ICPS – 2015

May 29-31, 2015, Tbilisi, Georgia

3rd INTERNATIONAL CONFERENCE on PHARMACEUTICAL SCIENCES

"Looking towards the future, honoring the past"

ABSTRACT BOOK

Notebook

Organized by:

Tbilisi State Medical University

TSMU I.Kutateladze Institute of Pharmacochemistry

TSMU Faculty of Pharmacy

Partners

Association of Scientists and Young Pharmacists of Georgia

Georgian Association of Pharmacists

Association of Professional Chemists of Georgia

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WELCOME

Dear Colleagues!

The Organizing Committee cordially greets you at the 3rd International Conference on Pharmaceutical Sciences "*Looking Towards the Future, Honoring the Past*" in Tbilisi.

We are glad to meet multi-disciplinary group of scientists from around the world to present and share most recent advances in major areas of pharmaceutical science here in Georgia – the middle of the ancient Silk Way – historical bridge between Asia and Europe.

We believe that ICPS-2015 will contribute to the development of modern pharmacy and give a push to new international linkages and joint researches.

We hope that you will enjoy the wonders of unique Georgian culture, delicious cuisine, and warm welcome of Georgian people, as well as the pleasant interaction with your colleagues.

The Organizing Committee thanks you once more for joining ICPS-2015 and will try to do its best to help you benefit from scientific and social parts of the Conference and carry home good memories of ICPS-2015.

Organizing Committee

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GENERAL INFORMATION

Conference Venue

Tbilisi State Medical University

Address: 33 Vazha Pshavela ave, Tbilisi, Georgia

Registration Desk

Conference Material and Name Badges will be distributed at the Regiatration Desk in the University hall left to main entrance.

Operating hours:

May 29 8:30 – 12:00

Secretariat and Information Desk

The Secretariat and Information Desk will operate in the registration area to provide any information regarding the Scientific Program, Social Events, transportation, lost & found, etc.

Operating hours:

May 29 8:30 – 17:30

May 30 9:30 – 17:00

SCIENTIFIC INFORMATION

For Oral Session Speakers

The session room is provided with LCD projector and laptop computer loaded with MS Office Power Point. Speakers are asked to bring their presentation (either on USB sick or CD) and load it during the break prior to the session. Staff will be available in the session room to assist with the operations.

Alloted time for Plenary Lecture will be 30 min, Invited Lecture – 20 min and Oral Presentation -15 min.

For Poster Presentations

The Poster Session will be held in the University hall. Authors are asked to mount posters on boards assigned by Secretariat during the registration. Mounting accessories will be available at the Information Desk.

PROGRAM

MAY 29, FRIDAY	
8:30 – 9:30	REGISTRATION
9:30 – 10:15	OPENING CEREMONY
	SESSION **1.** Chairmen: Assoc. Prof. Riad ELIAS, Dr. Malkhaz GETIA
10:15 – 10:45	PL-1. Recent Results in the Investigation of Bioactive Phenolics from Medicinal and Food Plants - *Prof. Cosimo PIZZA, Faculty of Pharmacy, University of Salerno, Italy*
10:45 - 11:00	OP-1. Secondary Metabolites from the Roots of Digitalis Ciliata Trautv. - *Dr. Alexander SKHIRTLADZE, TSMU I.Kutateladze Institute of Pharmacochemistry, Georgia,*
11:00 – 11:15	OP-2. In Vitro Antiproliferative Effect of Rubus Fairholmianus Gard. - *Dr. Blassan P. GEORGE, University of Johannesburg, South Africa*
11:15 – 11:30	OP-3. Novel Biomacromolecule from Medicinal Plants: Prospective Therapeutic Agent. *Dr.Vakhtang BARBAKADZE, TSMU I.Kutateladze Institute of Pharmacochemistry, Georgia*
11:30 – 12:00	COFFEE-BREAK
	SESSION **2.** Chairmen: Prof. Cosimo PIZZA, Dr. Alexander SKHIRTLADZE
12:00 – 12:30	PL-2. Medicinal Plants: Innovative Processes of Extraction. - *Prof. Riad ELIAS, Aix-Marseille Université, Faculté de Pharmacie de Marseille, France*
12:30 – 12:45	OP-4. Astragalus Species of Georgian Flora as Perspective Raw Material for Biologically Active Compounds. *Prof. Meri ALANIA, TSMU I.Kutateladze Institute of Pharmacochemistry, Georgia,*
12:45 – 13:00	OP-5. Development of Analytical Procedure for Quantification of Crude Extract from the Leaves of Hedera Colchica. *Dr. Malkhaz GETIA, TSMU I.Kutateladze Institute of Pharmacochemistry, Georgia*
13:00 – 14:00	LUNCH
	SESSION **3.** Chairmen: Prof. Athina GERONIKAKI, Dr. Maia MERLANI
14:00 – 14:30	PL-3. Multifunctional Polymeric Excipients in Mucosal Drug Delivery *Prof.Andreas BERNKOP-SCHNÜRCH, Institute of Pharmacy, University of Innsbruck, Austria*
14:30 – 14:50	IL-1. Application of Zeolites in Medicine *Dr. Vladimer TSITSISHVILI, Petre Melikishvili Institute of Physical and Organic Chemistry, Georgia*
14:50 – 15:10	IL-2. Counter-Current Chromatography, a Fast Method for Isolation of Natural Compounds. *Dr. Hamid-Reza ADHAMI, Tehran University of Medical Sciences, Iran*
15:10 – 15:25	OP-6. Modern Strategies in Treatment of Arterial Hypertension. *Dr. Maia OKUJAVA, Tbilisi State Medical University, Georgia*
15:25 – 15:40	OP-7. Pegylated Herbal Loaded Nanostructured Lipid Carriers for The Therapy of Cardiovascular Desease. *Dr. Lia TSIKLAURI, TSMU I.Kutateladze Institute of Pharmacochemistry, Georgia*
15:40 – 15:55	OP-8. Synthesis of Novel Chiral Sulfoxides and Investigation of Structure-Enantioselectivity Relationships in High-Performance Liquid Chromatography. *Rusudan KAKAVA, Tbilisi State University, Georgia*
15:55 – 16:25	COFFEE-BREAK
	SESSION **4.** Chairmen: Prof. Andreas BERNKOP-SCHNÜRCH, Dr. Lia TSIKLAURI
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16:55 – 17:10	OP-9. Camphor – as a New Scaffold for Antiviral Agents. *Dr. Olga I. YAROVAYA, Vorozhtsov Institute of Organic Chemistry, SBRAS, Russian Federation*
17:10 – 17:25	OP-10. Small Peptide Models of Metal-Amyloid **B** Binding Sites in Alzheimer Disease and New Bioactive Peptidomimetics. *Dr. Lili. ARABULI, Tbilisi State University, Georgia*
17:30	SOCIAL EVENT : CITY TOUR

MAY 30, SATURDAY	
	SESSION **5.** Chairmen: Assoc. Prof. Markus GANZERA, Dr. Vakhtang BARBAKADZE
9:30 – 10:00	PL-5. Novel Thiazolidin-4-One Derivatives With Potent Hiv-1 Reverse Transcriptase Inhibitory Activity. Divergence from Non-Competitive Inhibition Mechanism. *Prof. Athina GERONIKAKI, School of Pharmacy of Aristotle University of Thessaloniki, Greece*
10:00 – 10:20	IL-3. Biologically Active Azomethines. *Prof. Elizbar ELIZBARASHVILI, Agricultural University of Georgia, Georgia*
10:20 – 10:35	OP-11. ICL Catalyzed Synthesis of 3,6-Disubstituted-1,2,4,5-Tetrazines Under Microwave Irradiation: New Tool to Imaging Science & Cancer Therapy. *Dr. Nanjundaswamy HEMMARAGALA, University of Johannesburg, Lazer Research Center, South Africa*
10:35 – 10:50	OP-12. Screening of Adamantane Derivatives and Other Small Molecules Analogues for Antibacterial, Antiviral and Anthelminthic Activity. *Prof. Davit Zurabishvili, Tbilisi State University, Georgia*
10:50 – 11:05	OP-13. Synthesis of Bis-Pyridazinoindoles. *Natia KARCHAVA, Tbilisi State University, Georgia*
11:05 – 11:20	OP-14. The Biocomposite formulation Composed of 5-Fluorouracyl and a Biodegradable Polymer: the Preparation and Safety assessment. *Nino KUBLASHVILI, Georgian Technical University, Georgia*
11:20 – 11:35	OP-15.The Use of NMR Spectroscopy in Pharmaceutical Chemistry. *Dr. Mikheil LABARTKAVA, University "Geomedi", Georgia*
11:35 – 12:00	COFFEE-BREAK
	SESSION **6.** Chairmen: **Prof. Elizbar ELIZBARASHVILI, Dr. Karen MULKIJANYAN**
12:00 – 12:30	PL-6. DEVELOPMENT OF TECHNOLOGY AND DOSAGE FORM DESIGN FOR TARGETED DRUG DELIVERY - *Prof. Aliosha BAKURIDZE, Tbilisi State Medical University*
12:30 – 12:50	IL-4. Research and Development of Innovative Dosage Forms of Peptides. - *Prof. Rimma ABRAMOVICH, Peoples' Friendship University of Russia, Russian Federation*
12:50 – 13:05	OP-16. *Rhodopes Preparations in Dentistry: Results of Clinical Study. Dr.Natia NIZHARADZE, Tbilisi State Medical University, Georgia*
13:05 – 13:20	OP-17. *Needful Steps and Actions for Setting Up and Running A Pharmacovigilance and Adverse Drug Reaction Monitoring System in Georgia. Shota JIBUTI, State Regulation Agency for Medical Activities, Georgia*
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19:00	SOCIAL EVENT : GALA BANQUET

MAY 31, SUNDAY	
8:30	SOCIAL EVENT : EXCURSION TO GELATI-SATAPLIA

PLENARY LECTURES

PL 1. RECENT RESULTS IN THE INVESTIGATION OF BIOACTIVE PHENOLICS FROM MEDICINAL AND FOOD PLANTS

Cosimo Pizza

Dipartimento di Farmacia, Università degli Studi di Salerno, Fisciano (Salerno), Italy

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Phenolic compounds from medicinal and food plants include as main classes phenolic acids, flavonoids, tannins, phenylpropanoids, stilbenes, curcuminoids, coumarins, lignans, and quinones. Plant phenolics display a variety of biological activities including anti-inflammatory and chemopreventive properties such as antioxidant, anticarcinogenic, or antimutagenic.

In the present communication the main results obtained in the investigation of bioactive plant phenolics from medicinal and food plants are discussed.

Yucca schidigera (Agavaceae) known as yucca, is a plant native to the South-Western United States and Mexico. Two products, which possess GRAS label (Generally Recognized As Safe) given by FDA, obtained from the trunk of *Y. schidigera*, are available on the market: yucca powder and yucca extract.

Investigation of the phenolic fraction of *Y. schidigera* bark resulted in the isolation of the stilbenic derivatives trans-3,3',5,5'-tetrahydroxy-4'-methoxystilbene and trans-3,4',5,-trihydroxystilbene well known as resveratrol, along with larixinol, a spirobiflavonoid. Furthermore yuccaols A–E [1-2] and yuccaone A were isolated [3]. Yuccaols are very unusual spirostructures made up of C15 units probably derived from a flavonoid skeleton and a C14 unit corresponding to trans- 3,3′,5,5′-tetrahydroxy-4′-methoxystilbene linked via γ-lactone rings. Their similarity to resveratrol prompted us to evaluate for yucca phenolics some of the activities exerted by resveratrol, including antioxidant-, platelet activation inhibiting-, iNOS expression-inhibiting activities [4-6].

Continuing the study on species belonging to *Yucca* genus, in collaboration with the research group of Prof. Kemertelidze, Institute of Pharmacochemistry of Tblisi, Georgia, the investigation of *Yucca gloriosa*, a plant largely cultivated in eastern Georgia, was carried out [7]. In the past, the great interest in this plant was due to the very high content of steroidal sapogenins and their glycosides. The phytochemical investigation *Y. gloriosa* yielded phenolic derivatives, namely gloriosaols A-E and yuccaols C-E. Gloriosaols differ from yuccaols in the occurrence of two C15 units instead of one. Careful inspection of ROESY spectra revealed that gloriosaols C-E are stereoisomers of gloriosaols A and B, and the relative configuration of these compounds was derived according to an integrated NMR-quantum mechanical (QM) approach. Gloriosaols A-E exhibited potent antioxidant activity measured by the TEAC assay, showing the potential use of *Y. gloriosa* as a source of antioxidant principles [7].

Among natural polyphenolic derivatives there are examples of compounds with anti-STAT1 activity, such as epigallocatechin-3-gallate, other flavonoids, proanthocyanidins (PAs) or condensed tannins [9].

With the aim to screen a number of naturally occurring phenolics to identify anti-STAT1 compounds, investigations of *Garcinia cambogia* and *Guazuma ulmifolia*, sources of phenolic compounds, were carried out.

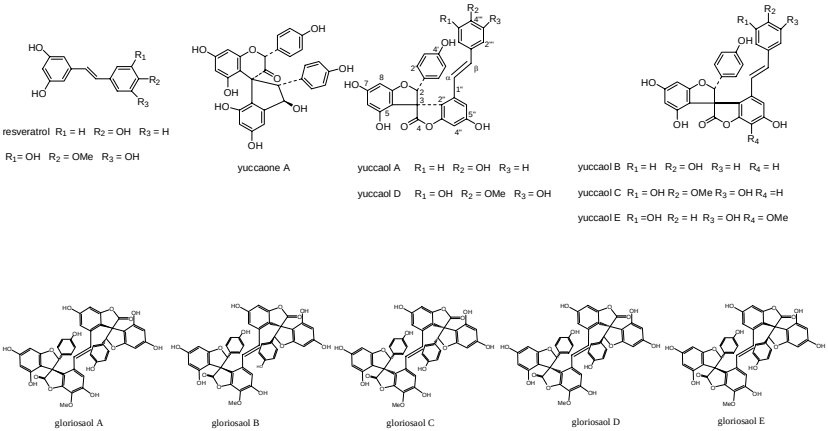


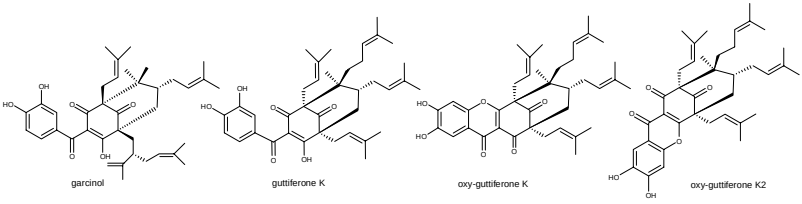
Fig.1 Phenolics of *Y. schidigera* and *Y. gloriosa*

Garcinia cambogia L. (Guttiferae) is a small or medium sized tree whose fruits, surrounded by a succulent aril, are used in the preparation of the well known *curry*. Our phytochemical study of the fruits of *G. cambogia* showed the occurrence of garcinol and guttiferones K, I, J, M, N, along with oxy-guttiferone K, M, K2, and I. Oxy-guttiferones are tetracyclic xanthones derived from the oxidation of the corresponding polyisoprenylated benzophenones [10,11]. The absolute configurations of oxy-guttiferone K, taken as a model of tetracyclic xanthones, and guttiferone M, as a model of polyisoprenylated benzophenones, have been determined by comparison of their experimentally measured circular dichroism (CD) curves with the TDDFT-predicted curves [7].

The affinity of garcinol, guttiferones K and M for STAT-1 has been evaluated by Surface Plasmon Resonance (SPR) and molecular docking studies. The equilibrium dissociation constants K_D obtained indicated that garcinol and guttiferones have a good affinity for STAT-1, when compared to that reported for ECGC. Molecular docking data of these compounds with the protein STAT-1 were in good agreement with the SPR results [12].

These compounds, by interaction with STAT-1, might interfere with cytokine signaling inhibiting the consequent induction of pro-inflammatory genes. Thus, the isolated compounds have been tested for their ability to modulate cytokine signaling in MDA-MB-231 and INS-1E cell lines.

The obtained data showed that garcinol was able to inhibit IFN-γ -induced STAT-1 as well as TNF-α -elicited NF-kB activation in both cell lines in a dose dependent manner, as assessed by evaluation of DNA binding of the two transcription factors by EMSA. Guttiferones K and M also exerted an inhibitory effect on cytokine signaling pathways, but with differences in different cell lines.



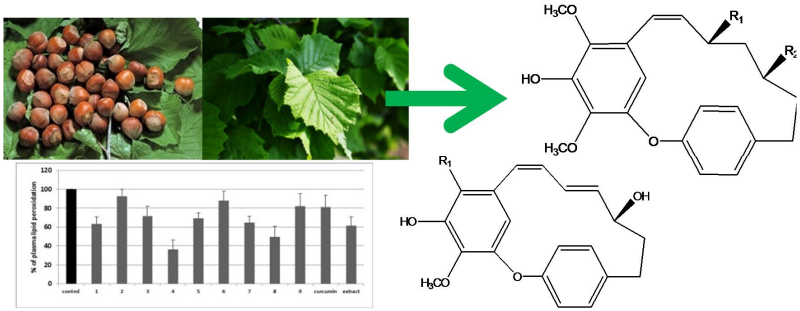
Flavanocoumarin derivatives, isolated from the bark of *Guazuma ulmifolia*, a plant used as anti-inflammatory remedy, showed affinity for STAT1, evaluated by SPR, comparable to that exerted by EGCG. Furthermore these compounds were able to inhibit STAT1 activation in human monocytic leukemia cell line TPH-1 [13].

More recently, our attention was focused on the tree affording the Italian PGI (Protected Geographical Indication) product "Nocciola di Giffoni" (*Corylus avellana* L.).

Hazelnut (*Corylus avellana* L.) is a plant belonging to the family Betulaceae. Hazelnut is known as a source of nutritious food with a high content of healthful lipids [14] Hazelnut skin, hazelnut hard shell, and hazelnut green leafy cover as well as hazelnut tree leaf do not have any commercial value and represent by-products with the potential to be valuable sources of bioactives.

The leaves of *C. avellana* have been used in traditional medicine for varicose veins and haemorrhoidal symptoms, and also for their slight antimicrobial effect. Previous investigations on the phenolic constituents of *C. avellana* leaves were focused only on the flavonoid and caffeic-acid derivatives [15]. Therefore, the phytochemical investigation of the MeOH extract of *C. avellana* leaves was carried out. Isolated compounds have been determined as cyclized diarylheptanoid-type molecules, characterized by oxygenated functions at different positions of the heptanoid chain, named giffonins A-I [16].

On the basis of the anti-oxidant activity reported for diarylheptanoids isolated from plants belonging to the Betulaceae family, the effect of the MeOH extract and giffonins A-I on human plasma lipid peroxidation induced by H_2O_2 and H_2O_2/Fe^{2+} have been tested and compared with the activity of the known antioxidant diarylheptanoid, curcumin. Lipid peroxidation has been quantified by measuring the concentration of TBARS. All compounds and curcumin have been tested at concentrations ranging from 0.1 to 100 μM. Most of the compounds were more active than curcumin at the same concentration.



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PL 2. MEDICINAL PLANTS: INNOVATIVE PROCESSES OF EXTRACTION

R.Elias, E. Ollivier, B. Baghdikian

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Extraction of natural products from medicinal plants was developed centuries ago by many ancient civilisations such as Egyptins, Greeks or Arabs. During these periods, innovative extraction methods, maceration or alembic distillation, were used for perfume, food and medicinal purposes.

The improvement of extraction methods and chemical knowledge led to the important use of organic solvents and energy which in turn resulted in the development of techniques such as soxhlet, heating under reflux, percolation….

These techniques require long extraction time and a large amount of organic solvents with a negative impact on the environment and human health.

Therefore tow decades afterwards the emergence of green chemistry aims to develop processes that reduce or eliminate the use of harsh organic solvents and to encourage the use of innovative processes of extraction known as green extraction.

Green extraction focuses on reducing energy consumption and using alternative solvents and renewable natural products so as to ensure safe and high quality extracted products.

Green extraction depends on six major principles:

1: Innovation by selection of varieties and use of renewable plant resources.

2: Use of alternative solvents and principally water or agro-solvents.

3: Reduction of energy consumption by energy recovery and using innovative technologies.

4: Production of co-products instead of waste to include the bio- and agro-refining industry.

5: Reduction of unit operations and favour safe, robust and controlled processes.

6: Aim for a non denatured and biodegradable extract without contaminants.

Currently the most used green extraction technologies are:

- Ultrasonic assisted extraction
- Microwave assisted extraction
- Pressurized liquid extraction
- Supercritical fluid extraction

Many examples using these technologies have been reported:

- Paclitaxel from Taxus baccata L.
- Curcumine from Curcuma longa L.
- Ginseng saponins from Panax ginseng C.A. Meyer
- Harpagoside from Harpagophytum procumbens (Burch.)DC.
- Glycyrrhizic acid from Glycyrrhizia glabra L.

- Cepharantine from Stephania rotunda Lour
- Essential oils from aromatic herbs

These examples illustrate that green extraction is an interesting concept for the 21st century. These ecological, economic and innovative technologies comply with environment and health protections and assure high quality and standardized extracts from medicinal plants. That is why It would be suitable that green extracts could be recognized by labeled organisms.

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PL 3. MULTIFUNCTIONAL POLYMERIC EXCIPIENTS IN MUCOSAL DRUG DELIVERY

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The potential of multifunctional polymeric excipients to improve the efficacy of drugs being administered by mucosal routes such as oral, nasal, ocular or vaginal has already been shown in numerous in vitro and in vivo studies. From the drug delivery point of view in particular mucus permeating, permeation enhancing, in situ gelling and mucoadhesive properties are of relevance. Particulate delivery systems are in most cases more efficient when they can permeate the mucus gel layer covering mucosal epithelia. On the one hand a prolonged residence time on the mucosa can be achieved and on the other hand the drug can be released in a more concentrated manner right on the absorption membrane. Polymeric excipients that can improve the mucus permeation properties of particulate delivery systems are PEGs, mucolytic enzymes such as papain and excipient combinations leading to a high density of positive and negative charges on the surface of mirco- and nanoparticles. Permeation enhancing polymers such as polyacrylates, chitosans and thiomers are able to open tight junctions on mucosal epithelia. In situ gelling polymeric excipients can prolong the mucosal residence time by increasing their viscosity once having reached the mucosa. Xanthan gum, gellan gum, poloxamers and thiomers exhibit this property. Furthermore, mucoadhesive polymers such as thiomers, carbomers and chitosans can also prolong the mucosal residence time.

