers in terms of β-amirin; a content of carotenoids was 0.94% in *V. teucrium* L. leaves and was 0.62% in flowers in terms of β-carotene.

By means of GC-MS, 48 components have been identified and quantified in essential oil of *V. teucrium* L. flowers and 49 components – in essential oil of leaves. Among identified compounds, mono-, norsesqui-, sesqui-, di- and triterpenoids, products of terpenoids oxidation, fatty acids, hydrocarbons and derivatives of these classes have been found. The content of essential oil was 0.07% (687.82 mg/kg) in *V. teucrium* L. leaves and was 0.24% (2407.11 mg/kg) in flowers. The content of terpenoids in the sum of identified components of essential oil was 21.95 % in *V. teucrium* L. leaves and was 11.78 % in flowers.

Conclusions

By means of a spectrophotometric method the content of iridoids, triterpene saponins and carotenoids have been quantified; by means of GC-MS the composition of essential oils have been identified and quantified in *V. teucrium* L. leaves and flowers. *V. teucrium* L. leaves and flowers can be considered as a BAS source for a development of pharmacological substances with target nootropic activity and antibacterial activity, this is due to iridoids and compounds of essential oils, respectively.

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CAIX role in human breast cancer cell migration and invasion

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Introduction

Carbonic anhydrase IX (CA IX) is a highly overexpressed membrane protein in numerous cancers and is well-recognized hypoxia marker with a promising diagnostic and therapeutic value [1]. CA IX regulates pH in hypoxic tumor cells and contributes to microenviromental acidosis, cell adhesion, migration and invasion [2]. To get better insight of CA IX role in the processes of cancer cells migration and invasion, we performed a genetic silencing of CA9 gene in triple negative breast cancer cells MDA-MB-231. Then the capabilities of cell migration and invasion were determined using 3D spheroid invadopodia formation method.

Materials and methods

Stable knockdown MDA-MB-231 lines, named CA9-1 (90% knockdown) and CA9-2 (0% knockdown, negative control), were generated using two Sigma lentiviral Ca9 shRNAs [TRCN0000349591 and TRCN0000319003] plasmids. Protein level was determined using Western blotting.

The invadopodia formation of modified cancer cells were evaluated in 3D cancer cell spheroids. Cell suspension was made in a matrigel and collagen mix and placed into 8-well chambers. The drops solidified after incubation for 1 hour at 37 °C, and the high-serum medium was added. After 6 days of incubation spheroids with invadopodia were formed, and they were imaged by fluorescent microscopy.

In inverse invasion assay, the cell suspension was placed on inverted transwell chamber containing collagen/matrigel/fibronectin inside. After the cells were attached to the filter, chambers were placed right way up in 24-well plates, containing free serum medium. On top of the matrix the medium with chemoattractant was added. After 7 days of incubation, the cells were stained with Calcein and imaged by confocal microscopy every 10 microns from the base membrane. All experiments were performed both in normoxia (21% O_2) and hypoxia (1% O_2) conditions.

Results and discussion

CA9 knockdown statistically significantly affected the invadopodia formation both in normoxia and hypoxia conditions. About 75% of MDA-MB-231 cell spheroids in hypoxia during 6 days of incubation formed invadopodias, and about 5% of them had migrated cells. Meanwhile, spheroids made from shRNA CA9-1 cells, did not formed any invadopodia, and majority of them were very loose and from 1.5 to 2 times smaller compared to spheroids formed from parent cell line. CA9-1 cell migration at a 20 μm distance from filter was statistically significantly lower compared to MDA-MB-231 cell line: 1.6 times less cells migrated in normoxia and 3.4 times less cells migrated in hypoxia conditions. Also, the distance of CA9-1 cell migration was 1.6 times shorter compared to the distance of migrated MDA-MB-213 cells in hypoxia.

Conclusions

Based on our results, CA IX may have an important role in cancer cell migration and invasion processes, therefore it could be a potential target in cancer therapy.

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Determination of protein quantity isolated from roots of *Glycyrrhiza glabra* L. and *Desmodium canadense* (L.) DC. during different vegetation phases

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Introduction

Glycyrrhiza glabra L. and *Desmodium canadense* (L.) DC – are the herbaceous perennial species of *Fabaceae* family. Both plants are commonly used herbs in ayurvedic medicine. Studies have shown that extracts of plants roots possess antibacterial, antioxidant, antimalarial, anti-inflammatory properties. It was found that preparations of both plants contain a big amount of proteins, but the quantity has not been studied during different vegetation phases. Proteins are primary plant metabolites, which perform important vital functions [1;2;3]. The aim of the experiment - to compare protein content in liquorice and Canadian tick-trefoil roots during different vegetation phases – regrow, intensive growing, bud, blooming and seed maturity.

Material and methods

1. Protein fractions were obtained from *Glycyrrhiza glabra* L. and *Desmodium canadense* (L.) DC raw root material by extraction and fractionation with ammonium sulphate and protease inhibitor - ε – amino-capronic acid.
2. The quantity of protein was measured by the Bradford method using bovine serum albumin BSA protein standards.
3. Results were analyzed with MS Excel.