PL 4. ALTERNATIVE APPROACHES FOR NATURAL PRODUCTS ANALYSIS

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Analytical sciences are steadily evolving, with new techniques and approaches being reported frequently. They often claim to be advantageous in respect to separation efficiency and selectivity, but as they are usually applied to well defined model mixtures only, they sometimes fail to convince in respect to practical use and applicability.

Two still rarely used techniques for natural products analysis are capillary electrophoresis (CE) and supercritical fluid chromatography (SFC). In CE separations are performed in narrow-bore silica capillaries filled with buffer, and with high voltage applied the analytes start to migrate because of the resulting electroosmotic flow (EOF). With published plate numbers of up to 500.000 this technique is extremely efficient, but at the same time economic and fast. SFC on the other hand utilises a supercritical fluid (mostly carbon dioxide) as mobile phase in a HPLC typical setup, resulting in low column pressure even at higher flow rates, increased solvating power and efficiencies. In this presentation it will be shown that all these general advantages can actually be transferred to practically relevant separations.

Lotus (*Nelumbo nucifera*) leaves are important remedies in traditional Chinese medicine to treat infections and liver ailments, the bioactive constituents are flavonoids and alkaloids. CE was ideal for the analysis of both compound classes. The separation of eight flavonoids was possible in nine minutes using a borax-based buffer system [1], for the analysis of different alkaloids (caaverine, isoliensinine, armepavine, etc.) non-aqueous CE was well suited [2]. CE-MS experiments were performed for peak identification, and they confirmed that nuciferine (0.34 – 0.63%) is the dominant compound regardless of geographic origin or harvesting season of the plant material.

The first protocol for the SFC analysis of isoflavones was recently reported [3]. It allowed the baseline separation of nine isoflavones (aglyca and glycosides) in 8 min, as well as their quantitative determination in relevant medicinal plants (*Glycine max*, *Trifolium pratense* and *Pueraria lobata*). For optimum results a 1.7 µm stationary phase was used and the mobile phase comprised supercritical CO_2 and methanol with phosphoric acid as additive. Method validation confirmed that the assay fulfils all required criteria and therefore in this case SFC has to be considered as an at least equivalent analytical alternative to established approaches.

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PL 5. NOVEL THIAZOLIDIN-4-ONE DERIVATIVES WITH POTENT HIV-1 REVERSE TRANSCRIPTASE INHIBITORY ACTIVITY. DIVERGENCE FROM NON-COMPETITIVE INHIBITION MECHANISM

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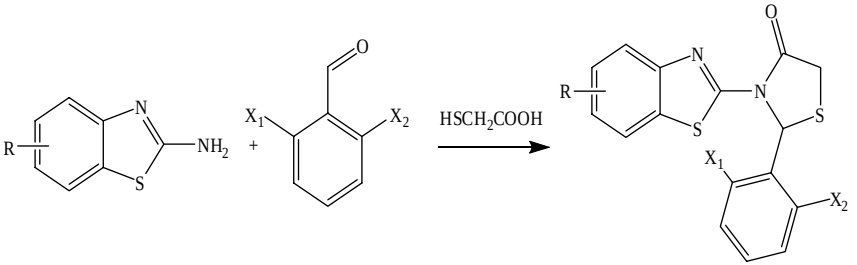
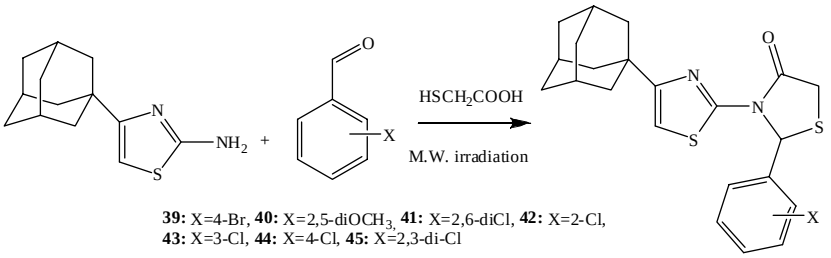
Human immunodeficiency virus (HIV) is a lentivirus that causes acquired immunodeficiency syndrome (AIDS), a condition in humans in which the immune system progressively fails and allows life-threatening opportunistic infections and cancers to thrive. Even if, 25 anti-HIV compounds have been formally approved for clinical use, no vaccine or cure is in sight. For this reason, there is an urgent need for the development of new therapeutic agents against HIV. Reverse transcriptase is one of the enzymes that play key role within the HIV replicative cycle and therefore it consists of the target of our research.

Based on the data from the literature regarding NNRTIs and taking into account the fact that cross-resistance is a common phenomenon, our aim is to design and synthesize new compounds, which preferentially exhibit a different mode of action, interacting with another part of RT. Two series of novel 4-thiazolidinones have been designed and synthesized, combining the thiazolidinone nucleus with benzothiazole or 4-adamantyl-thiazole groups, respectively.

Herein, we presented the synthesis and identification of 45 novel 2,3-aryl-thiazolidin-4-ones that could be divided into 2 groups: benzothiazole and 4-adamantyl-thiazole derivatives. All synthesized compounds were characterized by elemental analysis and spectroscopic methods (^{1}H NMR, ^{13}C NMR, HRMS).



Scheme 1. Reagents and conditions: (a) conventional method: toluene, reflux for 20–26 h, (b) microwave-assisted technique: absolute ethanol, 80–130 °C, power 50-200 W, 10–60 min.



Scheme 2. Reagents and conditions: microwave irradiation, absolute ethanol, 110–130 °C, power 100-200 W, 12–60 min

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PL 6. DEVELOPMENT OF TECHNOLOGY AND DOSAGE FORM DESIGN FOR TARGETED DRUG DELIVERY

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Malignant tumors are one of the leading causes of mortality. The number and incidence of new cancer cases are increasing annually. Chemotherapy is widely used along with other treatment methods. However, the shortcoming and the major problem is that chemotherapeutic drugs are not distinguished with selective cytotoxic activity and affect both tumor and normal cells.

Targeted delivery of medicinal substances to cancerous organs, tissues and cells significantly increases the effectiveness of the treatment and at the same time reduces drug side effects and toxicity [2].

Targeted delivery systems according to modern nomenclature include: liposomes, microcapsule, nanocapsuls, microspheres, nanospheres, nanosomes etc. Targeted drug delivery system consists of active substance interconnected with container with purpose to control targeted delivery and release kinetics [1, 3-5].

The goal of research was the development of technology and formulations of liposomal drug dosage forms.

Object of research was total alkaloids obtained from the bark of Magnolia species (*M. officinalis L. and M. glauca L.*) and Lipoid S75 (manufacturer - Lipoid AG, Steinhausen, Switzerland).

Based on biopharmaceutical researches the technology and formulations of liposomal drug dosage forms by modified shaking method and injection method were developed. Main characteristics: the dimensions of nanoparticles and zeta-potential have been determined using modern instrumental methods of analysis. Comparative analysis of the results showed that the liposomal nanoparticles prepared with the shaking modified method reveal the optimal characteristics.

Cytotoxic activity of total alkaloids obtained from barks of *M. officinalis L. and M. glauca L.* and their liposomal nanoparticles solutions was studied at the UQAC (Université du Québec à Chicoutimi, Quebec, Canada). Resazurin and Hoechst methods were used to determine cytotoxic activity against three cell cultures: A-549 (lung carcinoma), DLD-1 (colon adenocarcinoma) and normal skin fibroblasts (WS-1). The solution of liposomal nanoparticles expressed cytotoxic effect on the lung and colon adenocarcinoma cell lines. Total alkaloids of *M. glauca* and the solution of liposomal nanoparticles expressed predominant activity. Moreover, the liposomal nanoparticles are characterized with selective cytotoxic activity, and they do not affect skin fibroblasts.

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INVITED LECTURES

IL 1. APPLICATION OF ZEOLITES IN MEDICINE

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Zeolites are aluminosilicates ($Me_nSi_xAl_nO_{2(x+n)}\cdot mH_2O$, Me = Na, K,... ½Ca, ½Mg,...) with different three-dimensional framework structures that form pores and channels of molecular dimensions from 0.3 to 1.0 nm. Zeolites occur naturally but are also produced industrially on a large scale; over 200 unique zeolite frameworks have been identified, and over 40 naturally occurring zeolite frameworks are known. Molecular-sieve effect, sorption and diffusion at the surface and in pores or channels, the ability to undergo the ion exchange and to play a role of catalyst are the most important properties of zeolites giving a basis for their application as sorbents, ion exchangers and catalysts not only in industry, agriculture and protection of environment, but in medicine. Today zeolites are used for the treatment and prevention of many diseases and pathologies: there is a reduction of tumors and improved tolerability of chemotherapy; zeolites faster heal cuts, wounds and burns, they increase the efficiency of treatment of infectious diseases (e.g., tuberculosis); there were improvements in the treatment of diseases of the digestive tract; zeolite helps in combating the severe obesity and hair loss, it allows the body to quickly deal with the impellent diseases and helps in the fight against diseases of the thyroid and diabetes.

Application of synthetic zeolites in medical technique started in 1990's: anti-bacterial zeolite catheters, drainage tubes, etc., as well as silver zeolites in dentistry, filters for anesthetic vapours and medical-grade oxygen, contrast agent for magnetic resonance imaging, and other. Natural zeolites have been applied in surgery as antiseptic dehumidifiers and in preparation of hemostatic agents.

First zeolite drug, anti-diarrheic Enterex was developed in Cuba, but it was used for a short period and for local needs only. Megamin was developed in Croatia in the late 1990's and has been marketed throughout Eastern Europe, Germany, and Austria, but without success in the USA. In mid-2000's the "Zeolistas" was involving Croatia, the USA, China and Australia; experts considered operation of three teams: first, USA/Croatia headed by *Krešimir Pavelić*, designer of the Megamin, had no success at the American market, in spite or due to participation of "skilled" american experts. Second team was presented by *Harvey Kaufman*, the US patent-holder for "A method of treating epithelial cell cancer" by clinoptilolite, but Dr. Kaufman joined with the winner – team Deitsch/Wiora. First product of the Wiora company was Activated Liquid Zeolite (zeolite powder suspension in Ultra Purified Structured Water), cure-all, panacea, fifty dollars for fifty milliliter bottle. Later *Rick Deitsch* developed Natural Cellular Defense Zeolite, and his company Wiora created multilevel marketing net involving hundreds of individuals advertising and selling AL, NCD Zeolites, and other products, further comprising organic bio-active components.

Scientific researches in the field of "liquid zeolites" are still popular, recent reviews inform us about perspectives, and sales of the "defense" and "quantum" zeolites are not lowered. However and yet, today the company Wiora is not a monopoly in the USA market, there are other SMEs manufacturing and trading zeolite suspensions all over the world (British and Japan Liquid Zeolite Companies, zeoOne in Australia, etc.), but in a huge Chinese market, as well as in Europe, dominate powder products (Megamin, Detoxamin, etc.). Success in the development of methods to obtain ultrafine and nano-zeolites will enable the introduction of new products, both liquid and solid.

IL 2. COUNTER-CURRENT CHROMATOGRAPHY, A FAST METHOD FOR ISOLATION OF NATURAL COMPOUNDS

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One of the most crucial factors determining the safety and efficacy of any herbal medicine or natural product-based formulation is the quality of the raw material. The absence of readily available biomarkers (standards) is one of the hurdles which need to be overcome to develop robust and effective quality control protocols.

Extracts from medicinal plants are notoriously complex making the isolation and purification of the biomarkers a challenging and time-consuming process. Several chromatographic methods have been developed to isolate the biomarkers from herbal extracts for further studies.

Counter-current chromatography (CCC) is a liquid-liquid separation technique, which makes use of a support-free liquid stationary phase that is held in place by a rotating force field. In CCC, both the stationary and mobile phases are liquids while in other types of liquid chromatography, the stationary phases are solid. Thus through using CCC, decomposition and adsorption of compounds by the solid stationary phase do not occur making HPCCC ideally suited for the rapid isolation of natural products which is only one of many advantages HPCCC offers over conventional techniques [1, 2].

Isolation of biomarkers from Extra Virgin Olive Oil, *Aloe ferox* exudate, *Harpagophytum procumbens* tube extract and *Sceletium tortuosum* leaf extract by HPCCC are presenting.

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IL 3. BIOLOGICALLY ACTIVE AZOMETHINES

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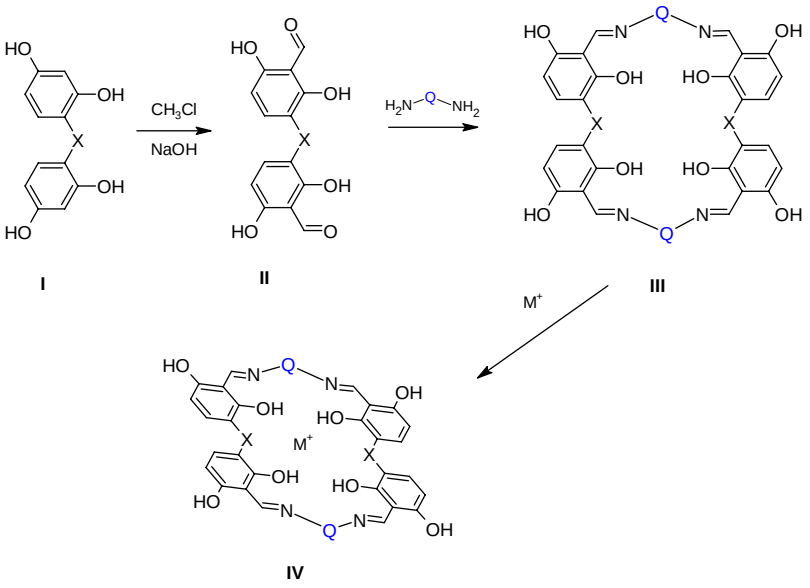
A large number of Schiff bases and their complexes are under intense study for their interesting and important properties, such as catalytic activity [1], photochromic properties [2] and complexing ability towards some transition metals [3]. Azomethines found application as a luminescent pigments as well as dioxygen carriers for modelling biological systems, fungicides, antimicrobial agents, etc.

There are a huge number of literature concerning to liner azomethines or 5-membered cyclic systems (oxazoles). Investigation on cyclic Schiff bases containing more than five atom in the ring still are rare.

We have announced a method for the synthesis 20-membered macrocyclic azomethines a few years ago [4]. Currently we are reporting the synthetic path of 24-membered macrocyclic Schiff bases and their metal complexes. In addition to establish the biological role of prepared Schiff bases and their metal chelates have been performed.

The synthesis has been carried out according to the scheme:



I II III IV

where: X is none or -N=N-, Q is none or $-CH_2-CH_2-$, M^+ is Cr^{3+}, Cu^{2+}

The key compounds are dihydroxyl derivatives of bisphenol or azonebzene. Carbonylation have been carried out under Reimer-Tiemann condition. Cyclisation have been performed by action hydrazine hydrate or ethane-1,2-diamine in the acetonitrile or methanol. For formation of complexes have been used cupper and chromium acetates.

The antibacterial activity of the Schiff base and its complexes against *E. coli, S. aureus,* and *P. aeruginosa* were studied. All the Schiff base complexes individually exhibited varying degrees of inhibitory effect on the growth of the tested bacterial species.

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IL 4. RESEARCH AND DEVELOPMENT OF INNOVATIVE DOSAGE FORMS OF PEPTIDES

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Drug technology evolves towards greater ease of use of medicines. Innovative dosage forms, such as oral fast dissolving tablets and films, allow to deliver active pharmaceutical ingredient instantly from the mouth into the bloodstream. This forms used especially in pediatrics and geriatrics, where it important [1,2,3].

Some of the major health problems of humanity include autoimmune diseases, which currently has about 80 species (psoriasis, lupus, etc.). These diseases is not only medical but also social problem.

One of effective immunosuppressive drugs is the original peptide - thymodepressin that is unparalleled in the world. thymodepressin is synthetic dipeptide. Chemical name: γ-D-glutamyl-D-tryptophan sodium salt.

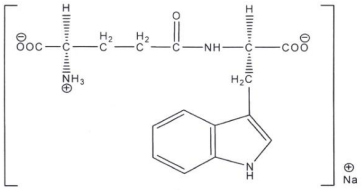

Figure 1. Structure of thymodepressin.

Currently, industrial production thymodepressin carried on by company "Tsitomed" and "Moscow Endocrine plant". The drug is available as a solution for intramuscular injection, the content in 1 ml of 0,001 g of the drug in the form of nasal drops of a 0,1% (0,1% solution for intranasal use), and nasal spray 0,25%, and 0,5% (1 contains a spray dose 250 ug and 500 ug of thymodepressin, respectively). In 2013 was sold more than 8 thousand packages of thymodepressin .

Due to the fact that drugs of peptide nature is preferred route of administration through mucous membranes, in particular through the oral mucosa, a goal of our research was developed fast-dissolving oral films (FDOF) of thymodepressin [4,5].

Oral dissoluble films have a number of advantages over conventionally used oral drugs:

1. The rapid dissolution in the mouth with a small amount of saliva (about 1 min);

2. No additional water and chewing;

3. High permeability of oral mucosa and a large area of interaction accelerates the flow of the drug directly into the bloodstream, bypassing the hostile environment of the stomach and liver enzyme systems;

4. High bioavailability provides rapid onset of therapeutic effect;

5. Ability minimum dosing and the almost complete absence of side effects;

6. Can be used in cases where the reception of syrups, tablets, suspensions and the other is impossible because of the patient coughs, swallowing problems, allergic edema, vomiting, diarrhea;

7. Can be used in pediatrics and geriatrics;

8. Exact dosing in each film compared with syrups and suspensions;

9. Local and resorptive effect in diseases of the oral cavity (teething, mucosal injury, herpes, etc.).

Experimentally was obtained formulation of FDOF which contains: a hydrophilic mucoadhesive polymer (multimodificated pea starch (MPS), a plasticizer (tween 80), sweetener (sodium saccharin, sorbitol), a solvent (purified water).

Conducted validation techniques quantifying thymodepressin in films. Developed and proven techniques eligibility criteria: specificity, system suitability, accuracy, precision, linearity and stability. Results of this investigations included in the project of specification.

Original technique was developed based on NMR ^{1}H spectroscopy of high resolution allows to determine the identity and assay thymodepressin in the solution of the FDOF without sample preparation.

Preclinical trials was carried out of acute and chronic toxicity of FDOF "Thymodepressin 10 mg." Both toxicity on laboratory animals of FDOF Thymodepressin not differ from similar parameters in comparison with other forms of thymodepressin (solution for intramuscular injection of 1 mg/kg) by company "Peptos Pharma" (Russian Federation).

In the study of the pharmacokinetics on experimental rabbits FDOF thymodepressin administration at 0,3 mg/kg determined that thymodepressin of the oral mucosa rapidly absorbed into the systemic circulation. Maximum concentrations recorded in the first 10 minutes and determined microgram amounts (3.70; 2.24; 0.34; 0.84 g/ml). After 24 hours application of the films is determined in a blood thymodepressin in minor amounts.

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ORAL PRESENTATIONS

OP 1. SECONDARY METABOLITES FROM THE ROOTS OF DIGITALIS CILIATA TRAUTV.

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For decades, cardenolides from foxglove occupy the leading place among the cardiac glycosides. Abundantly flowering and fructiferous *Digitalis ciliata* Trautv. is a strictly endemic plant for the Caucasus and is widespread in the Main Caucasian Ridge and its spurs. *D. ciliata* appeared to be high-grade medical raw material, in which almost all cardenolides described in *Digitalis* genus are biosynthesized. 24 cardenolides were isolated and characterized from the leaves. Cardiac glycosides, such as α-acetyldigitoxin, digitoxin and commercial preparations of *D. ciliata,* – Digicilen® and Digicil® containing standardized plant extracts, were used extensively for the treatment of heart failure. No less than 20 cardenolides were found in *D. ciliata* seeds - a rich source of the steroid glycoside digitonin, whose yield reaches up to 2% [1]. New furostanol and pregnane glycosides were isolated from the seeds and roots [2,3].

Continuing our study, sixteen secondary metabolites, including five furostanol, three triterpene and eight phenylethanoid glycosides were isolated from the methanolic extract of the roots using CC on Diaion HP-20 and silica gel, ten from them are new organic substances. The structures of isolated compounds were established by extensively use of ^{1}H and ^{13}C NMR (COSY, HSQC, HMBC), as well as mass-spectrometry experiments.

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OP 2. *IN VITRO* ANTIPROLIFERATIVE EFFECT OF *RUBUS FAIRHOLMIANUS* GARD. ON HUMAN COLORECTAL CANCER CELLS

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Cancer is a dreaded disease characterized by uncontrolled growth and spread of abnormal cells. The high mortality rate amongst cancer patients is an indication of limited efficiency of current therapies. Cancers that start in the cells lining inside of the colon and rectum are colorectal cancers (CRC). CRC is the third most predominant cancer worldwide and is the fourth most common cause of cancer mortality. It is significant that over 60% of currently used anticancer agents are from natural sources. Plants and plant derived products exert chemopreventive effects on various cancer cell lines by inducting cell death. Berries are rich in bioactive phytochemicals with anticancer properties due to scavenging free radicals, regulation of gene expression, cellular signalling and induction of apoptosis. *Rubus* species have been used in folk medicine. On the basis of previous pharmacological properties, we have selected *R. fairholmianus* to investigate the *in vitro* anticancer potential and track the possible cell death mechanisms, to put forward a scope to develop an effective drug.