Results

Proteins of *Glycyrrhiza glabra* L. and *Desmodium canadense* (L.) DC fresh root material was obtained in seven different vegetation phases. The biggest yield of protein was defined in liquorice root extract, while Canadian tick-trefoil root extract had a significant lower quantity of protein in all vegetation phases.
Protein quantity in fractions extracted from liquorice roots material had a tendency to decline till massive blooming phase, from that point the output started to grow. At the end of the vegetation, protein amount has become approximately stable.
Protein fractions, extracted from Canadian tick-trefoil roots, had the inclination to fluctuate. The biggest amount of protein was in seed maturity phase.

Conclusions

1. The quantity of protein fractions amount was changing during vegetation in both extracts: the highest content of protein was on regrow phase and at the end of vegetation.
2. Independently of the similarity of the botanical classification, analyzed plants had a highly different protein content in roots during vegetation.
3. During the start of the blooming phase, the protein amount of both plants became comparable.

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Evaluation of biological activity of different extracts from *Tanacetum parthenium* L. to glioblastoma multiforme C6 culture cells

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Introduction

Glioblastoma multiforme (GBM) is the most common and lethal primary malignancy of the central nervous system (CNS). GBM patients have a poor prognosis with survival rate of 14-15 months after diagnosis. [3] Radiotherapy in combination with temozolomide (TZM) is currently established as the first-line treatment for GBM patients. [1] However, due to major toxicities of TZM such as hematological effects and hepatotoxicity it is important to continue scientific studies in order to enhance efficacy of GBM treatment. Tanacetum parthenium L. is a medical herb also known as Feverfew and it is used as a folk remedy for the treatment of fevers, migraine and arthritis.[4] Parthenolide, a sesquiterpene lactone, is considered to be the primary bioactive compound in Feverfew and exhibits antitumor properties on glioblastoma cells. [2] The aim of this study is to determine total phenolic content of three different Tanacetum parthenium L. extracts, also assess and compare the effectiveness of three different extracts on rat glioblastoma cell culture viability.

Materials and Method0

Three different Tanacetum parthenium L. extracts (ratio 1:5) consisted of Feverfew's dry herb and of three different solvents - purified water, 10% of dimethyl sulfoxide (DMSO) and 40% of ethanol were produced by classical maceration method. Total phenolic content of prepared feverfew's extracts was evaluated using Folin-Ciocalteu's spectrophotometric method and calculated on the basis of the calibration curve of gallic acid. Cell viability was assessed by measuring the ability of C6 cells to metabolize MTT dye, additionally cell viability rated using Hoechst 33258 and propidium iodide assay.

Results and discussion

The highest content of total phenolic compounds was determined in ethanolic extract of Tanacetum parthenium L. and that is 1.6 times more than in DMSO extract and 2.5 times more than in aqueous extract. Results of both MTT assay, Hoechst 33258 and propidium iodide assay have shown the capacity of three different Tanacetum parthenium L. extracts to induce dose-dependent reduction in viability of C6 cells comparing to control groups. Also, Hoechst 33258 and propidium iodide assay has shown the ability of ethanol extract of Tanacetum parthenium L. to exert stronger cytotoxic effect on C6 cells than DMSO and aqueous extracts even though the same amount of polyphenolic compounds was added.

Conclusions

Three different Tanacetum parthenium L. extracts showed cytotoxic effect on rat C6 glioblastoma cells in a concentration-dependent manner and the effect of ethanolic extract compared with DMSO and aqueous extracts was stronger.

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The effect of *Solidago gigantea* L. extract on malondialdehyde (MDA) level in mice liver

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Introduction

It has been found, that *Solidago gigantea* L. leaves have the highest values of phenolic compounds compared with other *Solidago* L. species growing in different places of Lithuania [1]. Phenolic compounds have an antioxidant effect which determines activity against various degenerative diseases [3]. The aim of this study is to determine if the extract of *S. gigantea* leaves have an antioxidative effects in mice liver by measuring the MDA (malondialdehyde) concentration. MDA acts as an indicator of lipid peroxidation witch forms in organism when the oxidative stress occurs [4].

Materials and Methods

Experiments were done on 4-6 weeks old outbred mice weighing 20-25g. MDA concentrations were determined in the liver of laboratory mice after 21 days of *Solidago gigantea* L. extract intragastric administration via a stomach tube. Mice were randomly allocated to five groups with 8 mices per group to received following i.e., saline; *S. gigantea* extract; *S. gigantea* extract and $AlCl_3$; 10% ethanol (the same volume was used for extract); $AlCl_3$. MDA levels in liver samples were measured by the method which depends on the formation of MDA as an indicator of lipid peroxidation and reacts with thiobarbituric acid producing thiobarbituric acid reactive substances (TBARS). The absorbance was determined spectrophotometrically at 520 and 535 nm [2].

Results and discussion

The results showed that MDA level significantly ($p \leq 0.05$) increased by 349.19% in case of ethanol, by 47,52% in case of $AlCl_3$ and decreased by 50.77% in case of *S. gigantea* extract compared with control group. Whereas *S. gigantea* extract in combination with $AlCl_3$ had no effect on MDA level compared with control group with saline.

Conclusions

Our studies revealed that *S. gigantea* L. extract has an effect against oxidative damage in mice liver, because *S. gigantea* extract in combination with $AlCl_3$ insignificantly ($p \geq 0.05$) increase the concentration of MDA. Also, liver was most affected by ethanol, and aluminum also affected mice livers significantly. Antioxidative properties of *S. gigantea* extract counteract the negative Al effect protecting liver from lipid peroxidation.

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Issues compounding Levetiracetam preparations.

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Background

Levetiracetam is an anti-epilepsy medication [1] predominantly produced in form of coated tablets. An oral solution marketed by single company in EU is sadly unavailable for purchase in Latvia [2]. Compounding is a quick solution, for patients, who are unable to use tablets due to age restrictions and/or inadequacy of drug dosage. Although powders and solutions for internal use are easily compoudable, active pharmaceutical ingredients are not reimbursable [3]. As a result drug forms are compounded from crushed tablets, which produces a certain risk to the patients in a way of potential harm from excipients [4] i.e. colorants [5].

Methods

A retrospective analysis of compounded drug forms was performed in 3 pharmacies in Riga. Compounded levetiracetam preparations were cross referenced with EU guidelines for pediatric drug form manufacture as well as relevant scientific literature. Aim of the study was to gauge the safety of compounded drug forms for pediatric use.

Results

In total 3201 prescriptions were inspected. This amount included 470 drug forms for internal use. In the final analysis only 34 compounded drug forms were included, according to criteria of the study (drugs containing Levetiracetam). Analyzed drug forms presented as 5 suspensions and 29 powders for internal use. After theoretical evaluation 34 drug forms were considered to provide additional risk to the active ingredient [6-8], as they were produced from crushed tablets. As a result, 24 analyzed prescriptions contained minor discrepancies [7,9] and 10 drug forms were considered potentially harmful for children [10-12].

Conclusions

These findings suggest a quantitative study that would have possibility to determine the amount of risk, undertaken by compounding medicines from different active pharmaceutical ingredients. Drug reimbursement system in Latvia does not include active substances for compounding, which is an inhibitor for compounding safe and effective drug forms.

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Triple negative breast cancer cell sub-lines differences in MDA-MB-231 cell culture

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Introduction

Triple negative breast cancer (TNBC) includes a heterogeneous subgroup of tumors accounting for approximately 15-20% of all breast cancers. TNBC presents a more aggressive natural history and worse disease-specific outcomes than other breast cancer subtypes [1]. Cellular heterogeneity is observed in TNBC and represents a major hurdle for effective therapy [2]. Scientists found that in genetically homogenous cell populations, the phenotypic heterogeneity is frequently observed *in vitro* cell cultures even in a controlled environment [3]. Identifying the differences between populations and their response to anticancer drugs could help to predict the tumor resistance to chemotherapy and help rationalize the choice of appropriate antineoplastic drugs, paying attention to the nature and relationship of the phenotypic populations of the tumor [4]. The aim of the study was to isolate phenotypically different cell sub-populations (sub-lines) from the commercial MDA-MB-231 cell line.

Materials and Methods

Sub-line isolation was performed by multiple dilutions of the cell suspension and passage to a 96 well microplate. Based on the morphological differences of separate colonies, those cell sublines that were most distinct in appearance and in the density of the colony were selected. Also, sublines have been characterized by the expression of the CD133 receptor (immunofluorescence staining), their susceptibility to anticancer drugs doxorubicin (DOX) and paclitaxel (PTX) (MTT assay), and by ability to migrate (wound healing assay). All the experiments were done in at least triplicate independent measurements. Student's t-test was used, and p-values were calculated. A value of $p<0.05$ was considered as the level of significance.

Results and discussion

The expression of the CD133 receptor was more than 30% higher in the subline E7 compared to the commercial cell line MDA-MB-231. The susceptibility of cell sublines to the tested anticancer drugs was different. DOX most significantly reduced the viability of cell subline F5 (EC50 value after 72 hours was 58.9 ± 7.2 μM, whereas EC50 value for MDA-MB-231 cell line was 140.0 ± 23.2 μM). Subline H2 was the most resistant to DOX (EC50 value after 72 hours was 158.7 ± 33.3 μM). All sublines were from 2 to 4 times more resistant to PTX compared to the commercial line. The results of the cell migration study showed that majority of isolated sublines possessed higher ability to migrate compared to the parent line MDA-MB-231. The migration rate of sublines B7, F7, G5 and H2 was from 4 to 8 times higher in comparison to MDA-MB-231 line.

Conclusions

Sublines isolated from MDA-MB-231 show significant resistance to anticancer drugs DOX and PTX and higher migration ability compared to the commercial cell line. This study will facilitate the exploration of new therapeutic strategies based on the single-cell-derived clonal analysis in *in vitro* systems.

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Auranofin decrease progression of neurodegeneration by reducing Aβ peptide deposition in APP$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice

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Introduction

Neuroinflammation in the central nervous system contributes to several neurological disorders including Alzheimer's disease (AD) (*Madeira J. et al 2013*). Inflammatory cells, in particular microglia and astrocytes, are activated in brain areas affected by two main pathological hallmarks for AD, Aβ (1-42) peptide and tau protein hyperphosphorylation and aggregation (*De Deyn P.P. et al., 2010*). Several studies have proven that anti-inflammatory drug Auranofin (AR) has been used to reduce the neuronal loss caused by neuroinflammation *in vitro* studies (*Madeira J. et al 2013*). There are no effective clinical treatments to prevent or cease neuroinflammation. In this study we studied the efficacy of AR 1 mg/kg and 5 mg/kg to prevent the development of cognitive impairments and neuroinflammatory in the APP$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ AD model-mice model.