The effects of root acetone extract of *R. fairholmianus* (RFRA) on the proliferation of human colorectal cancer (Caco-2 cells) have been investigated in this study. The extract led to a dose dependent decrease in viability, proliferation and increased cytotoxicity using trypan blue exclusion, Adenosine 5′-triphosphate (ATP) and lactate dehydrogenase (LDH) assay. The morphological features of the treated cells were supportive for the antiproliferative activity. The Annexin V/Propidium iodide staining indicated that *R. fairholmianus* induced toxic effects in Caco-2 cells and the percentages of early and late apoptotic population significantly increased when compared with control cells. Also we studied the apoptosis inducing ability of the extract by analysing caspase 3/7 activity and the induction of cell death via the effector caspases was confirmed; the activity increased in treated cells compared with control. Moreover, the morphological alterations in the Caco-2 cells exposed to RFRA extract were suggestive for the apoptotic activities. The outcome of this study recommends the substantial antiproliferative activity of *R. fairholmianus* may be due to the caspase dependent apoptosis and the compounds of this extract may have promising use as a cancer chemotherapeutic agent.

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OP 3. NOVEL BIOMACROMOLECULE FROM MEDICINAL PLANTS: PROSPECTIVE THERAPEUTIC AGENT

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The high-molecular fractions from crude polysaccharides of the species of two genera (*Symphytum* and *Anchusa*) of Boraginaceae family *Symphytum asperum, S.caucasicum, S.officinale, S.grandiflorum* and *Anchusa italica* were isolated by ultrafiltration on membrane filter with cut-off value of 1000 kDa. The structure elucidation of the main structural element of these preparations was carried out using different techniques of NMR spectroscopy. According to ^{13}C, ^{1}H NMR, 2D heteronuclear $^{1}H/^{13}C$ HSQC spectral data and 1D NOE experiment the main structural element of these preparations was found to be a regularly substituted polyoxyethylene, namely poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA) or poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene]. PDPGA represents a new class of natural polyethers with a residue of 3-(3,4-dihydroxyphenyl)glyceric acid as the repeating unit. Most of the carboxylic groups of PDPGA from *A. italica* unlike the polymer of Symphytum species are methylated. The 2D DOSY experiment gave the similar diffusion coefficient for the methylated and non-methylated signals of *A. italica* PDPGA. Both sets of signals fell in the same horizontal. This would imply a similar molecular weight for methylated and non-methylated polymers. The NMR signals of both methylated and non-methylated carboxylic groups originate from the same poly[3-(3,4-dihydroxyphenyl)glyceric acid] polymer. Such caffeic acid-derived biopolymer to our knowledge was unknown and has been identified for the first time.

According to the results of *in vitro* and *in vivo* experiments PDPGA could be considered as potential anti-inflammatory, wound healing and anti-cancer therapeutic agent. The efficacy of PDPGA and its synthetic monomer *syn*-2,3-dihydroxy-3-(3,4-dihydroxyphenyl) propionic acid (DDPPA) against androgen-dependent and -independent human prostate cancer (PCA) LNCaP and 22Rv1 cells has been investigated. Both PDPGA and DDPPA suppressed the growth and induced death in PCA cells, with comparatively lesser cytotoxicity towards non-neoplastic human prostate epithelial cells. Furthermore, we also found that both PDPGA and DDPPA caused G1 arrest in PCA cells through modulating the expression of cell cycle regulators, especially an increase in cyclin-dependent kinase inhibitors (CDKIs) (p21 and p27). In addition, PDPGA and DDPPA induced apoptotic death by activating caspases, and also strongly decreased androgen receptor (AR) and prostate specific antigen (PSA) expression. Consistent with *in vitro* results, *in vivo* study showed that PDPGA feeding strongly inhibited 22Rv1 tumors growth by 76% and 88% at 2.5 and 5 mg/kg body weight doses, respectively, without any toxicity, together with a strong decrease in PSA level in plasma; and a decrease in proliferating cell nuclear antigen (PCNA), AR and PSA expression but increase in p21/p27 expression and apoptosis in tumor tissues from PDPGA-fed mice.

Thus, PDPGA and its synthetic monomer exerted anti-cancer efficacy in vitro and in vivo against androgen-dependent and -independent PCA cells via targeting androgen receptor, cell cycle arrest and apoptosis without any toxicity, together with a strong decrease in PSA level in plasma.

Overall, this study identifies PDPGA as a potent agent against PCA without any toxicity, and supports its clinical application.

OP 4 *ASTRAGALUS* SPECIES OF GEORGIAN FLORA AS PERSPECTIVE RAW MATERIAL FOR BIOLOGICALLY ACTIVE COMPOUNDS

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Astragalus L. - a large genus of about 2 500 species is known since the time of Dioscorides. The Scythians called *Astragalus* a «herb of immortality». *Astragalus* herb and roots have been used to treat diarrhea, anemia, leucopenia as well as kidneys, liver and heart diseases. To date only 3% of the *Astragalus* species are studied. Up to 1000 natural compounds of various chemical classes were isolated and identified from *Astragalus L*. Amongst them flavonoids and cycloartans are more attractive for the researchers due to high physiological activity [1, 2].

High content of flavonoids and triterpenes in *Astragalus* species, widespread in Georgia was established during the complex study started in the 60s of the last century [1].More than 140 compounds were isolated and identified from 12 of 73 *Astragalus* species growing in Georgia. 5 amino acids, 2 alcohols, 32 flavonoids (12 flavons, 15 flavonols, 2 isoflavones, 1 flavanonole, 1 chalcone, 1 aurone), 23 cycloartans, 2 triterpene saponins were isolated and identified from *Astragalus falcatus, A. galegiformis, A. caucasicus, A. microcephalus, A. bungeanus,* and *A. kadshorensis*. Among them 21 compounds appeared to be novel organic substances. The chemical structures of these substances were elucidated by chemical transformation derivatives and UV, IR, ^{1}H,^{13}C and 2D NMR spectral methods [3-5].

Study of trace elements revealed that *K* and *Fe* dominate in *Astragalus* species. Quantitative content of other elements decreases in the following order: *Na>Mn>Rb>Zn>Cu>Li*, while *Pb, Cd, Ni, Co* appear below the detection threshold [6].

In *in vivo* experiments crude flavonoids and individual compounds demonstrate hypoazotemic, diuretic, hypotensive, leucopoietic, hypoglycaemic, antioxidant, cardiotonic, hypocholesterinemic effects. Hypoazotemic, diuretic, hypoglycaemic remedy "Flaronin" was developed from *Astragalus falcatus* on the basis of flavonoid glycoside robinin and is used in medical practice [1].

The methods of standardization and pathways for the obtaining of biologically active substances were developed.

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OP 5. DEVELOPMENT OF ANALYTICAL PROCEDURE FOR QUANTIFICATION OF CRUDE EXTRACT FROM THE LEAVES OF *HEDERA COLCHICA*

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Colchis ivy - *Hedera colchica* (C. Koch.) (Fig.1) is an evergreen plant growing in Georgia (Fam. *Araliaceae*) [1]. In folk medicine the different species of ivies are known as plants possessing anticough, diuretic and spasmolytic activities. Recent pharmacological studies revealed anti-inflammatory, antibacterial, anti-bronchospasmodic, antimicrobial, antifungal, antihelmintic, memory improvement, antiprotozoal and contraceptive activities of triterpene saponins from *Hedera* species; antiviral and antioxidant activities of ivy's crude extract are also reported [2-6].

Crude extracts from the Colchis ivy leaves were obtained at the TSMU Institute of Pharmacochemistry (Tbilisi, Georgia) and appeared to have potent antiulcer activity [7].

Fig. 1. Colchis ivy - Hedera colchica (C. Koch)

Chemicals and reagents: HPLC grade acetonitrile of analytical grade were purchased from Merck & Co. Ultrapure water (18 for HPLC analysis was obtained from a Millipore Classic purification system. Hederacolchiside F (HCF) has been isolated from the crude extract of Hedera colchica and the purity determined by HPLC was 99,0% (Fig.2).

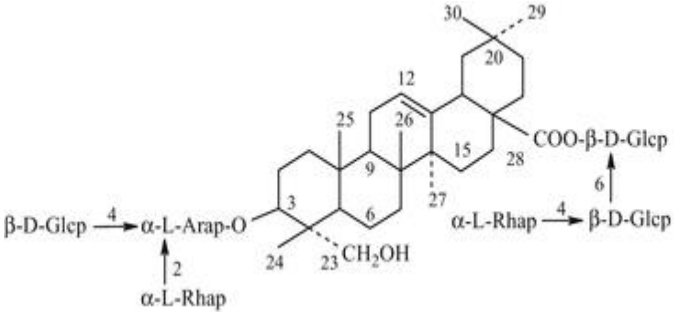

Fig.2. Chemical structure of *Hederacolchiside F*

NMR: Structure elucidation was carried out using ^{1}H NMR (Bruker Avance 400MHz), ^{13}C (Bruker Avance 100 MHz) [8].

Crude extract from the leaves of *Hedera colchica* (C. Koch) proved to have an antiulcer activity in pharmacological experiences. Hederacolchiside F (HCF) is being the chemical marker of this plant, a simple and reliable HPLC method is developed for the quantitative evaluation of the extract using this compound.

The chromatographic separation was achieved using an Eclipse XDB-Phenyl column C-18 (4.6 x 250 mm; 5 μm). The UV detection is performed at 205 nm. All separations were realized at 20°C. The proposed HPLC method is linear in the range studied (r^2> 0.999) for all the analytes. The method is precise with intra- and inter-day variations of less than 1.03%. Precision, sensitivity and linearity are satisfactory in the range studied. Accuracy 99,8±0,9%. The developed HPLC method can be used for the quality control of crude extract from the leaves of *H. colchica* [9-10].

Finally, a new, simple, sensitive and reproducible HPLC method has been developed and validated for the simultaneous quantification of HCF in the crude extract of Colchis ivy. Precision, sensitivity and linearity are satisfactory in the range studied.

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OP 6. MODERN STRATEGIES IN TREATMENT OF ARTERIAL HYPERTENSION

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Arterial Hypertension is one of the most distributed conditions and cause of cardiovascular [CV] events like stroke, myocardial infarction, sudden death, heart failure and peripheral artery disease, as well as end stage renal disease. Hypertension, likewise related diseases is important burden for health care systems of developed and developing countries. This in turn is the bases for elaboration and continues improvement of guidelines and protocols for hypertension management. Modern guidelines are based on best scientific evidences and propose the recommendations relating the goals, treatment thresholds and medications of hypertension [4,5].

During last four years 5 guidelines for high arterial blood pressure management were offered by different international organizations:

2011 NICE Guideline for the clinical management of primary hypertension in adults [6];

2013 ESH/ESC Guidelines for the management of arterial hypertension [5];

2013 AHA/ACC/CDC Science Advisory - An Effective Approach to High Blood Pressure Control [1];

2013 ASH PAPER Clinical Practice Guidelines for the Management of Hypertension in the Community - A Statement by the American Society of Hypertension and the International Society of Hypertension [2]

2014 Evidence-Based Guideline for the Management of High Blood Pressure in Adults Report from the Panel Members Appointed to the Eighth Joint National Committee [JNC 8] [4].

The goal of presentation is to highlight some new evidence-based recommendations in management of arterial hypertension.

In last guideline of JNC [4] was agreed the previous [6] definition of high blood pressure or hypertension as a systolic blood pressure greater than or equal to 140 mm Hg, a diastolic blood pressure greater than or equal to 90 mm Hg, or both. Herewith, there was detected strong evidence to support initiation of pharmacologic treatment in the general population aged 60 years and older to a blood pressure [BP] goal of less than 150/90mmHg.

For the general hypertensive population younger than 60 years, hypertensive adults with diabetes or nondiabetic chronic kidney disease [CKD] is recommended to initiate pharmacologic treatment to lower BP at less than 140/90mmHg [4].

Appropriate lifestyle changes are the cornerstone for the prevention and treatment of hypertension. Lifestyle changes recommendations include salt restriction, moderation of alcohol consumption, high consumption of vegetables and fruits and low-fat and other types of diet, weight reduction and maintenance and regular physical exercise. Smoking cessation is mandatory to improve cardiovascular [CV] risk and as well to prevent its acute pressure effect and raise of daytime BP. Appropriate lifestyle changes may safely and effectively prevent hypertension in non-hypertensive individuals and contribute to BP reduction in hypertensive patients [4, 5].

In accordance with JNC 8 recommendations angiotensin-converting enzyme [ACE] inhibitors, angiotensin receptor blockers [ARB], calcium channel blockers [CCB], or thiazide-type diuretics are initial drug treatment options in the nonblack hypertensive population, including those with diabetes. In the black hypertensive population, with or without diabetes, CCB or thiazide-type diuretics are

suggested as initial therapy. According to JNC 8 in general population β-blockers [BB] are considered for initial treatment of hypertension in patients with special conditions, in particular, when hypertension is associated with clinical CV events like previous myocardial infarction, angina pectoris, heart failure, aortic aneurysm, atrial fibrillation and ventricular rate control, while in ESH/ESC guidelines BB are presented in the group of drugs suitable for the initiation and maintenance of antihypertensive treatment, either as monotherapy or in some combinations. [4, 5].

For people aged less than 55 years initiation of antihypertensive treatment with ACE inhibitors and ARB is recommended, but combination of these two groups is not suggested. For people aged 55 years and older CCB are considered as step one antihypertensive treatment. If treatment with CCB is not suitable, use of thiazide-type diuretics is considered [6]. Initiation of antihypertensive therapy with an angiotensin-converting enzyme inhibitors or angiotensin receptor blockers shoved improvement of kidney outcomes in patients with CKD.

For effective management of arterial hypertension multidisciplinary team approach is required. Involvement of nurses and pharmacists in the management of hypertension, particularly, in patient education, behavioral and medical counseling, assessment of adherence to treatment, and interaction with physicians in the area of guideline-based therapy offer an important potential method for improvement of antihypertensive treatment compared with strategies involving physicians alone [5].

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OP 7. PEGYLATED HERBAL LOADED NANOSTRUCTURED LIPID CARRIERS FOR THE THERAPY OF CARDIOVASCULAR DESEASE

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Cardiac arrhythmias represent a major area of cardiovascular research, and for drug therapy, a large choice of antiarrhythmic agents have been available. However, clinical trials with antiarrhythmic drugs have recently indicated that serious side effects may considerably limit the use of various antiarrhythmic agents [1]. Herbal medicines have been widely used around the world since ancient times. For cardiovascular diseases, herbal treatments have been used in patients with heart failure, arrhythmia, etc. The effectiveness of medicinal plants depends on the supply of active compounds. Most of the biologically active constituents of extracts, such as alkaloids have low absorption, because they are unable to cross the lipid membranes of the cells, resulting in loss of bioavailability and efficacy. It has been widely proposed to combine herbal medicine with nanotechnology, because nanostructured systems might be able to potentiate the action of plant extracts, reducing the required dose and side effects, and improving bioavailability and activity [2].

The purpose of the present study was to formulate original anti-arrhythmic extract (Vingerbine) into PEGylated nanostructured lipid carriers.The sum of four alkaloids of aimaline derivatives has been obtained at the I. Kutateladze Pharmacochemistry Institute (Tbilisi, Georgia) from the plant *Vinca herbaceae* Waldstet Kit [3]. Pharmacological studies showed that cardioactive effect of Vingerbine is stipulated by specific activity of each component [4]. It was identified also that intestinal drug efflux mediated by P-glycoprotein limits oral bioavailability of vingerbine constituent alkaloids [5].

Several formulations of polyethylene glycolated nanoliposomes containing 5, 10 and 20% Vingerbine (Fig.1.) were prepared with cholesterol, native tetraether lipid, dipalmitoylphosphatidyl-choline (DPPC) and PEG-lipid - dipalmitoylphosphatidyl-ethanolamine (DPPE) bearing on the polar head poly(ethylene glycol) of average molecular weight 2000 (PEG:2000)at different molar ratios by ethanol injection and a solvent evaporation methods plus extrusion.

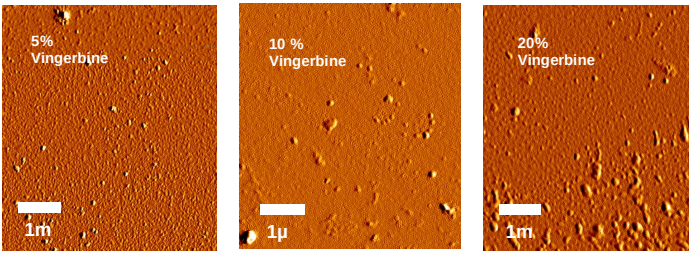

Figure.1. AFM images of Vingerbine PEGylated nanoliposomal forms

Then the liposomes were characterized according to their surface morphology; particle size parameters i.e., the average diameter size and polydispersity index using dynamic light scattering (DLS); zeta potential also determined using DLS; drug loading and release were measured by UV-Visible spectroscopy, and *in vitro* Vingerbine release was evaluated using dialysis bag diffusion technique under sink conditions. Based on *in vitro* results, the best formulationsprepared by thin film

hydration method were selected: nanoliposomes have the appropriate size, narrow polydispersity index, high drug encapsulation efficiency and low drug release.

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OP 8. SYNTHESIS OF NOVEL CHIRAL SULFOXIDES AND INVESTIGATION OF STRUCTURE-ENANTIOSELECTIVITY RELATIONSHIPS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Separation of enantiomers is a hot topic for academic research, as well as in modern pharmaceutical industry. The reason for this is that more than 50 % of the drugs currently in use are chiral compounds and significant part of them are racemates consisting of an equimolar mixture of two enantiomers, stereoisomers with differences biological activity. In many cases, one enantiomer is the active pharmaceutical ingredient while the other inactive enantiomer shows unwanted side effects or even toxic effects. Removal of the unwanted enantiomer from the racemic mixture improves drug efficiency and safety [1].

In order to investigate relationships between enantioselectivity and chemical structure of different chiral stationary phases (CSPs) and chiral compounds 17 chiral sulfoxides (some of them were not described in literature before) and 15 non-commercial cellulose trisphenylcarbamate-based chiral columns were synthesized. HPLC separation of enantiomers of synthetized chiral sulfoxides were performed with polar organic mobile phases such as methanol, ethanol, 2-propanole, acetonitrile, as well as normal-phase normal-phase eluents (n-hexane in combination with various alcohols) [2]. The effect of the structure of chiral selector and chiral analyte on analyte retention and enantiomer separation will be discussed.

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OP 9. CAMPHOR – AS A NEW SCAFFOLD FOR ANTIVIRAL AGENTS

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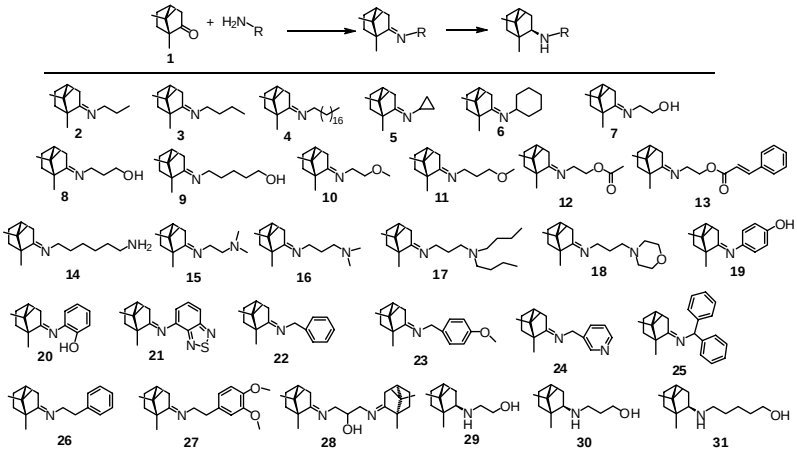
We previously reported the synthesis and antiviral activity of compounds based on camphor [1, 2]. Now we have tested a set of new imino-derivatives of camphor for their inhibiting activity against pandemic influenza virus A/California/07/09 (H1N1)pdm09. The cytotoxic and virus-suppressing activity was determined for each compound by microtetrazolium test and virus yield evaluated by hemagglutination reaction, respectively. Compound 7 have been shown to have high antiviral activity (SI > 500).



Camphor derivatives should be considered prospective for further development as potential antiviral able to overcome the resistance of currently circulating viruses to amantadine and rimantadine. Time-of-addition experiments revealed that the highest effect was reached when compounds were present in culture medium during the period 0-2 hours post infection. The compounds of this class, therefore, counteract to early stages of viral life cycle, such as absorbtion, endocytosis, uncoating, membrane fusion or nuclear transport of RNPs.