Materials and Methods

For this study, 14 months-old male APP$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice were used. Mice received once a day intraperitoneal injections for 30 days of either control (saline 1 ml/kg) or AR 1 mg/kg and 5 mg/kg. 21 days after the start of treatment, the animals were tested in behavioural tasks: object location task (OLT) and Morris water maze tests. For immunohistochemistry (IHC), we assessed macroglial marker glial fibrillary acidic protein (GFAP), GAD67, Homer-1, mouse anti-human Aβ$_{4–10}$ (W0–2 antibody) anterior cingulate cortex and striatum radiatum of hippocampal cornu ammonis 1 (CA1) region.

Results

IHC data shows that both injected doses of AR markedly reduce Aβ load in the CA1 region (1 mg/kg $p = 0.0004$ and 5 mg/kg $p = 0.02$) but not in cortex. Staining for the GFAP, GAD67 and Homer-1 did not show any significant differences among groups in average density, measured in both brain structures. There were no significant differences between treatment groups and control treated mice in the OLT and water maze tests.

Conclusions

We demonstrated that AR at the dosed of 1 mg/kg and 5 mg/kg reduced Aβ (1-42) peptide deposition in AD mice brain and may slow neurodegenerative processes. Therefore, further testing of AR in AD mouse models of neuroinflammation is needed.

Acknowledgements

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Terpenes profiling in *Cannabis sativa L.* from Lithuania by gas chromatography mass spectrometry (GC-MS)

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Introduction

The primary research focus of *Cannabis sativa L.* are cannabinoids which present abundance indications for medical treatment, however, they are not the only key compononets for the activity of cannabis extracts. [1] Terpenes play a significant role influencing some indications or inducing synergistical effects with cannabinoids. Terpenes shown many therapeutic effects like anti-inflammatory, anticarcinogen, sedative and plenty others. [1,2] Terpenes and chemical compositions of *Cannabis sativa L.* crucially depends on its agricultural environment and breed. Number of cultivars registered for the EU is 51. Since there are many different breeds it is important to find out their chemical constituents. [3] Two terpene profiles of different Cannabis sativa breeds (Fedora and Santhica) that grew up in Lithuania were compared in this research.

Materials and Methods

Cannabis sativa L. breeds Santhica and Fedora were collected from Joniškis, Lithuania. Both materials were air–dried and grinded separately. For oils extraction from raw materials hydrodistillation was used. 15,0 g of material was mixed with 500,0 ml distilled water and boiled on the oil bath (Heidolph) at 120 °C for 3 h. Essential oils were diluted with 1,0 ml cyclohexan and then this solution was used for further GC-MS analysis. Terpene analysis were conducted using a Shimadzu GC-MS – QP2010 Ultra with AOC – 5000 Plus autosampler, Restek Rxi – 5ms (Restek Corporation) capillary column (30 m long with 0,25 mm outer diameter and 0,25 μm liquid-stationary phase thickness) with a liquid stationary phase (5% diphenyl and 95% polysiloxane). The instrument and operating conditions were: split injection mode with 60,0 split ratio, Column Oven temp 50,0 °C, Injection temp 260,00 °C. Oven temperature program was 50 °C and 5,00 hold time, rate 2,00, 50 °C, 0,00 hold time, rate 15,00, 315,0 °C and 15,00 hold time. Main substances were determined by comparison with database mass spectra of compounds or analyzing ions characteristics of mass spectra.

Results and discussion

Terpene profiles of Santhica and Fedora breeds were analyzed by GC-MS. In Santhica 11 terpenes were found: 10 sesquiterpenes and 1 monoterpene. The quantative analysis of terpenes indicated that most abundant terpenes were those that took up the widest area of the chromatogram peaks area: Shyobunol (34,09%), Aromandendrene (26,57%), trans-α-Bergamotene (11,36%) and Isogeranial (11,14%). In Fedora 19 terpenes were found: 17 sesquiterpenes and 2 monoterpenes. Most abundant terpenes were β-Elemene (24,38%), trans-α-Bergamotene (16,21%), trans-Verbenol (16,30%) and β-Selinene (8,17%).

Conclusions

In Santhica and Fedora breeds sesquiterpenes dominated over monoterpenes. Fedora exposed greater variety and affluence of terpenes than Santhica. However, Santhica and Fedora terpene profiles had similarities, both had common Bergamotene, Famesene, Selinene and 5-α-10-β-Sibirene terpenes.

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Evaluation of safe medication use among older patients in Estonia and Finland living in nursing homes

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Introduction

Today, the use of medications has become common to most people. Advancements in healthcare, enable people living in nursing homes, to maintain a stable quality of life. However, older patients often face with problems connected to polypharmacotherapy – Inappropriate medication use, low compliance with treatment regimens, etc.

The purpose of this study was to evaluate safety aspects in medication use among older people living in nursing homes in Estonia, and to compare the results obtained with a similar study conducted in Finland.

Materials and Methods

The study sample included older adults aged 65 and older (n=303) who live in nursing homes in Estonia. The data about medication use was extracted twice for 6 months period (September 2016 and March 2017). The Finnish study started in 2015 (lasted for six months) and study sample consisted of patients (n=208) aged from 49 to 108 years. To evaluate the use of potentially inappropriate medications (PIM) and drug interactions, the analysis was performed according to the EU(7) – PIM list (1) and INXBASE database (2), respectively. The impact of demographic characteristics (gender, age) as well as number of medications to PIM use and drug interactions was calculated by statistical analysis of the Pearson Chi-Square test.

Results and discussion

Of the patients, 55.8% had 1 to 5 PIMs in the treatment regimen. A total of 154 Inxbase A-D class drug interactions, including 62.6% clinically significant interactions, were identified. Statistically significant correlations ($p<0,001$) were found with number of medications used, number of PIMs and drug interactions identified. Comparison of the results with Finnish study demonstrated similar level of PIM use among older patients – in Estonia 55.8% and in Finland 64.0%. More frequent PIMs as acetylsalicylic acid and PPIs were common in both countries. The results differed in terms of clinically significant D-class drug interactions, being higher in Estonia (7.4%) than in Finland (2.4%).

Conclusions

This was a first study in Estonia evaluating medication use of older patients using combined method of EU(7)-PIM list and drug interaction database Inxbase. Prevalence of PIM use was considerably high in both countries – Estonia and Finland. Clinically significant drug interactions were more identified in Estonia than in Finland. Different databases provide useful information to healthcare specialists about potential threats of medications used by specific patient groups. Further research is needed to find out how this information could be combined with treatment decisions for single patients to ensure safe and effective use of medications.

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Determination of buspirone, fluoxetine, escitalopram and paroxetine by HPTLC after SPE extraction

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Introduction

Patients with depression tend to abuse psychotropic drugs, take more than one antidepressant at the same time or at higher doses, use them as the suicidal agent [2]. In this case, there is an increased risk of poisoning, causing serotonergic syndrome or even death [1]. As a result, antidepressants are often found as an overdose cause in clinical practice. In order to determine which drug a person is poisoned with, it is useful to analyze the most effective and fastest ways to extract these drugs from plasma, separate and identify them. We analyzed buspirone (BUS), fluoxetine (FLU), escitalopram (ESCI) and paroxetine (PAR).

Materials and Methods

The SPE method (1 cm3 30 mg Oasis HLB reversed phase extraction columns, Waters, USA) was used to isolate the analytes from blood plasma. Separation and identification of analytes was obtained with using silica gel plates (HPTLC Silica gel 60 F254). The standards and test solution were injected by Camag Linomat 5 with volume 7 μL. Plasma solution was made by mixing plasma and each standard solution to equal parts. SPE eluting step was performed with methanol, propanol, trichlormetan, acetonitrile, 80% ethanol in water solution, 2% acidified with concentrated formic acid, 80% ethanol in water solution, 2% alkalined with ammonia solution. HPTLC mobile phase was made of acetonitrile:methanol:25%ammonia solution (85:10:5). The HPTLC determination was made with UV detector at the wavelength 254 nm.

Results and discussion

Therefore, the most appropriate eluent was found to be 80% ethanol in water solution, 2% acidified with concentrated formic acid for the best separation and identification of the analytes ESCI, FLU, PAR, BUS. HPTLC plate results showed the fast separation and indication of each drug, extracted from blood plasma. Spots from test solution corresponded to spots obtained from standard solutions. Rf of compounds BUS, PAR, ESCI, FLU was found as 0,92, 0,60, 0,72, 0,65 respectively. These results show an effective application of the methodology for BUS, PAR, ESCI, FLU analysis.

Conclusions

SPE procedure was found as suitable and specific method of investigation of analyzed compounds. Mixture of 80% ethanol in water solution, 2% acidified with concentrated formic acid is the most effective eluent to extract all analytes (buspirone, fluoxetine, escitalopram and paroxetine) from blood plasma. HPTLC is a fast and effective method for separation and identification of all four analyzed compounds. The developed method may be used for fast identification of these substances in the blood plasma.

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An analysis of the demand for additional pharmaceutical care services in a pharmacy

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Introduction. European healthcare policy pays particular attention to implementation of the programmes for prevention of chronic diseases, diagnostics of diseases and their effective treatment. In order to ensure provision and accessibility of qualitative healthcare services most countries develop additional pharmaceutical services in pharmacies. Pharmacists consult their patients on rational consumption of medicines and effectively solve the problems arising. By cooperating with doctors they prepare a treatment plan and help in the improvement of treatment results, realise the programmes of management and early diagnostics of chronic diseases, help to develop a healthy lifestyle and improve the quality of patient's life. In order to improve access to healthcare services in Lithuania's pharmacies it is very important to evaluate population's demand for the provision of those services in pharmacies, to reveal which services help to manage diseases and to establish the reasons restricting the development of those additional pharmaceutical services in Lithuania.

Materials and Methods. Research was carried out in the period of 2017–2019. To carry out research a quantitative method, i.e. an anonymous questionnaire survey consisting of 19 closed-ended questions, was chosen. During research, 500 questionnaires, 422 of which were suitable to the analysis, were distributed. The data obtained during the questionnaire surveying were analysed by using SPSS 25.0 version software packet.