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OP 10. SMALL PEPTIDE MODELS OF METAL-AMYLOID **B** BINDING SITES IN ALZHEIMER DISEASE AND NEW BIOACTIVE PEPTIDOMIMETICS

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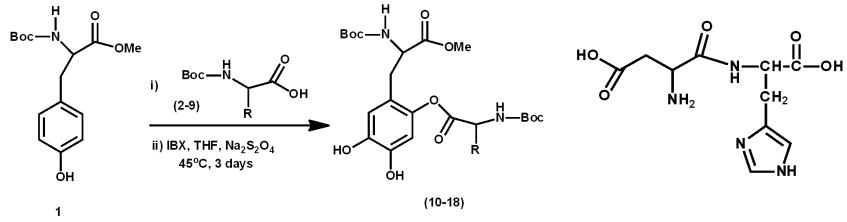
Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by the presence of amyloid plaques. The major constituent of AD plaques is the amyloid – β peptide, which is cleaved from the membrane-bound amyloid precursor protein via β/γ – secretase enzymes. Aβ is produced in health brains in a soluble, monomeric form, which is not toxic. AD risk factors are associated with abnormal production of amyloid beta. An increased Aβ production and/or accumulation lead first to the formation of Aβ oligomers, then to protofibrils and fibrils. Oligomers are supposed to be the most toxic species. On the other hand, high concentrations (~mM) of Fe^{3+}, Cu^{2+} and Zn^{2+} are observed in AD plaques, suggesting that Aβ aggregation could be mediated by some of these essential ions; These metals are involved in two key steps: 1) Cu and Zn are able to bind Aβ directly and modulate aggregation and 2) redox active Cu and Fe are crucial for the production of ROS (reactive oxygen species), though the mechanism of metal reduction and ROS production is still unclear. Therefore, elucidation of the coordination of metal ions to Aβ is important to understand their role in the aggregation of Aβ and in the production of ROS. The question is relevant to the mechanism and/or Cu^{2+} binding structure in Aβ aggregates. There are many different and sometimes controversial studies on the coordination environment of copper and zinc (iron is less studied) and different coordination modes are proposed; We have prepared and analyzed small peptides and their coordination compounds which are model to main binding sites of amyloid beta and metals (Zn, Cu) at different pH. On the other hand, 3,4-Dihydroxyphenylalanine – DOPA is the catechol derivative of tyrosine and the precursor to the neurotransmitter dopamine. Since 1960s, DOPA is the most successful therapeutic agent in the treatment of Parkinson's disease [1]. Moreover, it is the building block for design and for synthesis of biologically active compounds, thus, research in the design of novel synthesis of Dopa peptides has a great significance. Moreover, the authors have shown [2-4] that Dopa-containing molecules cross-link to their protein receptors by treatment of sodium periodate, in which Dopa is converted to an o-quinone intermediate that can be attacked by nucleophiles yielding a stable cross-linked products. As Dopa serves as an important neurotransmitter, a number of neurodegenerative diseases, such as Parkinson's, may involve in cross-linking of oxidized Dopa to receptors [5]. Cross-linking of tyrosine and cysteine residues in proteins via amino and sulfydryl groups by laccase and tyrosinase enzymes have been also reported [6-8]. It must be noted, that no published results and experimental data on the cross-linking between catechols and amino acids and/or small peptides via oxygen nucleophilic addition have not been reported up to now. For last few years, the IBX (2-iodoxybenzoic acid) was effectively used for synthesis of Dopa and Dopa peptides, as well as for hydroxytyrosol and its derivatives [9-11]. Herein we are first to report on the Dopa conjugation with amino acids via oxygen nucleophilic attack using IBX.

Selective oxidation of Tyrosine containing peptides using Tyrosinase enzyme and chemical oxidation were reported [9]. This synthetic approach has shown advances for preparation of Dopa and Dopa-containing peptides. The new strategy to this approach is kross-linking of Dopa-peptides with amino acids or small peptide sequences which implies oxidative transformation of substrates (monophenols) to cathecols and conjugation (cross-linking) with amino acid/small peptide in ortho-position of the aromatic ring, simultaneously. This synthetic novelty enables to produce kross-linked dopa-peptides

directly, without any step-wise reactions. This has served as a first synthetic methodology for such bioorganic systems proposed by us. No published results about cross-linking of 3,4-dihydroxyphenylalanine (DOPA) with amine-protected amino acids we have found. Herein we are first to report on the Dopa quinine intermediates conjugation with amino acids via oxygen nucleophilic attack using IBX. We analyzed the reactivity of N-Boc-Tyr-OMe 1 with a panel of N-protected amino acids, including N-Boc-Gly, N-Boc-Ala, N-Boc-Val, N-Boc-Leu, N-Boc-Phe, N-Boc-Pro, N-Boc-Trp and N-Boc-Met (Scheme 1). After reaction, the Dopa derivatives (comp.10-18) were obtained with different yields depending on the reaction conditions. The bioactivity of new synthesized cross-linked dopa derivatives to central nervous diseases and various types of cancers id under testing and preliminary results are indicating on potential clinical applications of synthesized DOPA conjugations.



Scheme. a. Cross-linking of Dopa with amino acids via oxygen atom b. Asp-His dipeptide

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OP 11. ICl CATALYZED SNTHESIS OF 3,6-DISUBSTITUTED-1,2,4,5-TETRAZINES UNDER MICROWAVE IRRADIATION: NEW TOOL TO IMAGING SCIENCE & CANCER THERAPY

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Heterocyclic chemistry is been playing major role in drug discovery. It is evident from the literature that more than 50 percent cancer drugs and other bio-molecules are heterocycles. Nitrogen is the most contributor and backbone of heterocycles and many other potent bio-molecules. 3,6-disubstituted-1,2,4,5-tetrazines are electro-active heterocycles and have drawn attention of chemists and biologists for their wide spectrum of applications and anti-tumor activity being the major point to highlight. Tetrazines have also been emerged as tool for labeling cells using fluorescent small molecule probes and to label bio-markers on cells with magneto-fluorescent nanoparticles[1]. The rapid cycloaddition of tetrazines is utilized in cellular microscopy, clinical point-of-care diagnostics, and *in vivo* imaging[2]. Recently, tetrazines have been coupled with methyl-cyclopropene dienophiles and these conjugated systems have been studied for imaging and labeling applications[3]. Researchers have also utilized fluorogenic tetrazine imaging probes via coupling chemistry to track the distribution of lipid analogs in live mammalian cells[2].

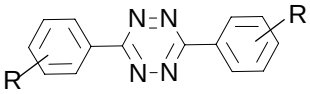
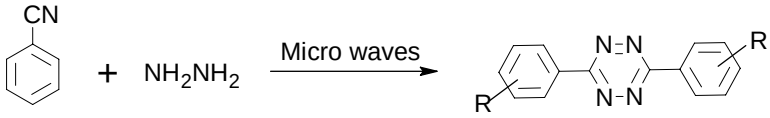


Fig.1. 3,6-Disubstituted-1,2,4,5-tetrazine

While scientists are striving to develop novel bio-orthogonal reactions as tools for addressing problems in biological imaging, the inverse electron demand cycloaddition between 1,2,4,5-tetrazines and strained dienophiles such as norbornene, cyclooctyne, and *trans*-cyclooctene has emerged as a valuable bio-orthogonal coupling tool[4]. Since the tetrazines act both as anti-cancer drugs and labeling tool, we have developed an elegant methodology to synthesize 1,2,4,5-tetrazines utilizing Iodine monochloride as catalyst under microwave irradiation. The method affords excellent yields of pure products in 5-10 minutes. This is of great importance in tetrazine chemistry and will uncover additional bio-medical applications especially in cancer therapy.



R = NH_2, OH, COOH, I, Br, OMe, Me

Scheme 1. Synthesis of tetrazines by irradiating nitriles and hydrazine employing ICl as catalyst

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OP 12. SCREENING OF ADAMANTANE DERIVATIVES AND OTHER SMALL MOLECULES ANALOGUES FOR ANTIBACTERIAL, ANTIVIRAL AND ANTHELMINTHIC ACTIVITY

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The adamantane derivatives are characterised by diverse biological activity including but not limited to antiviral, antimicrobial, anticarcinogenic, anti-cataleptic, immunotropic, neuro-psychotropic activities [1-3].

35 compounds (adamantane derivatives (N-adamantylanilides, 4-(1-adamantyl)anilides, adamantylbenzimidazoles) and analogues of salicylanilides) were synthesized in order to reveal prospective antiviral, antibacterial and anthelminthic agents.

The synthesized compounds were investigated against different Gram positive and Gram negative bacteria and virus strains in collaboration with U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID, S. Bavari, R. Panchal).

The different bacterial species and strains used in this study include *Bacillus anthracis* (Sterne), *B. anthracis* (Ames), *Staphylococcus aureus* ATCC 29213, MRSA 1094, *F. tularensis* (Schu4), *F. novicida. Mycobacteria smegmatis* ATCC 19420, *Escherichia coli* ATCC 25922, Acenitobacter baumanii complex, *Klebsiella pneumoniae* 5657, *Pseudomonas aeruginosa* PA01, *Yersinia pestis* CO92, *Y. pestis* (*pgm*-pST-), *Burkholderia mallei* ATCC 3344, *B. pseudomallei* DD503, *B. thailandensis*, and *B. cepacia* . The minimum inhibitory concentration (MIC) were determined by the broth microdilution method [4]. Several adamantane derivatives showed broad-spectrum antibacterial activity.

The different viral strains used for the study included the recombinant Zaire Ebola virus (EBOV) engineered to express the enhanced GFP (EBOV-eGFP), Marburg Ci67 (MARV) Venezuelan equine encephalitis virus (VEE), Eastern equine encephalitis virus (EEE) FL91, Rift Valley fever virus (RVFV) ZH501 and Crimean-Congo haemorrhagic fever (CCHF) [5]. The adamantane derivatives were tested at a single concentration of 20 μM against different virus families namely Filoviruses (EBOV, MARV), Bunyaviruses (Rift valley fever virus and Crimean-Congo haemorrhagic fever virus) and alphaviruses (Venezuelan equine encephalitis virus and Eastern equine encephalitis virus). A number of the adamantane derivatives exhibited a broad-spectrum antiviral activity. However, several of the identified hit compounds were also toxic to the cells as measured by reduction in cell number in our high-content imaging assays. Studies are in progress to first test the compounds for *in vitro* toxicity and then validate and determine the IC_{50} values of the identified hits.

The biological activity of compounds (GZ-042, 052, 067, 068, 069 and 072- from anilides group; GZ-105 and 109 –from benzimidazole group) were studied on 107 white rats, which were infested with adolescarias of Fasciola Hepatica. Compounds were administered orally as suspensions or boluses. From the compounds tested the highest efficacy was revealed for GZ-052 in doze 65 mg/kg, when using for sexually mature stage of Fasciola hepatica; its EE was equal to 75-100%, IE -90.4 and 100%. GZ-072 appeared effective for young forms (EE-80%, IE-90%) and for mature stages (EE-80%, IE-87.5) [6].

The new compound GZ-048 was synthesized and the high-yielding method of its obtaining was elaborated. Compositions GZ-050 and GZ-060 were prepared on its basis. GZ-048 has low toxicity and is effective in minimal dose (40 mg/kg) on sexually mature stage of Fasciola hepatica. In combination

with acemidophen (GZ-050) in dose 50-130 mg/kg, it completely set free animal from 5-6 week-age trematodes (EE=IE=100%). Preparations GZ-048 and GZ-050 are more effective than hexykhol, methylin and acemidophen. Preparation GZ-048 has manifested synergism with acemidophen and phenacetin (GZ-060) - decrease toxicity nearly twice and increase spectrum of the pharmacological action. On the basis of our investigation, it can be concluded that GZ-048 is very promising for treatment of sheep fascioliasis. It can be used in the design of herbicide, fungicide and anthelminthic compositions [7]. Obtained data show the perspectives of these compounds as anthelminthic remedies.

Novel adamantane derivatives synthesized by our group [8-10] were virtually screened using PASS software (www. Pharmaexpert.ru/passonline) and with high probability appeared to have antiviral (Influenza, Picornavirus, Adenovirus), anthelminthic, antineoplastic (brain cancer); cytostatic, antiparkinsonian and nootropic activities.

Acknowledgement. The present project was supported by Shota Rustaveli National Science Foundation (Grant FR/154/6-420/13).

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OP 13. SYNTHESIS OF BIS-PYRIDAZINOINDOLES

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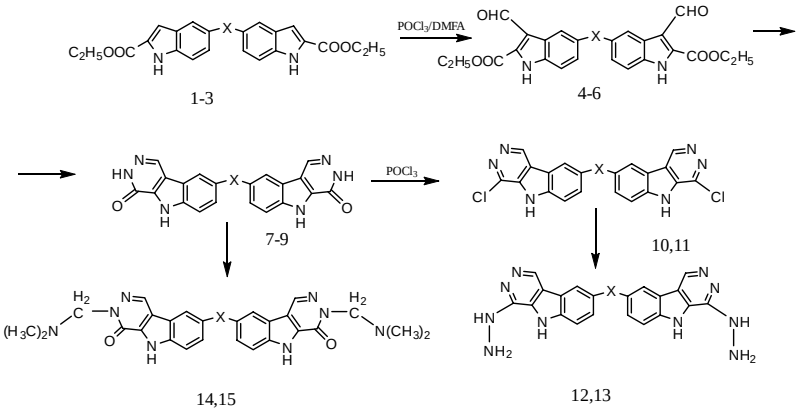
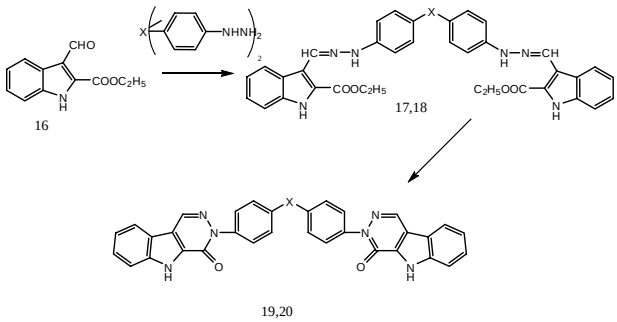
In 1987-88, a group of highly biologically active, anticancer antibiotics (Duocarmicine group) was extracted from the plant Streptomyces zebensis and from the bacteria Streptomyces. One of these antibiotics is CC-1065. It consists of three pyrroloindolic fragments. The biological activity of this compound noticeably exceeds activity of other known compounds, including Adriamicine, Distamicine A, Netrophsine, Nogalamicine etc, but the selectivity is extremely weak. Their high toxicity is the reason none of them is used in practice nowadays.

The discovery and research on Duocramicine group and its properties commenced new wave of studies directed toward synthesis of parts of these compounds and its structural analogues. Variety of new compounds with similar properties has been synthesized ever since, including bisindole and bispyrroloindole analogues. On the other hand, many pyridazino[4,5-b]indoles reveal similar biological activities and therefore, we were strongly interested in the synthesis of the corresponding symmetrical bis-tricyclic systems, in which the fragments of pyridazinoindoles are linked either by means of benzene or pyridazine rings.



1,4,7,10,12,14 X=CH_2
2,5,8,11,13,15 X=O
3,6,9 X=SO_2

Scheme.1



Scheme.2

The following modifications are perfomed on the basis of compounds 7 and 8: aromatization and dimethylaminomethylation by Manich. And from compounds 10 and 11 are obtained bishydrazones (12,13).

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OP 14 THE BIOCOMPOSITE FORMULATION COMPOSED OF 5-FLUOROURACYL AND A BIODEGRADABLE POLYMER: THE PREPARATION AND SAFETY ASSESSMENT

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Local delivery of chemotherapeutic remedies is an advanced method of treatment of malignant neoplasms. The essence of the method consists in the deposition (loading) of acarcinostatic preparation in a polymeric matrix that provides releasing the preparation into the target site. Preference is given to the biodegradable polymeric matrix which degrades by the erosivemechanism. This provides the sustained/controlled release of the preparation deposited in the matrix in accordance with the matrix erosionrate. The biocomposite preparation is placed just in the area of location of the neoplasm, where the release of the active agent into the surrounding tissues takes place. This provides a high local concentration of the carcinostatic preparation at a low integral dose, significantly decreasing in that way intoxication of the organism.

The goal of our study is the creation and practical application of a new anticancer biocomposite formulation composed of 5-fluoroacyl as a carcinostatic and a biodegradable matrix, called temporarily as “Fluorocol”. The amino acid based poly(ester amide) which represents the base of commercial preparation Coladerm® was used as the biodegradable matrix. The present study deals with the preparation of the new biocomposite formulation, as well as safety assessment by testing of the formulation for acute toxicity, cumulative action, allergic reaction and local irritation.

OP 15. THE USE OF NMR SPECTROSCOPY IN PHARMACEUTICAL CHEMISTRY

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Pharmaceutical analysis - the science of chemical performance and measurement of biologically active substances at all stages of production: from control of raw materials to evaluation of quality of finished drugs), study its stability, setting expiration dates and standardization of drugs. Pharmaceutical analysis has its specific features that distinguish it from other types of analysis. These features include the fact that the substances of different chemical nature are subjected to analysis: inorganic, element-organic, radioactive, organic compounds from simple aliphatic to complex natural biologically active substances. The range of concentrations of analyzed substances is extremely wide. Subjects of pharmaceutical analysis are not only the individual substances, but also mixtures containing a different number of components.

Methods for Pharmaceutical Analysis need systematic improvement in connection with the creation of new drugs and continuous improvement of quality requirements. Moreover, the demands as to the degree of purity of drugs and quantitative content are increasing. It is therefore necessary the widespread use to assess the quality of drugs not only chemical but also more sensitive physicochemical methods, one of which is the spectroscopy of nuclear magnetic resonance (NMR) - a method based on the registration of transitions induced by radio-frequency field between the nuclear magnetic energy levels of molecules of the substance, placed in a magnetic field. The method allows to study magnetic transitions of nuclei with spin quantum numbers greater than zero (nuclei ^{1}H, ^{13}C, ^{17}O, ^{19}F, ^{31}P). Set of signals of transitions between energy levels of nuclei of molecules involves the NMR spectrum. Each NMR spectrum is registered for one type of nuclei and specific for each compound.

Considering these parameters the qNMR methods can be validated with regard to linearity, robustness, parameters of accuracy (repeatability, comparability and measurement uncertainty), specificity, and selectivity. The linearity is often found to be characterized by a correlation coefficient higher than 0.999.7 Because of the fact that NMR signals are Lorentzian lines, the LOD and LOQ cannot be determined by means of the S/N ratio. Therefore, a calibration curve has to be studied using samples containing the analyte in the range of the LOD. The LOD based on the standard deviation of the response and the slope may be expressed as LOD = 3.3 /S, where is the standard deviation of the response and S the slope of the calibration curve. The LOQ may be expressed as LOQ = 10 /S. 12 As key prerequisites, the specificity and selectivity must be checked prior to qNMR investigations. Specificity means the ability to assess unambiguously the analyte of interest in the presence of other components; thus, all NMR lines have to be assigned to the structure of the analyte. The selectivity of a method is given by the ability to determine analytes to be examined in a complex mixture without interference from other components in the mixture. This can be checked by homonuclear correlation experiments.

The use of NMR for structural studies based on the fact that in addition to the external magnetic field on the core of a substance act various internal fields. They lead to a shift of the resonance frequency, the splitting of a few or a plurality of resonance lines, i.e. NMR spectrum formation, to change the shape of lines, the relaxation time. A study of NMR spectra suggests the chemical and spatial structure of various substances without chemical analysis. NMR is one of the most effective analytical methods With NMR we can provide information on: - the mobility of hydrogen nuclei and its distribution in the sample; - The ratio of the solid and liquid fractions in samples; - On the rate of

diffusion and self-diffusion; - On the proton density of the sample; - On the spectral distribution of chemically equivalent and non-equivalent protons.

NMR efficiency is due in particular to the fact that, to obtain the analytic signal from organic substances it is enough the availability of hydrogen atoms in the molecules of substances (1 H NMR spectroscopy) and carbon (13C), and for studying the effects of thinner cores nitrogen (15N), oxygen (17O), fluorine (19F), phosphorus (31P) . In the NMR spectra appear hydrogen or carbon atoms of all substances present in the sample specimen. Furthermore the area normalized under signal (area given to a single molecule, proton unit) does not depend on the order in which fragment are molecules or in which molecule present the analyzed atoms of hydrogen or carbon, and on the concentration of substances.

This NMR method compares favorably with other physical methods. The ratio of the normalized signal areas of different molecules is the ratio of the number of molecules of the substance in the sample under analysis. This property analytical NMR signal is very valuable for quantitative measurements. NMR spectra of the extracts represent a superposition of the spectra of the individual substances that make up the BAS in the respective weight ratios. The correlation of obtained NMR characteristics with the chemical composition of biologically active substances and literature data leads to the conclusion about the effectiveness of NMR spectroscopy in the study of the chemical composition and standardization BAS and substances in its composition. Methods of using NMR spectroscopy allows to trace the concentrations of all intermediate products of metabolism non-invasively in real time routine i the culture medium noninvasive real-time tracking of all concentrations of metabolic intermediates within culture medium. Data received by this method may be used for study of growth processes of various microorganisms. NMR spectra depend on the nature of the processes occurring in plants; - Modern electronic equipment and computers make it possible to obtain the parameters characterizing the phenomenon in handy for researchers and consumers NMR form (this is very important when it comes to practical use of experimental data). NMR experiments provide information about the structure and dynamic properties of the bio-molecules in aqueous medium make it possible to investigate protein-ligand interactions. NMR methods are applied for searching liganda with close bonds to proteins and for study factors defining specificity of interaction of liganda with biological target.

Relying on above mentioned we have conducted NMR spectroscopic research. ('H,^{13}C,^{17}O,^{19}F, ^{31}P) of more than three hundred biologically active compounds either isolated from plants (various glycosides, alkaloids, cardiac glycosides) or received with the methods of thin organic synthesis of steroids, deoxy-sugars, and their derivatives, the products of sugar reactions with various organic amines. The possibility of application of methods of spectroscopy NMR for definition of bio-identity of various medical preparations was displayed and proved.

Based on obtained data a catalogue on spectral data of studied compounds was compiled.

OP 16. RHODOPES PREPARATIONS IN DENTISTRY: RESULTS OF CLINICAL STUDY

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Improvement of treatment methods for mouth pathologies is one of the significant objectives of contemporary clinical dentistry. At the same time, viral infection-induced diseases remain the most urgent problem of contemporary medicine and the necessity of optimal combined treatment with antiviral and immunomodulatory preparations is undoubtable.

I. Kutateladze Institute of Pharmacochemistry fulfilled the study of phenolic compounds from endemic plant *Rhododendron Ungernii* (fam. Ericaceae). On the basis of the research carried out at the Department of Pharmacochemistry of Moscow Research Institute of Medicinal Plants and the Department of Biological Research of I.Kutateladze Institute of Pharmacochemistry a novel herbal remedy Rhodopes - "Ungentum Rhodopesum 5%" has been developed at I. Kutateladze Institute of Pharmacochemistry, passed State Registration (reg # 0011795) and was approved for treatment of Herpes virus-induced disorders.

The goal of present study was the establishment of clinical-experimental parallels of efficacy of the preparation "Rhodopes" for combined therapy of mouth mucosa membrane diseases caused by herpes virus. The study was held at the Department of Odontology of TSMU and at Dental clinic, Training and Research Center Unident.

Patients with herpes simplex, recurrent herpes simplex and herpes zoster were involved in the clinical trials to evaluate efficacy of Rhodopes. The study proved the advantage of Rhodopes and its finished formulation "Ungentum Rhodopesum 5%" over traditional drugs "Zovirax" and "Interferon" in case of complex treatment of mouth herpes infections, particularly in remission terms.

Condition of immune homeostasis of the patients with herpes infection before and after treatment was studied, and obtained immunological data were offered as criteria for treatment quality and outcome forecast.

For the optimization of treatment process, we have initiated the development of novel Rhodopes formulations: adhesive ointment and polymer films. Adhesive ointment "Ungentum Adhesivum Rhodopesum 5%" was manufactured by I. Kutateladze Institute of Pharmacochemistry, whereas polymer films - in the Research Centre of Medical Polymers and Biomaterials – RSMPB. Both formulations prolongatethe release of active substance and create high concentration depot in the damaged area, thus increasing the efficacy of preparation.

In vivo study carried at the Pharmacological Department of I. Kutateladze Institute of Pharmacochemistry revealed expressed keratoplastic effect of preparation in treatment of traumatic lesions. Later the the advantage of Rhodopes polimer films over the adhesive ointment in terms of keratoplastic efficacy has been detected and proved in clinical trial.

Rhodopes preparations are effective in children, adults and immunocompromised persons. Application to skin and to the mouth mucous membrane causes neither systemic adverse effects, nor local irritative and allergic reactions.

Pronounced antiviral activity, keratoplastic, immunostimulating and anti-recurrent properties suggest successful application of Rhodopes finished formulations in dental practice.

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OP 17. NEEDFUL STEPS AND ACTIONS FOR SETTING UP AND RUNNING A PHARMACOVIGILANCE AND ADVERSE DRUG REACTION MONITORING SYSTEM IN GEORGIA

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Pharmacovigilance principles and safe drug use promotion activity is a one of the main problems of many countries, because description, registration and taking of appropriate measures during deterioration of the patient's health conditions due to administration of the pharmaceutical products that is associated with specific organizational aspects.

Following measures are necessary for the developing pharmacovigilance system in Georgia:

1) To sign the Service Level Agreement for Maintenance and Support of Vigilyse between State Regulation Agency for Medical Activities (herein the Agency) and UMC;

2) To send the Vigiflow application from the Agency for the inclusion in this program;

3) To send the reminder letters from the Agency to all medical institutions and centers and recall their obligations, which comes from the Law of Georgia on Drugs and Pharmaceutical activities;

4) To send same kind of letters to the representatives of the Pharmaceutical companies of Georgia;

5) To organize the meeting between Agency and representatives of pharmaceutical companies and medical institutions, hospitals and centers;

6) To organize the meeting between Agency and NGOs and experts in pharmaceutical and medical fields, in aim to reconciling the all opinions and proposals related to PV activity;

7) To modernize the Laws and orders for the harmonization of European legislations;

8) To change the ADRs form for simplifying the procedures for the filling;

9) To inform the population of Georgia for the new activities to rise the level of information;

10) To train co-workers of Agency in PV;

According to the above-mentioned activities, we plan to receive and resend ADR forms to UMC and become a full member of WHO program for International Drug Monitoring, which gives the opportunity to protect further the health condition of our people.

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OP 18. TREATMENT OF CERVIX UTERUS BY POLYMER DEPOSITED MITOMICINE

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Efficient treatment of cervix uterus is one of the significant challenges of modern therapy. Relatively efficient method of therapy is local administration of chemotherapeutic drugs.

The essence of this approach is that cancerostatic drug is placed (deposited) in a biodegradable polymer matrix (such system is called polymer biocomposite preparation). Biocomposite preparation is placed directly in the amputation area, where the polymer matrix suffers decomposition through erosion mechanism, which is accompanied by infinite release of the active source (cancerostatic) to the adjoining tissues. Such mechanism of release provides high concentration of a drug in cancer localization zone, which can exceed hundreds times the concentration achieved by systemic administration. Besides, integrated quantity of drug is low, which significantly decreases intoxication of a body.

The research pursued obtaining of drug-containing polymer bio-composite that would possess anticancer properties and study of kinetics of release of anticancer preparation deposited in it.

To fabricate the bio-composite preparation pursued by us, polymer matrix used by us was polyester amide obtained on the base of derivatives of biodegradable and biocompatible amino acids leucine and phenyl alanine, a component of the preparation Koladerm produced by the pharmaceutical company "Neopharm" in Georgia. Koladerm, which is 7% alcohol solution of the above referred polyester amide, when applied on body surface, forms thin polymer film, which sticks closely to the surface and is characterized by bactericidal and protecting properties; it speeds up regeneration and epithelization of damaged tissues, doesn't incite allergic reaction and is not characterized by toxic impact on the body.

Study of kinetics of release of deposited anticancer preparation showed that the preparations which contain protease ferments seem more perspective since alongside with high rate of release of Mitomicine, positive role of protease ferments in therapy of oncologic diseases is known too.

POSTER PRESENTATIONS

PP 1. SOME SECONDARY METABOLITES AND MICROSTRUCTURAL FEATURES *OF ASTRAGALUS BUNGEANUS* BORISS. LEAVES

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The 72 species of *Astragalus* are widespread in Georgia. *Astragalus bungeanus* grows in the east of Georgia and is endemic plant for Caucasus [1].

Since ancient times *Astragalus* species were used in folk medicine for treatment of various diseases . as a diuretic, tonic remedies also against leukemia, nephritis, diabetes, tumors [2-4]; some plants of this genus are used as officinal raw material [4].

Recently, the significant content of flavonoids and cycloartans was found by preliminary analysis of *A. bungeanus* leaves .

Oral administration (10 mg/kg) of the aqueous residue of the alcoholic extract from the aerial parts of *A. bungeanus* exhibited pronounced hematopoietic activity in mice cyclophosphamide-induced leucopenia, causing three-fold increase of total WBC when compared with untreated animals.

The chemical composition of *A. bungeanus* leaves was studied in order to identify the substances responsible for the biological activity. Some flavonoid compounds were isolated and identified from leaves and flowers: flavones – cosmosiin, apigenin [4]; flavonols – astragalin, trifolin, isoquercitrin [4]; anthocyanins - gesneridin-7-0-β-D-glucopyranoside, luteolidin-7-0-β-D-glucopyranoside; coumarins - scopoletin and scopolin; cycloloartans - cyclocantoside E and cyclogaleginoside A [5, 6].

The microstructural characteristics of *Astragalus bungeanus* leaves were studied (Fig.1).

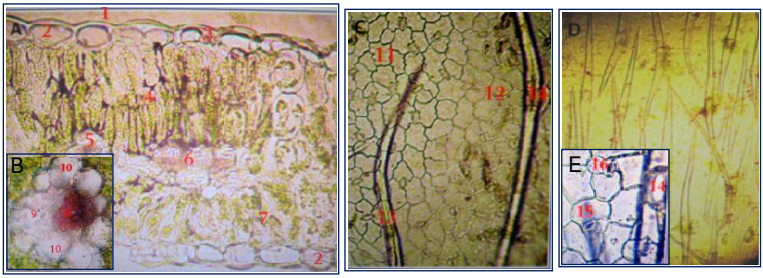

Fig.1. Microstructural features of *Astragalus bungeanus* leaves: A: Mesophyll of leaf ; B: Collateral vascular bundle; C: Upper epidermis; D: disposition of trichomes; E: Lower epidermis 1.Cuticle; 2.Epidermis; 3.Stomata; 4.Palisade parenchyma; 5.Vascular bundle; 6.Anastomosis; 7.Spongy parenchyma; 8. Xylem; 9.Phloem; 10.Tissue around of vascular bundle; 11,15.Crooked linear cell; 12.Anomocitical type stomata; 13.Trichome; 14.Marks of cutting trichomes; 16.Hemiparacytical stomata.

The leaf of is bifacial with dorsiventral mesophyl and is ampistomatical according to the disposition of stomata; the various intensity of spherical and branched (T-shaped) trichomes disposition was observed on the both surfaces of the leaf. Epidermal tissue is characterized by the uniform cells embedded in one row. The collateral type bundles and anastomosis are located between the in two rows embedded palisade mesophyll and spongy mesophyll cells.

According to the classification [7] the basic cells of the upper and lower epidermis of leaf belong to the crooked linear cell type. The stomata principally are anomocitical type at the upper epidermis of leaf, also there is noted the hemiparacitical type aeration system. Marks of cutting trichomes are clearly expressed. Also two types of stomata are observed on the lower epidermis, hemiparacitical type stomata prevails over anomocitical type; the gap between the guard cells is spindle-shaped; stomata are characterized by chaotic location.

The trichomes with low pedicle located parallel to the main vein of the leaf are conical, branched (T-shaped) on the lower epidermis of *A. bungeanus* leaf.

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PP 2. TO DEVELOPING THE TECHNOLOGY OF PRODUCING BIOLOGICALLY ACTIVE SUBSTANCES OF HALOSTACHYS BELANGERIANA BOTSCH. AERIAL PART

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Natural biologically active substances have a broad spectrum of medicinal and pharmacological properties therefore they are of great interest for medical practice.

Halostachys belangeriana Botsch.(Chenopodiaceae) collected in Kyzyl Orda district (Kazakhstan) was chosen for the research [1]. We determined indices of high quality of the aerial part of the plant material in % on the mass: humidity 9; ash content 16; the amount of extractive substances of the aerial part 13,03 on the methods of the State pharmacopoeia XI [2].

The mineral composition of ash residues of *H. belangeriana* Botsch. aerial parts was studied using atomic emission on a Profile Plus inductively-coupled plasma optical emission spectrometer (USA) [3]. 43 macro- and microelements were found for *H. belangeriana* Botsch.. The biogenic elements included phosphorus, potassium, sodium; the essential (necessary for life) elements were manganese, iron, copper, zinc, chromium, molybdenum, and cobalt; conditionally toxic and toxic - aluminium, cadmium, lead, beryllium, barium, bismuth, germanium, silver, titanium, tellurium, uranium, tungsten, tin, and zirconium. High amounts of sodium (48.04%) and potassium (33.20%) were detected in *H. belangeriana* Botsch..

Various types of extraction are used to improve the efficiency of extraction nutrients from plant material. We studied the optimal extraction methods of bioactive substances from the aerial part of *H. belangeriana* Botsch.. Nonpolar (hexane, petroleum ether, benzene, chloroform) and polar (ethanol, propanol-2 containing water from 30 to 70%) solvents were used as extractants. 70% ethanol appeared the optimal extractant of bioactive substances. We fixed optimal conditions of extracting: mass ratio of the plant material – extractant 1:8, duration of the process – 72 hours, temperature - up to 20-25^{0}C, extracting aliquot - 2-3 times.

The composition of volatile compounds of the aerial part of *H. belangeriana* Botsch. was studied using GC/FID and GC/MS methods. 28 compounds of *H. belangeriana* Botsch. representing 88% of the total volatiles detected were characterized. The major volatile compounds appeared to be hexadecanoic acid (24.3%), hexahydrofarnesyl acetone (14.5%), tricosane (7.3%), pentacosane (7.1%), heptacosane (5.1%), heneicosane (2.7%), 4-vinyl guaiacol (2.6%), 3,4-dimethyl-5-pentylidene-2(5H)-furanone (2.2%), docosane (2.2%).

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PP 3. BIOLOGICALLY ACTIVE SUBSTANCES OF HALIMODENDRON HALODENDRON VOSS. LEAVES

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The search for new sources of bioactive substances and herbal medicines with a wide spectrum of pharmacological activity, low toxicity and lack of side effects is an urgent problem.

One of the promising plants Ching silver (*H. Halodendron*) refers to plants of the genus *Halodendro*n legume family (*Fabaceae Lindl*.) [1]. This monotypic species is known as the silver *Halimodendron*.

In the literature there is only fragmentary information on the chemical composition of leaves and fruits of *H. Halodendron* [2-4], therefore it was interesting to carry out phytochemical research of leaves of this plant. The extraction using solvents of different polarity and the procedure of extract group separation was applied. Terpenes, saturated and unsaturated hydrocarbons, alcohols, sterols, free acids were detected in hexane fraction. In this fraction alkane - n-nonacosane (11.07%), diterpene alcohol - phytol (15%), free acids – linolenic acid (29.27%), palmitic acid (11%), dodecanoic acid (6.38%), sterol derivatives - β-sitosterol (5.05%), sitostenone (4.45%) present in a huge amount. Polar (methanol) fraction was studied with High Performance liquid chromatography method with UV detection. The main components of the polar (methanol) fraction are combinations of the group of flavonoids such as luteolin, lutein, isoramnetin, avikularin. The resulted sum of flavonoids exhibited marked ability to neutralize the reproduction of the flu H7N1 virus.

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PP 4. DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF CARVEDILOL IN BULK AND PHARMACEUTICAL FORMULATION

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Carvedilol is a third-generation nonselective chemical drug for controlling different problems of heart having chemical formula (±)-[3-(9H-carbazol-4-yloxy)-2-hydroxypropyl][2-(2-methoxyphenoxy) ethyl] amine (scheme 1) and molecular weight (406.474).

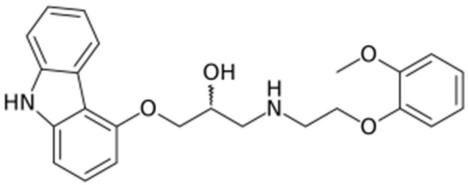

Fig. 1

It has been identified as most effective and non-selective α1 and β-adrenoreceptor (β1, β2) antagonist in the treatment of systolic heart failure, possesses both reactive oxygen species (ROS) scavenging and ROS suppressive effects. It showed protective effects against daunorubicin- (DNR-) induced cardiac toxicity by reducing oxidative stress and apoptosis1,2. Its antioxidant effects are attributed to its carbazole moiety.

The purpose of this study was to develop a fast, simple, reliable, selective, sensitive and inexpensive UV spectrophotometric method for the determination of Carvedilol in bulk drug and commercial pharmaceutical formulations as tablet. The proposed method was developed and validated according to the validation parameters. The developed method was applied to the determination of Carvedilol in pharmaceutical formulations without the necessity of sample pre- treatment. UV Spectrophotometric is selected because it is simplest and less time consuming. Compared to HPLC, as it takes more than an hour to make a single analysis and its calculation. The method was validated as per the ICH guidelines.

For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. The spectrum of 10ppm solution of the Carvedilol in methanol was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength was observed. The spectra of Carvedilol were showed maximum absorbance at 240nm. The linearity was obtained in the concentration range of 5–30 μg/ml, the mean recovery was 100.91 ± 1.77, Low values of %RSD for intra- and inter-day precision suggested reproducibility of the method. Satisfactory values of percent recovery indicated accuracy of the method. Sensitivity of the method was proved by low value of Limit of Detection and Limit of Quantitation. Assay results of marketed formulation were found to be 101.92%.

Results suggest that the proposed method can be applied in routine quality control studies for assay of carvedilol in bulk and tablet dosage forms.

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PP 5. EVALUATION OF NOVEL CORE-SHELL POLYSACCHARIDE-BASED CHIRAL COLUMNS FOR SEPARATION OF ENANTIOMERS OF *TRANS* –STILBENE OXIDE IN HIGH-PERFORMANCE LIQUID CHROMATOGRPAHY

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UHPLC has proven to be an effective way to reduce analysis times without losing separation efficiency through the use of small particle and core-shell column technologies. The use of higher column temperatures and shorter column lengths has allowed the analysis speed of UHPLC to be further increased. A number of highspeed UHPLC applications and conditions will be presented which now allow up to four analytical runs to be completed in only a one-minute timeframe. Although a few studies have been published on the use of core-shell silica particles for enantioseparations in HPLC, their potential as supports for the preparation of chiral stationary phases (CSP) has not been adequately studied yet [1-3].

The use of core-shell silica-based stationary phases has been successfully demonstrated in various separation modes (RP, HILIC, etc.) and for both small and large molecules. While fast mass-transfer is expected to have a more significant impact in the analysis of large molecules (e.g. proteins), in practice core-shell silica is used extensively for the analysis of small molecules (such as pharmaceuticals, environmental pollutants, etc.). This success in the analysis of low relative molecular mass species has been attributed primarily to a significant decrease in the value equivalent to a theoretical plate (HETP) brought about by limited eddy dispersion and molecular diffusion contributions for columns made with core-shell particles. In this study the separation performance of chiral stationary phases (CSPs) made of polysaccharide-based chiral selectors coated onto superficially porous (core-shell or fused-core) silica supports were evaluated. High column performance reaching 200 000 plates per meter was observed.

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PP 6. SUBSTITUTED NITROQUINOLINES AND ISOQUINOLINES: SYNTHESIS AND ANTIFUNGAL ACTIVITY

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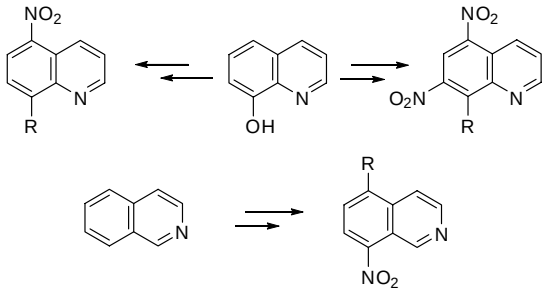
Quinolines and isoquinolines represent perspective classes of heterocyclic compounds. Numerous derivatives of these heterocycles (including nitro compounds) have large synthetic potential and are used for the synthesis of biologically active substances.

The quinoline and isoquinoline moieties were found in numerous alkaloids, natural compounds, are part of pharmaceuticals, and cyanine dyes [1-4]. For example, 8-hydroxy-5-nitroquinoline (Nitroxoline) is an antibiotic that has been in use in Europe for about fifty years, and has proven to be very effective at combating biofilm infections. The chelating activities of nitroxoline have also been used in an anticancer setting.

Here we report on the synthesis of different nitroquinolines and nitroisoquinolines and study of their biological activity. The target compounds were synthesized starting from commercially available 8-hydroxyquinoline and isoquinoline.



R= SBn, OBn, NHBn, SPh, 4-NO_2-C_6H_4NH, Alk_2N

The antibiotic activity tests were conducted on cultures: *Pseudomonas aeruginosa, Escherichia coli, Staphilococcus aureus, Candida albicans* and *Aspergillus niger* by agar diffusion method, and the serial dilution method. It was found that the test substances have a selective effect on the fungal microorganisms. The best activity was shown by a number of 5,7-dinitroquinoline derivatives containing at position 8 N-alkyl or N-aryl substituents. It should be noted that to date there is an acute shortage of selective antifungal antibiotics. At the same time, existing drugs have mainly only two action targets. In addition, they are highly toxic, which greatly limits their applicability. In this connection the results we have obtained can be used in drug discovery research.

Thus, we synthesized a number of functionalized nitroquinolines and isoquinolines. A selective effect on the fungal organisms: *Candida albicans* and *Aspergillus niger* was revealed

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PP 7. STEROIDAL COMPOUNDS FROM THE STEM OF YUCCA GLORIOSA L.

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Steroidal sapogenin-tigogenin from the leaves of *Yucca gloriosa L*. is recognized as a suitable raw material for the synthesis of steroidal hormonal drugs. 150 ha plantations of *Yucca gloriosa L.* have been set in the Eastern Georgia to provide raw material for tigogenin manufacturing [1]. About 40 spirostanol, furostanol and cholestanol glycosides, among them 23 new origanic compounds, were isolated from the leaves, flowers and underground parts of *Yucca gloriosa* L. [2,3].

The leaves drying on the lower tier of the living plant contain only spirostanol glycosides that reveal fungicidal activity against yeast and dermatophytes. Fungicidal remedy named "Gloriofucin" is recommended for clinical approval [4].

Effective agricultural plant growth stimulant – Alexin, was developed on the basis of steroidal glycosides of the flowers *Yucca gloriosa L.* [5].

The roots and bark of rhizomes and stems are rich in phenolic constituents. Stilbenes of rare spirostructure possessing high antioxidant and antiproliferative activitiy were isolated from the abovementioned parts of the plant [6].

Yucca gloriosa L. produces a strong stem, which compiles 30-35% of whole plant weight. Data on their steroidal composition is presented below.

From the barkless air-dried powdered stems of *Yucca gloriosa L.*, collected in the experimental field of the I.Kutateladze Institute of Pharmacochemistry, 9% crude steroidal glycosides consisting of about 15 spirostanols and furostanols were obtained. 2 steroidal glycosides: (25R)-5β-spirostan-3β-ol 3-O-β-D-Glcp (1→2)-O-β-D-Galp (Yuccaloeside A) and (25R)-26-O-β-D-Glcp-5β-furostan-3β,22α,26-triol,3-O-[β-D-Glcp-(1→2)-O-β-D-Galp] were identified. Yuccaloeside A was earlier isolated from the leaves of Yucca aloifolia by our group.

Acidic hydrolysis of the extract from the stem gives sum of sapogenins, from which 4 steroidal sapogenins were isolated by absorption chromatography. Based on physical-chemical constants and spectral analysis they were identified as smilagenin - 5β, 25R-spirostan-3β-ol, tigogenin - 5α, 25R-spirostan-3β-ol, hecogenin - 5α, 12 keto-25R- spirostan-3β-ol and neogitogenin - 5α, 25S- spirostan-2α-3β-diol.

Thus it was established, that the stems of *Yucca gloriosa L.* are rich in steroidal glycosides and the main constituent is Yuccaloeside A, dominant sapogenin is smilagenin, content of the latter in raw material is within 1%. To our knowledge, neogitogenin was isolated from the *Yucca gloriosa L.* for the first time. It was shown that 5β-steroids are synthesized in stems and rhizomes of plant, whereas 5α-steroids - in leaves and flowers.