Results and discussion. Our survey results showed that 74,4 % of the respondents in a pharmacy purchased prescription and 87,4 % non-prescription drugs. However, a considerable number of the respondents consult on the indications of medicines 36,7 % and their side effects 36,7 %, their possible interactions 36,5 % and accurate dosing 36,3 %. The results obtained from research correspond to the conception of additional pharmaceutical services described in the (Švarcaitė, 2014) article. The main reason, because of which the respondents refer to a pharmacist, is an ability to consult on minor complaints 87,7 %. The results obtained from research analogously (Awad AI et al., 2017) correspond to the data of research, which show that patients' appropriate healthcare is ensured the ability to solve health problems effectively. Besides, the obtained results indicate that only 27,7 % of all respondents use additional services. Basing on the obtained results it may be assumed that infrequent use of these services is determined because these services are not rendered constantly and this is not accessible for most of the population. However, in the opinion of the respondents, extension of prescriptions 74,8 %, revision of medicines 73,1 % and measurement of blood cholesterol 72,9 % and glucose 72,8 % would be the most significant services in the management of diseases as well. It has been revealed that 40% of the respondents have suffered from the side effects of medicines and prescription of wrong medicines 29,9 %, and even 84.8% of the respondents have noted that rendering of additional services would solve these problems. Through research it was revealed that the essential factors preventing from the development of these services in the pharmacies were premises intended for an individual consultation and lack of confidentiality 77,1 %, a shortage of special measures and equipment 72,7 % as well as busyness of a pharmacist 72,5 %.

Conclusions. The results of our survey have shown that rendering of additional pharmaceutical care services in pharmacies would be relevant to the most of the respondents and that would help in solving issues related to the consumption of medicines and controlling the course of the disease. In terms of the most respondents, extension of prescriptions, revision of administrated medicines and assessment of their compatibility, measurement of blood cholesterol and glucose and home delivery of medicines would have the largest effect on the management of diseases. The main reasons restricting the development of additional pharmaceutical services in pharmacies are separate premises and lack of confidentiality, a shortage of special measures and equipment and pharmacist's busyness.

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Survey of Latvian pharmacists on implementation of additional pharmaceutical care services

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Introduction

Worldwide community pharmacies are shifting their role in the healthcare system from simple medication dispensers to health care providers. Although pharmacists have always had involvement in providing health services, this trend has increased in recent years. In last decades the scope of services introduced in pharmacies is growing, moreover new legislation that defines the range of services has been set in some of European Union countries. Study targets exploring pharmacist attitude to potential services offered in other European Union countries, addressing possible boarders for introduction of these services in community pharmacies of Latvia from pharmacist perspective.

Materials and Methods

The study utilized a quantitative design, and data were collected through a survey of closed-ended questions. The survey was designed and conducted by University of Latvia. The questionnaires were distributed to pharmaceutical specialists in Latvia – pharmacists, pharmacist assistants. The data were collected by 107 questionnaires under the supervision of 2 field-work coordinators.

Results and discussion

Opinions regarding additional pharmaceutical services differ greatly among respondents: 42% of pharmacists support implementation of services, however 58% of respondents are against it. The employees of Latvian pharmacies are the most interested in an in-depth patient consultation on new medications (57%), and they support programs for weight loss (48%) and smoking cessation (47%). The most negative attitude of responders was expressed regarding the service of vaccination (67%) and first aid for small injuries and wounds (66%) in pharmacies. The practitioners as the main reasons against implementation of additional pharmaceutical care service mentioned: Community pharmacy has not private space for counselling; A lack of standards of additional pharmaceutical care services; Pharmacists long working hours and shortages of experienced, trained staff at the pharmacy. Previous studies have shown, that by implementation of the advanced pharmaceutical care the increase in patient loyalty could be achieved and thus strengthen competitiveness of pharmacy businesses[1].

Conclusions

Community pharmacies present several benefits for its customers. Due to an extended business hours and no appointment needed in advance, the pharmacies can be more accessible than other health care providers. In has been found that customers could be interested in wider range of services available in pharmacies that are currently provided in other health care facilities[2].

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Development of assay method for analysis of a novel Kardiazol substance by HPLC

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Introduction

Kardiazol ([3-allil-4-(41-metoxi fenil)-3N-thiazol-2-iliden]-(3²-trifluoromethylphenyl) amin hydrobromide) is a novel substance that was synthesized in Danylo Halytsky Lviv National Medical University. Quality of medicines depends from many factors. One of the main is quality of its components and first of all active pharmaceutical ingredients (API). Therefore, developing assay method method for analysis of Kardiazol is quite necessary as far as it is promising substance that has cardioprotective [1], inta-inflammatory, analgetic, hypolipidemic and antioxidant effects.

Materials and Methods

HPLC analysis has been carried out with usage of Shimadzu Nexera X2 LC-30 AD (Japan). Separation was performed on ACE 5 C8 (250*4.6 mm, particle size μm). The binary solvent system of the mobile phase was used: the solvent A (water) and the solvent B (acetonitrile). The following linear gradient elution profile was used: 55% A/45% B–0 min, 55% A/45% B–3 min, 20% A/80% B–7 min, 20% A/80% B–14 min, 55% A/45% B–16 min and 55% A/45% B–20 min. The flow rate was 1mL/min and injection volume was 10 μL. The column temperature was constant 35 ˚C. The chromatograms were recorded at 300 nm. The weights of substance was diluted in acetonitrile with final concentration 400μg/ml.

Results and discussion

In suggested conditions retention time of the investigated component was about 13.9 min. The column performance was determinate for its main indexes such as theoretical plate number (more then 65000) and symmetry factor (about 1.00).