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PP 8. EVALUATION OF NOVEL CORE-SHELL POLYSACCHARIDE-BASED CHIRAL COLUMNS FOR SEPARATION OF ENANTIOMERS OF FLAVANONE IN HIGH-PERFORMANCE LIQUID CHROMATOGRPAHY

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The concept of pellicular particles was imagined by Horváth and Lipsky fifty years ago. They were initially intended for the analysis of macromolecules. Later, shell particles were prepared. The rational behind this concept was to improve column efficiency by shortening the pathways that analyte molecules must travel and, so doing, to improve their mass transfer kinetics. Several brands of superficially porous particles (core-shell) were developed and became popular in the 1970s. However, the major improvements in the manufacturing of high-quality, fully porous particles, that took place in the same time, particularly by making them finer and more homogeneous, hampered the success of shell particles, which eventually disappeared. Recently, the pressing needs to improve analytical throughputs forced particle manufacturers to find a better compromise between the demands for higher column efficiency that require short diffusion paths of analyte molecules in columns and the need for columns that can be operated with the conventional instruments for liquid chromatography, which operate with moderate column back-pressures. This lead to the apparition of a new generation of columns packed with core-shell particles, which bring chromatographic columns to a level of efficiency undreamed of a few years ago. Few papers were also published on the application of core-shell particles for separation of enantiomers in various liquid-phase separation techniques [1-3]. In the present work application of polysaccharide-based chiral stationary phases made by using core-shell silica particles for separation of enantiomers of flavanone are reported in high-performance liquid chromatography. High plate numbers per unit of time, as well as ultrafast enantioseparation are reported.

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PP 9. BIOLOGICALLY ACTIVE STILBENOIDS OF SAPERAVI GRAPE (*VITIS VINIFERA L.*)

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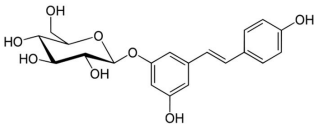
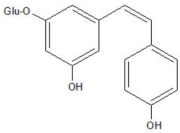
Introduction. Within the broad class of phenolic compounds, stilbenoids represent the distinguished group with diverse biological activity. Resveratrol, the monomer representative of stilbenoids, and its derivatives were discovered in red grapes and wine both as *cis*- and *trans*-isomers [1,2]. It was established that stilbenoids have antioxidant, antibacterial and anticarcinogenic properties and therapeutic and prophylactic efficacy in cardiovascular and other diseases [3-6]. It was proved that *trans*-stilbenoids have superior biological activities over *cis*-stilbenoids. Stilbenoids in red grapes, being potent antioxidants, are accordingly relocated in red wine and thus significantly determine its functional purposes [7,8].

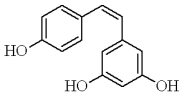
Saperavi is unique Georgian sort of red vine. It is rich in flavonoid and non-flavonoid phenolics. Among them *trans*-resveratrol, epsilon-viniferine, *trans*-piceid, *cis*-piceid and tetramer stilbene were identified and determined [9-11]. Stilbenoid profile is considerably more complicated, therefore in present study we aimed to get a new knowledge on stilbenoids from Saperavi grapes.



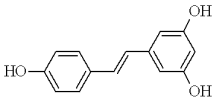
trans-piceid



cis-piceid



cis-resveratrol



trans-resveratrol

Objects and methods. Juice, skin, seeds and stem of Saperavi were chosen as objects for our study. Extracted stilbenoids were implemented with ethyl acetate and then the total product was separated using adsorption chromatography.

Qualitative analysis was carried out by thin-layer chromatography with chloroform:methanol (80:20) eluting system. Chromatograms were developed with diazotized sulfonic acid. UV and IR spectra of the extracted individual substances were recorded on "VARIAN" CARRY 100; "THERMO NIKOLET" AVATAR 370 instruments.

Results and discussion. Parts of Saperavi grapes turned out to be different from each other by qualitative composition of stilbenoids. Mainly, *trans*-resveratrol was not detected in grape juice, but there were found a number of resveratrol glucosides, *cis*- and *trans*-piceid, and the last one being the dominant. In grape skin *trans*-resveratrol, *cis*- and *trans*-piceid, epsylon-viniferine and others were

found. Noteworthy, *cis*-piceid in the skin dominates among piceids. Quantity of polymerized forms of resveratrol increases in the skin→stem→seed direction. Along with known stilbenoids, individual forms of unknown stilbenoids were detected and their identification is currently in progress. In order to determine sustainability of stilbenoid glucosides, the alcoholic fermentation of Saperavi juice with natural microflora was carried out. After the fermentation the piceids were partly split, producing *cis*- and *trans*-resveratrol.

Conclusion. It was established, that quantitative and qualitative stilbenoids composition varies in different parts of Saperavi grapes. Some unknown individual stilbenoids were isolated, and their identification is in progress. Profile of biologically active stilbenoids of Saperavi is very important in order to explain functional purposes of grapes and wine, in terms of therapeutic and prophylactic effects.

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PP 10. ALGORITHM OF NANOSIZED SUBSTANCES QUALITY CONTROL METHODS DEVELOPMENT

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The pharmaceutical development of medicinal preparations is inextricably linked to the quality control methods. Without the development of quality control methods on the nanostructured substances, including such indexes as methods of identification and quantitative determination, we can not investigate the stability of drugs during storage and accordingly to define their shelf life.

Nanomaterials are not sufficiently studied yet and described in the scientific literature, so the development of pharmaceutical preparations with nanosized substances should be based primarily on experimental studies.

At the department of Drug Technology and Biopharmaceutics of Danylo Halytsky Lviv National Medical University are conducted studies considering pharmaceutical development of antimicrobial preparations with nanocompositon in which as active pharmaceutical ingredients are used nanocompositions of silver with metronidazole and levofloxacine, that are made by "Laboratory of Electron-Ray Nanotechnology of Inorganic Materials for Medicine" of E.O.Paton Electric Welding Institute NAS of Ukraine and A. A. Bogomoletz National Medical University.

We elaborated algorithm of nanosized substances quality control methods development, which primarily includes study of the structure of nanomaterials, for example, by X-ray crystallography method. It makes possible to define including of metal nanoparticles in antimicrobial substances or location on the surface of crystals and investigate the nature of the chemical bond. Further selection of quality control methods should be based on the results of studies of nanomaterials structures. When the diffraction pattern of initial substances does not change after applying metal nanoparticles can be considered pharmacopoeial methods of analysis for the initial substances as potential methods for quality control of their nanocompositions with silver nanoparticles. If metal nanoparticles incorporated into the structure of antimicrobial substances it is necessary to develop new methods of quality control. In addition for the identification and quantitative determination of silver nanoparticles in substances it is advisable to use a method such as inductively coupled plasma atomic emission spectrometry.

This approach to nanosized substances quality control methods development is perspective and allow to develop effective quality control methods, what in turn will help to intensify research associated with the creation of medicinal preparations based on nanomaterials.

PP 11. ADSORPTION OF PIROMELLITIC ACID AT THE MERCURY ELECTRODE FROM AQUEOUS SOLUTIONS

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The aim of our investigation is electrochemical behaviour of piromellitic acid, which belongs to the class of organic compounds known as tetracarboxylic acids containing exactly four carboxyl groups in C-1, C-2, C-4 and C-5 position. Benzenepolicarboxylic acids are widely applied in medicine and pharmacochemistry, namely, for synthesis of blood maintenance substances, for orthopedic and stomatological materials. Piromellitic acid exhibits anti-tumor activity [1]. Consequently, it is very interesting to study the adsorption of those acids in double electrical layer, especially for pharmacochemisry.

Adsorption of piromellitic acid was studied at the mercury electrode in 0.5M $NaClO_4$ aqueous solutions ($c = 8 \cdot 10^{-4} \div 6 \cdot 10^{-3}$M) by measuring the differential capacity (C) as a function of electrode potential (E) by means a.c. bridge (C,E-curves) (Fig.1). Hanging mercury drop electrode was used for the study, which was updating by small moving glass shovel located in the electrochemical cell. The value of potential was taken respectively to the saturated calomel electrode potential. All chemicals were purified according to the well-known methods. Measurements were performed in the thermostatic electrochemical cell at 20 ± 0,5^{0}C temperature in the inert atmosphere.

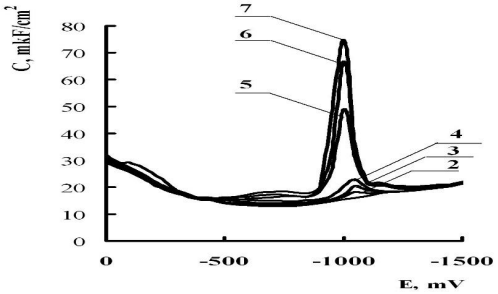

Fig.1. Differential capacity curves of Hg electrode in a 0,5 M $NaClO_4$ aqueous solution containing piromellitic acid in the following concentrations: 1-0; 2- $8.2 \cdot 10^{-4}$; 3-$1.6 \cdot 10^{-3}$; 4-$2.4 \cdot 10^{-3}$; 5-$3.1 \cdot 10^{-3}$; 6-$4.5 \cdot 10^{-3}$; 7-$5.9 \cdot 10^{-3}$M.

Apparently from (fig.1), adsorption of piromellitic acid happens generally at low values of the negative electrode charge. During anode polarization of an electrode there is a π-electronic interaction of adsorbate molecules with positively charged electrode cover. According to the literary data in the diluted solutions of piromellitic acid large aromatic ions are present. Therefore, during anode polarization of the electrode both π-electronic interaction and electrostatic effect simultaneously influence the adsorption of piromellitic acid. At the E=-1000mV on the C-E-curves of piromellitic acid are observe desorption peaks which height of them rise with increase of concentration. Before of desorption peaks at the fig.1 (curves 2÷6) are looked a small maxima, which

height increase is depending of concentration. This effect conceivably can occur due to bond destruction in the molecular scceleton.

The obtained data were processed accordingly to Frumkin-Damaskin theory for adsorption of organic substances [2]. Mathematical treatment of experimental C,E-data was done in order to obtain complete picture of the process. Electrode charge values (q) were received by integration of C,E-curves for the "base" solution (0.5M $NaClO_4$ aqueous solution) and then q,E-curve for the "base" solution was. q,E-curves were plotted on the basis of reverse integration of C,E-curves for different concentrations of piromellitic acid. Integration constant value was obtained from peak values of negative potential. The surface charge density, σ, has been obtained by integration of q,E-curves and electrocapillary σ,E-curves were plotted.

On the basis of the surface charge density at peak potential (E=-475mV) the surface pressure $\Delta\sigma$ was calculated and constructed the surface pressure dependence on from concentration logarithm ($\Delta\sigma$,lgC-curve). Data were processed at $\Delta\sigma$ less 3mN/m, assuming that in this area adsorption of a piromellitic acid corresponds to Henry's equation:

$$\theta = Bc, \quad (1)$$

Henry's isotherm corresponds to the state equation.

$$\Delta\sigma = R\,T\,\Gamma_м\,Bc \quad (2)$$

The values of the adsorption equilibrium constant B=26,46 kJ/mol and the free adsorption energy - ΔG_A=24,8 kJ/mol for piromellitic acid were received, as well as values of coverage by adsorbate molecule **Г**, saturation coverage **Гм** and the other adsorption parameters for the piromellitic acid were calculated according to Frumkin-Damaskin's theory: attraction constant, adsorption equilibrium constant, value of limiting adsorption, standard adsorption free energy.

The form of curves and the calculated parameters from them indicate the adsorptive ability of piromellitic acid on the mercury/aqueous solutions interface, that is important from both scientific, and pharmacological viewpoints.

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PP 12. ON THE MECHANISM OF INDOLIZATION REACTION

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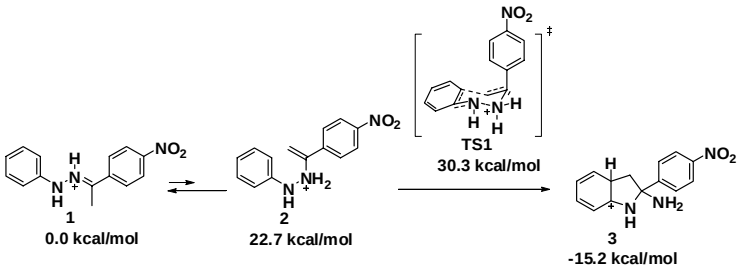
Nitrogen atom containing natural products and synthetic compounds have wide range of pharmacological activity. Among those indole compounds are especially important, therefore it is highly desirable to develop and elaborate relevant new systems and their derivatives. The acid catalyzed cyclization of arylhydrazones known as E. Fischer reaction still remains one of the most popular method for the synthesis of Indole core structure. New C-C bond formation step is one of the key step of the mechanism of Fischer reaction, it is considered as [3,3']-sigmatropic rearrangement, o-benzidine rearrangement, intermolecular nucleophilic reaction, intramolecular electrocyclic reaction etc. by different authors. It is well established in the literature that electron withdrawing substituents in hydrazine fragment hinder the reaction, while donor substituents facilitate it. The information about influence of carbonyl fragment on indolization reaction is scarce in the literature, with the exception of a few publications highlighting the fact that indolization of acetophenone derived phenylhydrazones is significantly facilitated by substituents, especially by electron withdrawing groups [1,2].

The presented report covers the results of quantum-chemical calculations on the cyclization reaction of unsubstituted and p-nitroacetophenone derived phenylhydrazones. Calculations were carried out by using modern quantum chemistry software package "Gaussian O9W" at scs-MP2/def2-SVP//M06-2X/def2-SVP+ZPE level of theory.

A comparative analysis of the possible intermediate resonance chemical structures has shown that the highest energy is required for the first stage of the reaction - hydrazone conversion into enhydrazine - 22-24 kkal/mol. The lower energy activation barriers correspond to the following steps of the process.

Fig.1.



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PP 13. DETERMINATION OF IN VITRO OUTLET OF LIZINOPRIL CONTAINING MEDICAL PREPARATION "LIZINOCOR" AND ITS ANALOGUE USING METHOD OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ACE Inhibitors represent the first class of antihypertensive agents that was designed and developed on the basis of a well-defined physiopathological axis of arterial hypertension, a vascular disorder that is now becoming one of the major causes of morbidity/mortality, not only in developed societies but also in the highly populated developing countries.

An angiotensin-converting enzyme (ACE) inhibitor the carboxylalkyl compounds derivated lizinopril is a pharmaceutical drug, used primarily for the treatment of hypertension(elevated blood pressure) and congestive heart failure [1.3].

Purpose of investigation was a comparison of in vitro outlet of 5 mg tablets of "Lizinocor" containing Lizinopril, produced by the Georgian pharmaceutical company "GMP" and its analogue "Zestril" using method of high-performance liquid chromatography [2.4.5].

Table 1. Determination data of in vitro outlet of lizinokor and zestril

Sample, outlet time	a	d	P	S0	S1	S2	V1	V2	outlet %	outlet (mid) %
lizinocor 5,0 mg (120031013)(5min)	20,0	5,0	100,0	8809,65	8809,65	290,21	100,00	500,0	65,88	60,71
	20,0	5,0	100,0	8809,65		244,60	100,00	500,0	55,53	
lizinocor 5,0 mg (120031013)(10min)	20,0	5,0	100,0			395,23	100,00	500,0	89,73	89,73
	20,0	5,0	100,0			395,28	100,00	500,0	89,74	
lizinocor 5,0 mg (120031013)(15min)	20,0	5,0	100,0			413,70	100,00	500,0	93,92	94,80
	20,0	5,0	100,0			421,47	100,00	500,0	95,68	
lizinocor 5,0 mg (120031013)(30min)	20,0	5,0	100,0			428,24	100,00	500,0	97,22	97,65
	20,0	5,0	100,0			432,06	100,00	500,0	98,09	
zestril 5,0 mg (62604) (5 min)	20,0	5,0	100,0			241,31	100,00	500,0	54,78	55,78
	20,0	5,0	100,0			250,07	100,00	500,0	56,77	
zestril 5,0 mg (62604) (10 min)	20,0	5,0	100,0			307,71	100,00	500,0	69,86	68,64
	20,0	5,0	100,0			296,95	100,00	500,0	67,41	
zestril 5,0 mg (62604) ((15 min))	20,0	5,0	100,0			352,61	100,00	500,0	80,05	83,31
	20,0	5,0	100,0			381,36	100,00	500,0	86,58	
zestril 5,0 mg (62604) (30 min))	20,0	5,0	100,0			421,56	100,00	500,0	95,70	95,88
	20,0	5,0	100,0			423,09	100,00	500,0	96,05	

According to the received results average percent quantity outlet of "lizinocor" is 97,65 %, "Zestril" – 95, 88%;. Inclination in comparison with Zestril is 1,85 %, (norm ± 5 %). "Lizinocor" 5 mg tablets containing Lizinopril produced by the Georgian pharmaceutical company "GMP" are characterized by good outlet quality.

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PP 14. SYNTHESIS OF POTENTIALLY BIOACTIVE 4-HYDROXYMETHYLISOINDOLINIUM SALTS AND THEIR INTRAMOLECULAR RECYCLIZATION

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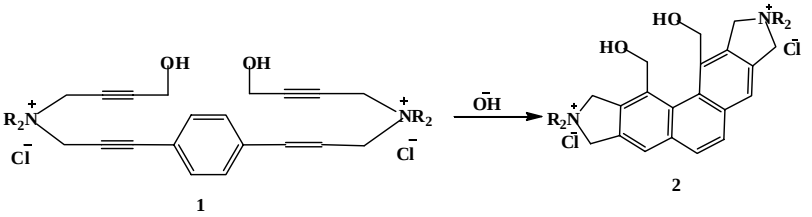
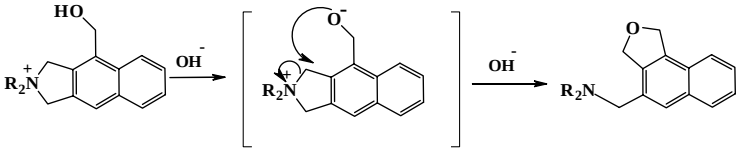
On the basis of base catalyzed intramolecular cyclization of unsaturated ammonium salts discovered for the first time by acad. A. T. Babayan, E. O. Chukhajian and co-authors [1,2] was work out the accessible method for the synthesis of 4-hydroxymethylbenzo[f]-, naphth [f]isoindolinium salts [3-5]. Among the analogues of these salts there are representatives with expressed cardiovascular, hypotensive activity and analgetic action of non-narcotic nature. The activity was defensed by Soviet Union Copyrights and Patents of Republic of Armenia. It was established that salts 1 in base catalysis conditions simultaneously undergo double cyclization which lead to the above mentioned salts 2 and prototropic isomerization [6].



1 2

Scheme 1. Cyclization of dichlorides of *n*-bis[3-(dialkyl-4-hydroxy-2-butinyl)ammonio-1-propynyl]bezene

In 2003 by E. O. Chukhajian and co-authors was discovered intramolecular recyclization of 4-hydroxymethylisoindolinium salts in water base cleavage conditions [7]. The recyclization includes the stages of nucleophilic attack of alkoxy anion on partly positively charged carbon atom of isoindolinium cycle, destruction of N-C bond and formation of C-O-C bond.



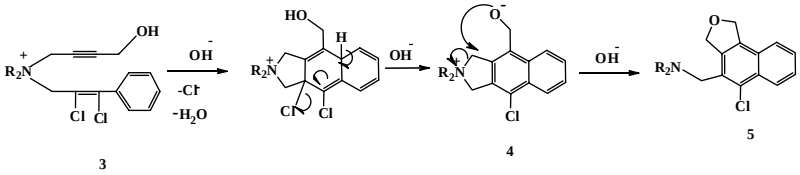
Scheme 2. Recyclization of 2,2-dialkyl-4-hydroxymethylbenzo[f]isoindolinium chlorides

These transformations have a dominate character and lead to formation of compounds in molecule of which dihydrofuranic cycle is condensed with aromatic ring.

The phenomenon of recyclization of 4-hydroxymethylisoindolinium salts absolutely new direction in organic chemistry.

It was established that in the presence of voluminous substituents at nitrogen atom [5,6] and also increase of number of cycles in molecule of 4-hydroxymethylisoindolinium salts favorable act on recyclization [5,6].

More recently it was established that salts 3 in water base medium predominantly undergo dehydrochlorination leading to the formation of salts 4 which in water base cleavage reaction conditions undergo recyclization with formation of amines 5.



Scheme 3. Cyclization-dehydrochlorination of 2,2-dialkyl-4-hydroxybutyn-2-yl(3-phenyl-2,3-dichlorallyl)ammonium chlorides and recyclization of cyclic products.

Investigations carried out in the field of intramolecular recyclization besides fundamental have also preparative significance. It is necessary to note that furanic cycle enters in composition of molecules of alkaloids.

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PP 15. DISCLOSURE OF THE PHENOMENON OF ISOMERIZATION IN -3a,4-DIHYDROISOINDOLINIUM SALTS

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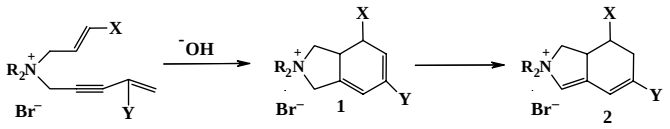
It was established that during base-catalyzed intramolecular cyclization of ammonium salts containing group of allylic type alongside with 3-alkenylpropyn-2-yl group, instead of expected -3a, 4-dihydroisoindolinium bromides (1) were obtained their isomeric forms bromides of -2,6,7,7a-tetrahydro -1H-isoindolium (2) in high yields [1-3].