The method was validated according to Ukrainian Pharmacopoeia [2] and ICH guidelines [3] in terms of specificity, linearity, precision and accuracy. The validation data of proposed method met all requirements. The linearity was made and expressed by the following quadratic equation: R^2=0.9998 (y=0.9956x+0.05031), the linearity range 80-120% of standard solution concertation. The precision was found to be satisfactory, since all the obtained relative standard deviation (RSD) values were lower than 1.0%.

Conclusions

A specific and accurate HPLC method for determination of novel substance Kardiazol has been developed and validated. It may be used for future investigation the substance as a component of different dosage forms.

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Artemisia afra Jacq. as a medicinal plant for malaria and skin diseases?

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Introduction

According to the World Health Organization about 70–95% of the world's population in developing countries relies mainly on medicinal (aromatic) plants (MAPs) for their primary health care [3]. MAPs are the renewable resources of biologically active compounds. Most of natural compounds possess several biological activities: antioxidant, antimicrobial, anti-inflammatory and play a certain physiological role in human organism. Therefore, it is very important to look for the new natural compounds and study less investigated MAPs as a possible source for such compounds. Object of this review - *Artemisia afra Jacq.* (*A. afra*).

The aim of this review was therefore to collect all available scientific literature published on *Artemisia afra Jacq.*

Materials and Methods

In this review, we have compiled data of recent literature (2010–2018) on essential oil composition, antimicrobial, insecticidal and antioxidant activities of *A. afra*.

Results and discussion

The genus *Artemisia* L. consists of about 500 species, occurring throughout the world. Some very important drug leads have been discovered from this genus, notably artemisinin, the well-known antimalarial drug isolated from *Artemisia annua* [4]. *Artemisia* L. genus is also known for its aromatic nature and hence research has been focused on the chemical compositions of the volatile secondary metabolites [2]. In the southern African region, *A. afra* is one of the most popular and commonly used herbal medicines. It is used to treat various ailments ranging from coughs and colds to malaria, diabetes and for dermatological inflictions - skin diseases [1].

Conclusions

Artemisia afra Jacq. is widely distributed in the southern parts of Africa. It is therefore one of the most utilized plants in the African ethnopharmacology. This review aimed at describing the different uses of *A. afra*, either alone or in combination with different substances or species, in treating various ailments. The main focus of this review was however to bring together all available scientific research that has been conducted on this species. The identification of the volatile secondary metabolites obtained from this species received a lot of attention as is reflected by the number of papers that has been published on this matter.

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Selling of OTC medicines at general sales stores - public's and pharmacy specialists' perspective

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Introduction

The regulation of selling OTC (over-the-counter) drugs varies across various European Union (EU) member states, e.g. in France Pharmacists still retain a strong control over selling of medications[1]. However, regulatory reforms were introduced in countries like the Netherlands, Poland, Sweden, Norway, Denmark, Ireland and UK to allow selling of selected non-prescribed drugs at General Sales Stores (GSS) [2]. Lithuania is one of the latest countries and the first of the Baltic States to have this regulatory reform introduced into its law (effective date 1st January, 2019). Hence, there are not many studies available regarding the population's and Pharmacy specialists' attitude towards this issue.

Aim – to analyze the opinion of selling of non-prescription drugs at GSS from Lithuanian public and Pharmacy specialists' points of view.

Tasks:

- To collect surveys from public in Lithuania;
- To collect and compare surveys from Pharmacy specialists from Lithuania and Estonia;
- To compare the answers of Lithuanian public with the results of PMR Research carried in Poland in 2011[3].

Materials and Methods. Two different questionnaires (for public and for Pharmacy specialists) were prepared. After collecting the data, analysis was performed.

Results and Discussion. Most of the Lithuanian Pharmacy specialists disagree (87%) with the selling of non-prescribed drugs at GSS while public's opinion is less rigid (35%), however, most of the respondents agree that they would lack a consultation from a specialist (36%). As one of the main advantages public sees the ability to buy non-prescribed drugs at GSS in emergency cases which in contrast to PMR Research was the least important priority (less than 5%). According to preliminary data, Pharmacy specialists from Estonia share similar views to Lithuanian Pharmacy specialists.

Conclusions:

- Public in Lithuania is more likely than Pharmacy specialists to agree with selling of non-prescribed drugs at GSS.
- Results of our public survey have some similarities with PMR Research survey.

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Frequency in use of medications with clinically relevant drug-drug interactions – a sample of nursing home patients in Estonia

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Introduction

Today health of the large number of people is directly related to the use of medications. Every year the number of medications consumed by patients is increasing and this leads also to increase of drug related problems. The objective of this study was to identify and evaluate potential risk of medications and their various combinations with clinically relevant drug-drug interactions in the medication list of patients living in nursing homes in Estonia.

Materials and Methods

A list of 12 medications with clinically relevant drug-drug interactions was created based upon the previous research (1-4). Identified potentially hazardous medications were searched in the medication list of the nursing home patients (n=416) aged 23 to 103 years. Potential drug-drug interactions were identified by using drug interaction databases Inxbase and Drugs.com.