Scheme 1. Cyclization of (3-alkenylpropyn-2-yl)(allyl- or crotyl- or -3-phenylallyl)pyrrolidinium, piperidinium and morpholinium bromides

It has been shown that cyclization of allylic salts (X=H) at room temperature proceeds with rapid self-heating [1] even in the presence of base in a molar ratio of salt / base=13/1, resulting to formation of salts -2,6,7,7a-tetrahydro-1H-isoindolium (2) with 85-87% yields. Cyclization of crotyl analogues ($X=CH_3$) was carried out with moderate self-heating even in the presence of 0.2 mole base per mole of the starting salt [2]. In these cases was formed a mixture of isomeric salts 1 and 2 with 72-76% overall yield. The maintenance of -2,6,7,7a-tetrahydro-1H-isoindolium bromides (2) in the mixture is 80-82%. By adding a small quantity of aqueous base to the aqueous solution of -3a,4-dihydroisoindolinium bromides (1) they rapidly isomerized to -2,6,7,7a-tetrahydro-1H-isoindolium bromides (2).

With moderate self-heating also accomplished the cyclization phenylallyl ($X=C_6H_5$) analogues (even in the presence of 0.2 mole base per 1 mole salt), however in this conditions during cyclization of above mentioned salts are formed only -2,6,7,7a-tetrahydro-1H-isoindolium bromides (2) with 80-90 % yield [3].

Isomerization of -3a,4-dihydroisoindolinium bromides (1) to -2,6,7,7a-tetrahydro-1H-isoindolium bromides (2) includes moving of multiple bond from β, γ- position to α, β.

On the basis IR spectral investigations which were realized for establishment of mechanism of cyclization of ammonium salts, containing group of allylic type [4], as well as on the results of water-base cleavage reaction of salts 2 [1,2] was established that during cyclizationof above mentioned salts firstly were formed -3a,4-dihydroisoindolinium bromides (1) which in base medium were underwent isomerization leading to the formation of salts 2. By abinitio quantum chemical calculation method was shown that bromides 1 have 6 kkal / mol more energy compared with their isomers (2) [1], which apparently is the driving force for discovered isomerization. This isomerization have a general character and except theoretical have also preparative significance, because includes possibility for synthesis of potentially bioactive -2,6,7,7a-tetrahydro-1H-isoindolium bromides (2) with quantitative

yields, compounds, synthesis of which by other chemical methods is difficult realized. It is known that the derivatives of -1H-isoindol have antihypertensive, anti-inflammatory activity, stimulating and sedative activity [5-7].

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PP 16. SOCIAL RESPONSIBILITY ASPECTS OF PHARMACEUTICAL BUSINESS IN GEORGIA

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Business activity is part of certain social and political-cultural environment, therefore it's perfectly natural that it cannot neglect the interests of the society. On the other hand, social stability is a necessary condition for the effective business activity. Hence compliance with the principles of social responsibility (PSR) enhances the effectiveness and viability of the business environment [1-3].

The goal of current study was to reveal the attitude of representatives of both entire society and pharmaceutical business towards PSR as well as to determine the level of protection of these principles by Georgian pharmaceutical business.

100 respondents were polled using predefined questionnaires in order to determine the compliance of pharmaceutical business with PSR in terms of:

a) safety; b) stimulation of the remuneration; g) additional medical and social insurance of the employees; d) professional trainings and skill enhancing programs for employees; e) provision of assistance to workers in critical situations; v) sponsorship and charity; z) preservation of the environment; t) interaction with local social groups and public organizations; i) readiness for crisis situations; k) quality of commodities and services, manufacturing of high-quality safe and effective medicines.

The results of the survey are represented in Fig. 1

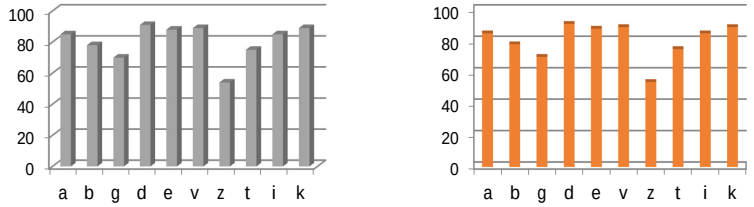

Fig 1. The results of the survey: left - businesspersons; right - society representatives

Large pharmaceutical companies and the population, in general, positively appraise the business environment in the country today and the dynamics of its development. Most businesspersons fix that the situation improved over the past three years. From corporate PSR point of view lack of awareness about the social responsibility is mentioned as important constraint, as well as the lack of proper planning experience and knowledge for the determination of priorities and management of socially responsible initiatives.

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PP 17. STANDARDIZATION OF HYDROPHOBIC AND HYDROPHILIC SUBSTANCES OF GEORGIAN PROPOLIS

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According to literary data, a wide spectrum of propolis pharmacotherapy is conditioned by the presence of mixtures of various chemical structures. At present, modern methods of analysis made it possible to detect more than 300 substances [1,3].

Propolis of Georgian origin is characterized by high content of biologically active compounds, especially polyphenols and flavonoids [2] .

Recently, immumodulating properties of propolis, its hepatoprotective efficacy and successful treatment of ulcers were reported [5].

Anticancer and antioxidant properties are of special interest. Some scientists believe that a trustworthy correlation between these two effects exists. Hydrophobic preparations of propolis substance revealed good results when treating of breast and prostate cancer [6,7

According to above stated, in last few years, there has been heightened the interest not only in alcohol-containing balms, but to hydroalcoholic and aqueous extracts as well, which were obtained by cold extraction method and characterized by a higher antioxidant index AA%-99.9 [2], in comparison with alcoholic extract.

The goal of present research was to separate hydrophobic and hydrophilic substances obtained from propolis samples spread in Western Georgia (Tskhaltubo, Tkibuli, and Chiatura) and to identify the dominant flavonoids using UV-Vis and GC/MS spectrometry.

It was detected that both the alcoholic tinctures and aqueous solutions have the same absorption maximum at λ290 nm that is typical of polyphenols. Out of 3 samples, the Chiatura's one had a relatively high optical density. Two flavonoids: pinocembrin (m.w. 256) and pinostrobin (m.w. 270) were detected in high amounts, especially in hydrophilic substance fractions of propolis. Presumably, this also explains higher antioxidant activity of a propolis aqueous solution. The studies in this direction are in progress.

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PP 18. ANALYSIS OF GEORGIAN PHARMACEUTICAL MARKET CONCERNING MEDICINAL DRUGS OF PLANT ORIGIN

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During the last decade, Georgian pharmaceutical market expanded and nowadays, commodity nomenclature of Georgian pharmaceutical market increases rapidly. This trend is also valid for medicinal drugs of plant origin – so-called plant based drugs.

In present study we aimed to analyse Georgian pharmaceutical market of both prescription and non-prescription plant based drugs.

It is obvious that the main goals of market analysis of plant based drugs are: obtaining of deep understanding of the drugs assortment, determine consumers' needs of and raise the awareness of the competitiveness of drugs.

According to the Drug Registry published by the State Regulation Agency For Medical Activities of Ministry of Labour, Health and Social Affairs of Georgia 11150 pharmaceutical products were registered in 2015, and among them more than 427 preparations are of plant origin (including 180 paramedical remedies); 197 kinds of pharmaceutical products of plant origin are produced in Georgia, among them 7 preparations are manufactured at TSMU I.K. Institute of Pharmacochemistry [1].

27 countries are present in Georgian pharmaceutical market of plant based drugs. Georgia is recognized as a leading producer – with 46,1%; then comes Pakistan- with 11,5%, Germany – 10,77% and India with – 9,1%. Georgian (LTD Neopharmi and LTD Naturpharm) and foreign (Herbion Pakistan (Pvt) Ltd" (Pakistan), „UNIQUE PHARMACEUTICAL LABORATORIES (A Div. J.B. CHEMICALS&PHARMACEUTICALS LTD. India) companies hold top positions among manufacturers. It is worth mentioning that plant based drugs have different drug formulations: capsules – 55, tablets – 108, granules – 16, syrups – 58; lyophilized powders – 5, powders – 3, tinctures – 34, drops – 29, creams – 14, extracts – 10, medicinal plants – 48, oils – 3, pastilles – 4, suppositories – 3, teas – 4.

Medicinal preparations developed and manufactured by TSMU I.Kutateladze Institute of Pharmacochemistry are presented in various drug formulations: tablets, capsules, drops, lyophilized powder, tinctures and some others. A few months ago, the permissions for manufacturing ointments and pharmaceutical substances were obtained.

Commodity assortment has particular socio-economic importance, because it ensures to meet the users' needs. Determination of optimal nomenclature of the plant based drugs is important for the pharmaceutical companies and defines their optimal economic efficiency. Among main competitive factors the therapeutic efficacy, safety, availability, rational formulations, optimal dosage, packaging etc. should be considered.

The survey of both patients and physicians revealed the increased demand for plant based drugs forms; therefore, this factor should be included as one of main indicators of competitiveness, when evaluating the competitiveness of drugs of similar compositions from different manufacturers.

During a comparative market analysis it is highly recommended to consider therapeutic efficacy, side effects, drug formulation and dosage regimens as important competitive factors.

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PP 19. SOME BIOLOGICALLY ACTIVE COMPOUNDS FROM THE BARK OF *BETULA MEGRELICA*

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Gen. *Betula* L. (Betulaceae) in Georgian flora is presented with 7 members. Among of them, *Betula megrelica* Sosn. (Fig. 1) is an endemic plant of Georgia [1]. The species of Gen. *Betula* L. are known for constituent triterpene and phenolic compounds. Triterpenes are presented as derivatives of pentacyclic lupane and oleanan [2-5]. The Betulinic acid has clearly expressed anticancer activity. The arilbuthanoids, diarilheptanoids, lignans and phenolic compounds [6-8], possessing high antioxidant, cytotoxic, anti-cancer and antiviral activity [9, 10], are isolated from the other species of *Betula* L.

Fig.1. Betula megrelica Sosn.

Material and Methods: The object of our research is a Georgian endemic plant *Betula megrelica* Sosn. [1]. Bark of the plant was collected in August 2013, at north side of Mt. Migaria (Georgia). Fractionations of ethanol extract using column chromatography and preparative HPLC allowed isolating triterpene and phenolic compounds. The structures of compounds were determined by ^{1}H, ^{13}C NMR (COSY, HSQC, HMBC) and MS spectrometry. The purity of tested compounds was evaluated by analytical HPLC.

Results and discussion: On the base of various chromatographic techniques, from the crude ethanolic extract of the bark of *B. megrelica* were isolated more than 20 individual compounds; among of them, were claimed presence of following compounds: Lupane, Oleanolic acid, Betulin Catechin, Catechin-7-O-Glycoside, 4-(p-hydroxylphenyl)butan-2-ol and 4(e)-1,7-bis(4-hydroxylphenyl)4-hepten-3-on. Their structures have been determined on the base of Mass-spectral, 1D and 2D NMR spectral evidences. NMR data of all compounds were in agreement with the data reported in the literature [11, 12].

The anti-inflammatory, anti-oxidant and anti-cancer activities of the crude extract and enriched fractions from the bark of *B. megrelica,* were determined.

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PP 20. DIFFERENT METHODS OF PHARMACOTHERAPY FOR PREVENTION AND TREATMENT OF PARACETAMOL TOXICITY

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Drug-induced liver injury is of the current importance among safety problems related to pharmacotherapy. Nowadays, hepatotoxicity is identified for more then 1000 medications in use and their amount progressively increases every year [2,7]. Similarly with drugs, some metabolites have the ability of liver injury as well [2].

Paracetamol by itself, like some other drugs, is not hepatotoxic, but during first phase of its metabolism, by action of cytochrome P450 is produced toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI), wich has the property of damaging the liver cells. Conjugation with glutathione groups detoxifies this metabolite. Overdose of paracetamol causes accumulation of high concentration of NAPQI, causing disturbance of oxidative phosphorylation in mitochondria and development of different degree centrilobular necrosis of liver cells [2, 4,6,7].

Without dependence from disease etiology, in almost all cases, oxidative stress is common pathologic chain of liver injury [2,6,7]. Therefore, antioxidants are especially significant for treatment of drug-induced hepatotoxicity, causing optimization of energy exchange and amplified deactivation of toxic metabolites.

Against paracetamol hepatotoxicity efficacy of antioxidant compound acetylcysteine is known. Acetylcysteine is glutathione's precursor (indirect action). They both protect cells from damaging action of free radicals. Protective activity of acetylcystaine is provided by maintaining or restoring glutathion's content and conjugation with toxic intermediate metabolites [1].

For prevention of paracetamol overdose we proposed combination of acetylcysteine with antioxidant drug corvitin, the water-soluble form of kvercetin. Corvitin, as well as its active metabolite has antioxidative, immunomodulating, antiischemic, antihypoxic, hypolipidemic and antiatherosclerotic actions [5]. It is registered and accepted for intravenous use during myocardial infarction.

The toxic metabolites of paracetamol are causing disturbance of energy exchange and so, use of antioxidant compound L-carnitin is desirable. L-carnitin is natural substance similar with vitamins of group B. Mainly it is involved in transportation of free lipid acids from cytosol to mitochondria [42,45] and protective action is provided trough increased synthesis of antiproliferative, antiinflammatory and antioxidative molecules. Also L-carnitin is preventing induction of apoptosis [3].

Based on the overview of mechanisms of hepatoprotective action of antioxidant drugs, we set a goal to investigate the hepatoprotective action of acetylcysteine, corvitin and L-carnitin and their combinations during experimental hepatitis caused by paracetamol.

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PP 21. NANOMATERIALS AND APOPTOSIS

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In the last decade, the nanotechnology advancement has developed a plethora of novel and intriguing nanomaterial application in many sectors, including research and medicine [1]. Studies have revealed that the same properties that make nanoparticles so unique could also be responsible for their potential toxicity [2, 3]. To date, much of the nanotoxicology literature has focused on the assessment of cell viability or cell death. The many different pathways of programmed cell death offer numerous targets for engineered nanomaterials. Insights regarding nanomaterial-induced perturbation of cell death pathways may be of relevance for biomedical applications of nanomaterials and could be harnessed for therapeutic purposes [4].

Nanomaterials are described as triggers of extrinsic and intrinsic apoptotic pathways [5]. Metallic nickel nanoparticles induced apoptotic cell death through an FAS/caspase-8/BID mediated, cytochrome c-independent pathway in mouse epidermal cells [6]. Cells exposed to silicon oxide (SiO2) nanoparticles show caspase activation and cell death via apoptosis but the suggested pathways leading to apoptosis differed [7]. One study concluded that lysosomal destabilization was the initiating factor [8], whereas two others concluded that loss of mitochondrial membrane integrity was the predominant cause of cell death [7]. Of particular interest for nanoparticle-induced apoptosis is the fact that lysosomal disruption with release of lysosomal proteases (cathepsins) also engages the mitochondria-dependent pathway of apoptosis, in part through the proteolysis of Bid [9]. The oxidative stress paradigm of nanomaterials-induced cell death linked to intrinsic apoptotic network is by far the most accepted in fact many in vitro studies have identified increased ROS generation as an initiating factor of toxicity in nanomaterials exposed cells [10]. Various toxicity studies have shown that nanomaterial-induced ROS generation causes lysosomal membrane destabilization, DNA damage and mitochondrial membrane potential; leading to increases in the activation of p53, caspase-3 and caspase-7, and eventual cell death via apoptosis [1]. Chronic or unresolved endoplasmic reticulum (ER) stress can also cause apoptosis [11]. Zhang et al. and Tsai and collaborators reported that nanoparticles may exert cytotoxic effects through modulation of ER stress; this could also, in turn, lead to activation of mitochondria-dependent apoptosis[12, 13]. In spite of the recent advances in our understanding of cell death mechanisms and associated signalling networks, much work remains to be done before we can fully elucidate the toxicological behaviour of the nanomaterials as well as understand their participation in the determination of cell fate. More and accurate results are needed for apoptosis [1, 14]. In fact, in these areas, the use of many kinds of manufactured nanoparticles products is in development, such as metal oxide nanoparticles (cerium dioxide, cupric oxide, titanium dioxide, zinc oxide [15], etc.), metal nanoparticles (gold, silver, platinum, palladium, etc.), [1, 16] C60 fullerenes nanocrystals, carbon nanotubes (CNTs), and quantum dots [1]. A lot of contradictory literary data , are mainly stipulated by discrepancy of nanomaterials and experimental models engaged. Additionally, relationships between the responding cell type and nanomaterial properties are not well understood yet [1, 3] .

Thus, more precise definition of the molecular mechanisms by which nanosized particles activate cell death signalling pathways will actually contribute to the development of prevention strategies to minimize the cytotoxicity of nanomaterials.

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PP 22. THE EFFECT OF BASIC AND ACIDIC ADDITIVES ON SEPARATION OF β-BLOCKER ENANTIOMERS ON POLYSACCHARIDE-BASED CHIRAL COLUMNS AND ACETONITRILE AS A BULK MOBILE PHASE

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Polysaccharide-based chiral selectors (columns) are established as most useful materials for analytical- and preparative-scale separation of enantiomers in liquid chromatography and several related techniques, such as super-/sub-critical fluid chromatography, nano-liquid chromatography and capillary electrochromatography. In spite of wide application of polysaccharide phenylcarbamates and ethers in liquid-phase separation of enantiomers, the chiral recognition mechanism of these materials is still poorly understood. Although many efforts involving various experimental and computation techniques have been made in the past, at present we are still far from the status allowing us to develop a tailor-made chiral selector for separation of enantiomers of a given chiral analyte, to predict the most useful separation mode, mobile phase and mobile phase additives not to mention at all the enantiomer elution order (EEO).

Polysaccharide phenylcarbamate-based chiral selectors were initially proposed for separation of enantiomers in high-performance liquid chromatography (HPLC) in combination with hydrocarbon-alcohol mobile phases. In very first article on this topic published 30 years ago Okamoto et al. mentioned about some separations in water-ethanol mixture used as a mobile phase but especially stressed the usefulness of these materials in combination with hydrocarbon-alcohol mobile phases thus assuming the hydrogen bonding between the chiral selector and chiral analyte to be the most important contributor to the chiral recognition. Initially, polysaccharide phenylcarbamate-based chiral selectors were not recommended to be used in combination with pure polar organic mobile phases, such as alcohols or acetonitrile, although the earlier article on the application of cellulose triacetate as the useful chiral selector for liquid chromatographic separation of enantiomers reported the use of pure ethanol as a mobile phase. In 1980-1990s few articles have been published on the application of polysaccharide phenylcarbamates for HPLC separation of enantiomers in combination with some alcohols. However, these studies do not stress any potential advantages of polar-organic mobile phases for HPLC separation of enantiomers. More systematic studies in this area were published since early 2000 and at present the polar-organic mobile phase mode has been well established for analytical, as well as for preparative-scale separation of enantiomers. Major advantages of this mode include short analysis time, high plate numbers, favourable peak shape and commonly higher solubility of the analyte in the mobile phase. The last is important for preparative-scale separation of enantiomers. The potential of bulk acetonitrile, acetonitrile with minor basic or acidic additives, or acetonitrile with the hydrocarbon (n-hexane) additives as a mobile phase for HPLC separation of enantiomers in combination with polysaccharide-based chiral selectors has been evaluated in several studies. Interesting effects of basic and acidic additives in this separation mode were reported for some beta-blocker drugs in our previous study.

In the present study the separation of enantiomers of 16 beta-blocker drugs was investigated on 6 different polysaccharide-type chiral columns and acetonitrile as the bulk mobile phase. The emphasis was made on the effect of minor basic and acidic additives to the mobile phase on resolution and elution order of enantiomers. Out of the studied chiral selectors, amylose phenylcarbamate-based ones more often showed a chiral recognition ability compared to cellulose phenylcarbamate derivatives. An interesting effect was observed with formic acid as additive on enantiomer resolution

and enantiomer elution order for some basic drugs. Thus, for instance, the enantioseparation of several β-blockers (atenolol, sotalol, toliprolol) improved not only by the addition of a more conventional basic additive to the mobile phase, but also by the addition of an acidic additive. Moreover, an opposite elution order of enantiomers was observed depending on the nature of the additive (basic or acidic) in the mobile phase (Fig. 1).

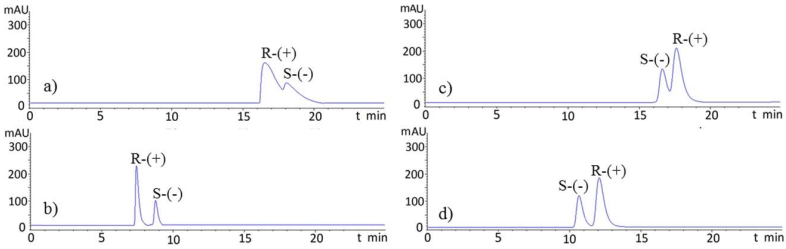

Fig.1. Separation of enantiomers of atenolol (1:2 ratio of S- and R-enantiomers, respectively) on Lux Amylose-2 column with the following mobile phases: ACN (a), ACN+0.1% DEA (b), ACN+ eq. FA (c), and ACN+0.1% DEA+ eq. FA (d); Y axis – absorbance at λ 220 nm; X axis - time.

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PP 23. STUDY OF ENANTIOMER MIGRATION ORDER OF CHIRAL IMIDAZOLE DERIVATIVES USING CAPILLARY ELECTROPHORESIS WITH CYCLODEXTRIN-TYPE BUFFER MODIFIERS

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Many imidazole derivatives are widely used or recommended for pharmaceutical use as antimycotics (clotrimazole, miconazole, enilconazole, etc.), antineoplastic agents (dacar- bazine), antiepileptics (nafimidon, denzimol), cytostatics (erbulozol), thromboxane synthetase inhibitors (dazoxiben). etc. [1]. Some of the pharmaceutically used imidazole derivatives contain a chiral carbon atom and therefore exist as racemic mixtures of the enantiomers. Substantial differences in pharmacokinetic, pharmacodynamic and toxic properties between the enantiomers of many chiral drugs are well established [2-4].

This study was conducted in order to evaluate systematically the resolution of chiral drugs containing an imidazole moiety by capillary electrophoresis (CE). Enantiomeric resolutions and enantiomer migration order of some chiral pharmaceuticals containing the imidazole (1,3-diazole) moiety were carried out using capillary electrophoresis.