Results and discussion

Medications from the developed list were used by 52.3% of the patients (n=219). Of these patients 79.9% (n=175) were over 65 years old and 25.6% (n=56) used more than one potentially hazardous medication. Based on the analysis of drug interaction databases there were identified 86 potential clinically relevant drug interactions that may have occurred 223 times in 48.4% of the patients (n=107). More frequently used risk medications were NSAIDs, PPIs, Digoxin, Furosemide and Amitriptyline. Of 219 patients 4% (n=9) may have experienced severe Inxbase D-class drug interactions

Conclusions

Study results demonstrated that even carefully controlled patient treatment regimens might contain potentially hazardous medications and these cases should be treated with special attention. In the studied sample half of the patients used medications with clinically relevant drug-drug interactions and quarter may have experienced drug interaction symptoms. More detailed analysis of treatment regimens might be needed to increase safety and effectiveness of drug treatment.

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The amount of chlorophyll *a* and *b* in various conifers

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Introduction

Chlorophyll is one of the most important chelates in nature. It could have a wide application in medicine and pharmacy to treat acne, help to heal wounds, it could be used as a deodorant or one of the blood building compounds. The purpose of research was to identify the amount of chlorophyll *a* and *b* in various conifers, while using methods from literature and recognise the most valuable species.

Materials and Methods

Needles of 6 taxa of genus *Picea* (*Picea abies*, *Picea abies 'Barryi'*, *Picea pungens*, *Picea gemmata*, *Picea pungens 'Hoopsi'*, *Picea mariana)* and 8 taxa of genus *Pinus (Pinus strobus*, *Pinus sylvestris*, *Pinus ponderosa*, *Pinus cembra*, *Pinus banksiana* × *Pinus contorta*, *Pinus koraiensis*, *Pinus parviflora 'Glauca'*, *Pinus mugo*) growing in Vilnius University (Lithuania) Botanical garden were investigated. Fresh needles of investigated trees were extracted with N, N-dimethylformamide five days; analysis of chlorophyll *a* and *b* carried out with UV spectrophotometer.

Results and discussion

Chlorophyll *b* was prevalent over chlorophyll *a* in all investigated taxa: in most cases amount of chlorophyll *b* was even 1.5–2 times higher than amount of chlorophyll *a*. The highest amounts of chlorophyll *b* and chlorophyll *a* were established in needles of *Pinus koraiensis*, the lowest – in needles of *Picea abies*. Results showed also, that variation of amount of chlorophyll *b* between 14 investigated taxa of conifers was higher in comparison with variation of chlorophyll *a*. The average amount of chlorophyll *b* was higher in genus *Picea*, while average amounts of chlorophyll *a* in genus *Picea* and *Pinus* were similar. After biologically active food supplements with chlorophyll extracts are produced from needles of *Picea abies*, however present results demonstrated that taxa of genus *Pinus* could be also promising in preparation of chlorophyll extracts .

Conclusions

Chlorophyll *b* was more abundant in all taxons of genus *Picea* and genus *Pinus* in comparison with chlorophyll *a*. Amounts of chlorophyll *b* were very similar in both genera, while amounts of chlorophyll *a* were established higher in taxa of genus *Pinus*.

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Investigation and comparison of the chemical constituents in green and black tea *(Camellia sinensis)* using physicochemical analysis methods

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Introduction. Green and black teas are one of the most consumed beverages in the world, both containing a wide range of active substances and proved to have beneficial effect to tea consumers' health. Green and black tea are produced from the same plant *Camellia sinensis (L.)*, but the further preparation of the tea leaves differ. [1,2] In this study the main objective was to analyze quantitative and qualitative parameters of aqueous black and green tea extracts, and to determine the conditions (by changing the water temperature and the extraction time) under which the amount of chemical constituents is the highest. The chosen active substances were caffeine, theobromine and theophylline.

Materials and Methods. Black and green tea samples were purchased from the same manufacturer in a community shop in the form of loose tea leaves. Both teas' origin country is Sri Lanka, Ceylon region. The samples were grinded separately and weighed into sample sizes of 2,0 g each. Each sample was mixed with 50,0 ml distilled water of the chosen temperature. Chosen temperatures were 70°C and 100°C and the extraction times were 3 min, 5 min, 10 min, 15 min. One sample of each tea was made using room temperatured distilled water and left in room temperature for 22 h. After the extraction of the determined period of time samples were filtered through a paper filter and chilled samples were used for further HPLC analysis. Analysis was conducted using a Waters 2695 chromatographer, 2998 PDA detector, ACE C18 (250mm × 4,6mm) column, sorbent particle size of 5 μm, gradient pump mode, mobile phase consisting of trifluoroacetic acid (TFA) and acetonitrile (ACN). The amounts of caffeine, theophylline and theobromine were determined by comparing sample results with standard solutions of the chosen substances.

Results and discussion. Caffeine, theobromine and theophylline determination was conducted by HPLC. In green tea extracts the highest amount of caffeine (2,696 mkg/ml) and theobromine (89,598 mkg/ml) was determined with 70°C water and extraction time of 3min. The only sample where theophylline was detected (2,821 mkg/ml) was the one that was kept in room temperature for 22hours. Among green tea extracts the highest amount of theobromine (159,754 mkg/ml), theophylline (18,994 mkg/ml) and caffeine (2494,058 mkg/ml) was detected in a sample, which was made using 70°C and brewed for 15min.

Conclusions. The highest amounts of theobromine and theophylline were determined in black tea sample, which was made using 70°C water and brewed for 15min. The highest quantity of caffeine was determined in green tea sample that was brewed with 70°C water for 3 min.

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