Various native cyclodextrins (α-, β- and y-cyclodextrin), heptakis(2,6-di-O-methyl)-□-CD and heptakis(2,3,6-tri-O-methyl)-β-CD were used as chiral buffer modifiers. The effects of the cavity size and the structure of the selectors on the chiral recognition ability and the enantiomer migration order were evaluated.

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PP 24. ENANTIOSEPARATION OF FMOC-AMINO ACIDS USING POLYSACCHARIDE BASED CHIRAL STATIONARY PHASES UNDER POLAR ORGANIC MOBILE PHASE CONDITIONS

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N-Fluorenylmethoxycarbonyl (FMOC) α-amino acids are important building blocks for the solid phase synthesis of peptides [1]. After the development of FMOC/tBu strategyfor solid phase peptide syntheses, FMOC α-amino acids have become the raw materials of choice for the preparation of synthetic peptides [2]. Using this methodology, peptides can be prepared in a few days with high yield using a peptide synthesizer. As the number of amino acids residues increases the final purity and overall yield of the peptide produced is directly affected by the chemical and chiral purity of the protected amino acids used. Currently, for standard protected α-amino acids, the expected enantiomeric purity is > 99% ee and sometimes even > 99.8% ee. This level of precision can only be achieved by very few analytical techniques, chiral HPLC being one of them. The main advantages of chiral HPLC analysis over other techniques are speed (run less than 20 min), detection level and ease of use. HPLC is also used on a regular basis by the peptide community for the analysis of purified fractions and peptide purity. In this presentation, we will report the chiral separation of the 19 standards FMOC protected ?-amino acids under reversed phase and polar organic separation modes using polysaccharide based chiral stationary phases [3]. As the mobile phase alcohols (methanol and ethanol) and acetonitrile with 0.1% (v/v) additive of formic acid were used. Five different polysaccharide-based chiral columns of Lux series, such as Lux Cellulose-1, Lux Celluose-2, lux Cellulose-3, Lux Cellulose-4 and Lux Amylose-2 from Phenomenex (Torrance, CA, USA) were used. The separations were performed using Agilent 1260 HPLC instrument equipped with a binary pump and variable wavelength UV-detector. In alcohols as mobile phases the best results were obtained on Lux Cellulose-1 and Lux Cellulose-3 columns. In particular, the enantiomers of almost all FMOC-amino acids were separated on these columns. In acetonitrile as a mobile phase Lux Amylose-2 was a preferable column. Few examples of enantiomer elution order reversal depending on the chiral selector and the mobile phase were observed.

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PP 25. DEVELOPMENT OF NOVEL FINISHED DOSAGE FORM FOR WOUND HEALING

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Two Caucasian species of comfrey – *Symphytum asperum* and *S. caucasicum* widespread in Georgia were chosen as a vegetable raw for the development of novel effective wound healing remedies due to expressed antioxidant, antinflammatory and antiproliferative activity. Water soluble crude polysaccharides (WSCP) obtained from the roots, stems and leaves from *S.asperum* contain a high-molecular (> 1000 KDa) fraction - the principal constituent, responsible for the aforesaid effects [1].

The aim of present study was an attempt to choose optimal carriers and excipients for novel ointment on the basis of biopharmaceutical investigations.

Eight compositions were designed (Tab. 1) and studied for i) determination of optimal ointment base; ii) establishment of ointment stability; iii) Biopharmaceutical study.

Table 1. Ointment compositions.

Constituents (g) / Composition #	1	2	3	4	5	6	7	8
Active principle								
WSCP *from S.asperum*	10,0	10,0	10,0	10,0	10,0	10,0	10,0	10,0
Excipients								
Petroleum jelly	90,0	64,0						75,0
Anhydrous lanoline		18,0						
Distilled water			73,0		80,0	88,0	81,0	20,0
Sodium carboxymethylcellulose		8,0	7,0					
Polyethylene glycol -1000				60,0				
Glycerin			10,0	30,0	10,0			
Sodium alginate						2,0		
Askana Clay							9,0	
TWEEN-80								5,0

Stability of ointment was evaluated using centrifugation and thermal assays. All compositions were centrifuged at 6000 rpm for 5 minutes and then stored at 0ºC, +20ºC, and +45ºC –at for 24 hours and centrifuged once more. Compositions 1, 2, 3 and 6 passed the test, whereas in compositions 4, 5, 7 and 8 the stability was slightly affected by centrifugation.

The biopharmaceutical study was carried out using the agar diffusion assay [2] (Fig.1,2).

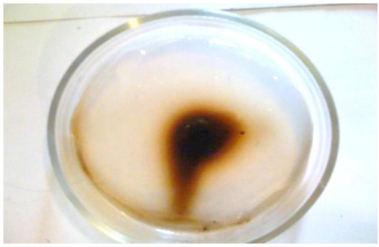

Fig. 1. Liberation of active principle in agar diffusion assay. (composition #6).

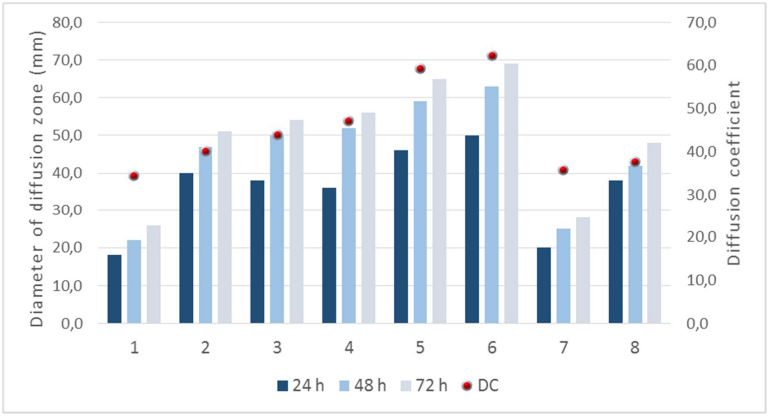

Fig.2. Liberation of active principle in agar diffusion assay at 32ºC. X-axis – sample compositions 1-8.

Based on the obtained results the optimal ointment formulation has been justified and formulated: WSCP from S.asperum/ Distilled water/Sodium alginate - 10:88:2. The composition remains stable both right after manufacturing and during storage at different temperatures for 24 hours with following centrifugation. Biopharmaceutical studies established that the maximal release of WSCP from the ointment was achieved when sodium alginate was used for an ointment base.

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PP 26. THE MAIN FEATURES OF THE COMPOSITION AND TECHNOLOGY OF DIALYSIS SOLUTIONS

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Dialysis therapy is widely used at the terminal stage of chronic kidney disease. Peritoneal dialysis and haemodialysis are the two main modalities of renal replacement therapy, which employ solutions of the requisite quality and composition. Ideally, the dialysate electrolytes concentration should be individualized to meet specific patient needs. Thus, dialysis centers should have solutions of various compositions for providing specific patient needs. In Ukraine national commercially available solutions are restricted in a list according to concentrations of their components. Therefore, research of pharmaceutical development of dialysis solutions is of current interest.

Peritoneal dialysis solutions (PDSs) are introduced into the peritoneal cavity for the purpose of removing toxic substances, normally excreted by the kidney, across the semipermeable peritoneal membrane (PM). The latter is very sensitive to various factors. PDSs are hypertonic solutions with the different extent of tonicity and contain electrolytes in concentrations approximately close to ones in the blood. These solutions should adjust the disorder of the electrolyte composition of the blood at chronic renal failure. The drug substances with buffer properties are incorporated into PDSs to adjust acid-base status. Commercially available PDSs contain lactate, hydrogen carbonate or their mixture. Hypertonicity is made with the different dextrose strengths for the purpose of avoiding absorption of water from a dialysis solution into the circulation and removal excess of fluid from organism. Commercially available peritoneal dialysis solutions contain dextrose in concentrations 1.36-4.0 %. However, dextrose is involved in local toxicity towards mesothelial cells PM. Besides, it forms degradation products during heat sterilization. This degradation is strengthened in the presence of sodium lactate. As literature data and our research have shown, pH of a solution before sterilization, the composition of a solution, sterilization temperature, the time of heating autoclave for reaching sterilization temperature affect the extent of forming dextrose degradation products (DDPs). 3,4-dideoxyglucosone-3-ene, as a product of separation of two molecules of water from dextrose, has been identified as the most toxic. In addition, PDSs are injected into peritoneal cavity in very large volumes from 7 up 40 L per a day. Thus, they have to be sterile, apyrogenic, and to contain the minimum level of DDP. The content of bacterial endotoxins should be less than 0.25 EU/ml. For assurance of sterility these solutions have to undergo steam sterilization. Therefore, during pharmaceutical development of PDSs it is necessary to select the composition and pH of the solution before sterilization, and optimal sterilization conditions for the purpose of assurance of both sterility and low content of DDPs.

Haemodialysis is also used to remove toxins and excess of fluid from the blood by means of shunting the blood through an artificial dialyzing membrane bathed in an electrolyte solution of the requisite composition. These solutions are used in huge volumes. A 4-h haemodialys procedure requires at least 120 L of a dialysis solution. Therefore, they are produced as concentrated solutions which are diluted before administration. There are three types of concentrated solutions according to the British Pharmacopeia. The main feature of technology of solutions for haemodyalisys is the preparation of a concentrate with the requisite concentration of electrolytes, pH level, the highest microbiological quality, and low level of bacterial endotoxins (0.5 EU/ml of the solution diluted for use). The requisite level of pH is dictated with adjusting acid-base balance of organism and avoiding precipitation of calcium carbonate in a dialysis machine during use of the mixture of diluted

concentrated acid solution and sodium hydrogen carbonate. Sodium hydrogen carbonate is supplied in a separated container and must be added immediately before use to a final concentration of not more than 45 mmol/l.

PP 27. INFLUENCE OF PHENOL VAPOR INTOXICATION ON CYCLIC NUCLEOTIDES AND PROSTAGLANDINS RATIOS

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Effect of majority hormones on the target cells is realized by universal substances-cyclic nucleotides:- cyclic adenosine-3^1,5^1-monoposphate (c-AMF) and cyclic-guanosin-3^1,5^1-monoposphate (c-GMF) - so called second messengers. As cyclic nucleotides are the second messengers so are prostaglandins. They are similar of hormone substances, which are found in most tissues and organs. Cyclic nucleotides and prostaglandins can change activity of enzymes and influence on the hormones synthesis in the tissues, provoking various diseases. [1; 2; 5]

The purpose of the work is to establish the possible correlation between the changes in cyclic nucleotides (c-AMF, c-GMF) - and some groups of prostaglandins (PgE, PgA, $PgF_{2\alpha}$) under intoxication by phenol vapor. [7]

Experiments were performed using 90 male rats (body mass 200-250 g.). 10 animals were in the control group. During the experiment, each animal was exposed to phenol vapor intoxication in a closed box separately. Vapor concentration was 1280-1520 ppm. The exposure was terminated as the animal obtained the lateral decubitus position. Thereafter, each rat was relocated in its individual box. [6, 3]. Experiments were approved by Institutional Animal Care and Use Committee on bioethics in accordance with [8].

After the procedure, two groups of animals were distinguished: group I–with lethal outcome and group II–survived rats.

In group I, the life-span of rats was from 1h 50min up to 7h after the inhalation. Estimation of cyclic nucleotides and prostaglandins was performed 5 min after cessation of inhalation and every 2, 4, 7h or just before the death of rats. 0.5 ml of blood was collected from the tail vein. The concentration of hormones in blood plasma was determined by radioimmunoassay. [4]

Concentration of c-AMF increased by 40% in group I, but in the group II (survived) - decreased to 20% of starting level. Subsequently, in group I c-AMF varied within 120-150% of starting level till the death of the animal. In group II the concentration of c-AMF remained decreased from the starting level till 20-30%.

The concentrations of prostaglandins PgA and PgE, the second messenger for which is c-AMF, were performed in the following way: the level of PgA varied in the group I, but remained at 200% of starting level and only before death dropped down to initial level. Though, arising of the concentration in group II was less expressed quantitatively and then dropped to 80% from starting level.

Change of PgE concentration of in both groups had similar trend, but we observed the differences in degree of increase. In group I PgE concentration's rose was sixfold, whereas in group II only twofold higher than the starting level.

As opposed to c-AMF c-GMF concentration in both groups changed similarly. In group I c-GMF concentration varied within starting level and dropped only before the death of animal. In group II, already at early stage it dropped to 20% of initial level and remained unchanged.

Observed variations in $PgF_{2\alpha}$ concentrations allows to conclude, that the dynamics of change was different. The concentration of $PgF_{2\alpha}$ increased in group I and by the end of experiment reached 500%, but in group II it fluctuated within 80% from the starting level.

In our experiments we studied the dynamics and the mechanism of change in cyclic nucleotides and some groups of prostaglandin's concentration under the intoxication by phenol vapor. The experimental data indicate that after the inhalation by phenol vapor activation of cyclic nucleotides is directly proportional to the hormones secretion second messengers of which they are. In particular, the high concentration of c-AMF in group I is caused by elevated level of PgA and PgE, so as the increase of c-AMF is a result of elevated concentration of $PgF_{2\alpha}$. Accordingly, in group II – survived animals - the organism passed on unsparing regiment and secretion of prostaglandins is carried out in little amounts, conditioning low levels of cyclic nucleotide's concentration.

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PP 28. DETERMINATION OF DOXEPINE AND DESMETYLDOXEPINE IN BLOOD USING HLPC

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Doxepin is a tricyclic antidepressant from the group of dibenzoxepines. It is chemically known as 1-propanamine,3-dibenz[b,e]oxepin-11(6H)-ylidene-N,*N*-dimethyl hydrochloride. This substance is widely used for treatment of depression and anxiety. It displays a potent central anticholinergic activity and can inhibit both norepinephrine and serotonin (5-HT) reuptake in brain synapses [1, 2]. Doxepine is quickly metabolizing in the body. The main metabolite N-desmethyldoxepine also has an antidepressant effect [3]. The drug is usually administered by oral route in daily doses of 50–150 mg. At high concentrations, severe adverse effects and toxicity can appear sometimes with fatality intoxication cases. Death can occur of consciousness due to either cardiac effect or respiratory arrest [4, 5, 6].

The aim of this research was the development of rapid and sensitive methods of determination both doxepine and N-desmethyldoxepine in whole blood by HPLC with UV detection, which can successfully be applied for routine chemical-toxicological analysis.

Methodology: Samples of citrated human whole blood was obtained from Lviv station of blood transfusion and verified drug free. The samples of the blood were kept frozen at -20 °C. 10 mL human whole blood was spiked with doxepine and N- desmethyldoxepine at five concentrations: 0.20; 0.5; 1.0; 5.0 and 10.0 μg/mL each compounds. The spiked blood was sonicated for 15 min at 20 °C temperature. Aliquots (2 ml) of spiked blood added the internal standard (100 μL aqueous solution of quetiapine fumarate 100 μg/mL). In all samples of blood were added 1 mL of phosphate buffer (pH 7.4) and 0.5 ml 20 % solution of sulfosalicylic acid. Then the samples were vortex mixed for 1 min, and the mixtures were centrifuged at 5000×g for 15 min. The whole liquid was transfered on column for solid phase extraction (SPE).

For SPE extraction was applied Oasis HLB cartridges 60 mg (Waters Corporation Milford, Massachusetts USA). The SPE column was activated with 2 mL methanole and 1 mL water. Then test solution of blood skipped. The cartridges were washed with 2 mL of phosphate buffer (pH 6.85) and with 2 ml of methanol aqueous solution (1:9). After the cartridges were dried 2 min using weather stream in the light mode (~ 27 kPa) of vacuum pump VacElut 20®. The analyzed substances were eluted with 2 ml of methanol. Prepared eluates were dried in a stream of nitrogen at 40° C and dissolved in 200 μl of methanol. 10 μl of analyzed solution was injected in HPLC column.

High performance liquid chromatography was used for the identification and quantification of antidepressants extracted from blood. A liquid chromatograph „Waters 2690“ and diode array detector „Waters 996“ were used. The most acceptable eluent for HPLC analysis was a mixture of 0.1 % trifluorethane acid (A) and acetonitrile (B). Elution conditions: linear gradient flow at 1 ml/min from 0 min: A – 95 %, B – 5 % to 20 min: A– 45 %, B – 55 %. The substances were detected at 210 nm. The best results of chromatographic separation were achieved on “ACE C18” (250 x 4.6 mm; 5 μm) column. Components of the mixture were completely separated ($R \geq 2$) at these conditions.

Retention time was 9.57 min, 15.95 min and 17.15 min for Quetiapine, doxepine and, N-desmethyldoxepine, respestively

Peak areas and peak area ratios were determined using Emprove software for quantification of doxepine and N-desmethyldoxepine applying an internal standard. The calibration curve was linear within 0.20 - 10.0 μg/mL for both doxepin and N-desmethyldoxepine. The linear correlation was Y =

3.1402·X + 0.0339, the correlation coefficient was 0.9998 (for doxepine) and: Y = 3.0147·X + 0.04178 (for N-desmethyldoxepine, r= 0.9989).

The limit of doxepine detection (S/N of 3:1) in blood treated on Oasis columns, was 0.02 μg/mL, and N-desmethyldoxepine – 0.024 μg/mL. The limit of quantification was 0.05 μg/mL and 0.058 μg/mL for doxepine and N-desmethyldoxepine respectively.

Under these conditions 87.0 % - 89.3 % of doxepine and 82.4% - 85.2 % N-desmethyldoxepine were isolated from the samples of blood.

The conditions of HPLC are available for the identification of other metabolite of doxepine investigating blood from femoral vein and heart from Doxepine-related death.

Conclusions:

- Doxepine and N-desmethyldoxepine separation and identification procedure by high-perfomance liquid chromatography was established.
- Optimal conditions for Doxepine and N-desmethyldoxepine isolation from whole blood using solid phase extraction were established.
- HLPC techniques for separation, identification and quantitative determination of components isolated from blood by HLPC was elaborated. The method is effective, simple, and reliable and can be used for determination of doxepin in whole blood.

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PP 29. DEVELOPMENT AND USE OF APPLICATION FORMS FOR THE TOPICAL TREATMENT OF DENTAL DISEASES.

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The treatment of dental patients with lesions of the oral mucosa involves complex medical scheme with the use of local medications to restore soft epithelium and periodontal tissues.

Local treatment in periodontology is complicated by the dissolution of the traditional soft-consistency dressings by saliva, which constantly washes out the medications.

To enhance the effectiveness of drugs, we offer a new type of dosage application forms for the topical treatment – medicinal dental films (MDF).

MDF belongs to transdermal therapeutic systems that are used in the form of applications on the mucosal lesions, where local protection from exposure to mechanical, thermal and chemical stimuli is required. MDF create artificial temporary barrier and correct oral microflora, particularly in the periods of lowered resistance of the organism (lack of immune response).

MDF consists of hydrophilic polymer base, which includes active pharmaceutical ingredient (API). Under the influence of oral fluid (saliva) polymer-based MDF gradually dissolves resulting in controlled and sustained release of an API into the oral epithelium by the way of diffusion. The new medicinal form – MDF increase bioavailability and efficacy of administration of API, protects active substances from saliva and at the same time allows the use of substances with different therapeutic and physical-chemical properties in one polymer base that is an advantage when compared with other medicinal forms such as ointments, creams, gels, pastes, rinses and sprinklers.

MDF are prepared by the method of polymerization, ex tempore: by the way of irrigation on plates of various shapes and then being dried.

Clinical observations were carried out with the consent of patients aged from 18 to 46 years: the main group (10 patients) treated with MDF and the group of control (10 patients), where traditional course of treatment was carried out. Among them 4 patients had mechanical trauma of oral mucosa and 6 patients suffered from aphta and stomatitis. The course of treatment lasts for 1-2 weeks, and comparative observations were performed with the patients of the control group in one and 3 months.

Observations showed that patients of the main group experienced subjective improvement already in two days, and a noticeable improvement in clinical condition was achieved in 3-5 days of treatment. The sensitivity of oral mucous reduced significantly, tissue swelling disappeared in 2-4 days. In the group of control improvement in clinical condition was observed with a delay of 3-6 days when compared with the main group [1].

MDF in periodontal dressings, alternative methods in comparison with the traditional forms of treatment of oral mucous membrane. Thus, it can be concluded that MDF – used to accelerates process recovery on the oral mucous membrane in the treatment of dental diseases.

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PP 30. A REVIEW OF MEDICATIONS APPLIED IN DENTISTRY IN XIX CENTURY (FROM THE BOOK WRITTEN BY THE PROFESSOR OF LVIV UNIVERSITY KARL PROKIP KALIGA (1838)

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The aim of present investigation was evaluation of the level of dentistry, equipment and medications in XIX century. Dental manual "About teeth diseases and facilities for their treatment", ("Über die krankheiten der Zähne und die Mittel sie zu heilen"), published in Wien, 1838, by professor of dentistry of Lviv King's University Karl Prokip Kaliga was analyzed in our work. This book was soon republished in Lviv in polish language – "O chorobach Zębów I środkach lezcenia takowych" and in 1841 professor Kaliga managed to publish this book in Italian, in Milan [1, 2, 3].

The work includes description of old medicinal compositions with different ingredients, used by the author according to the demands of pharmacopeia of that time, scientific articles, reference books of different countries from Europe and Asia, as well as author's recipes, scientifically enriched and proved. Medicinal recipes are written in Latin in classical form and style. Analysis of recipes, published in the issue testified to highly professional scientific attitude of professor Kaliga to his work. The publication includes indications about the composition and ways of preparation of each separate recipe, short recommendations for use and means of taking the medicine, proved in medical experiments and given in rational, available for that time medicinal form. The publication, in addition to therapeutic scheme and ways of rendering of medical help, presents compositions of medicines in the form of recipes, prepared ex tempore, what is especially informative not only for dentists but for pharmacists as well. In the investigated scientific document a lot of combinations of medicinal substances are described, the most rational technology of different medication forms as powders, pastes, ointments, rinses are depicted and multi vector attitude not only in the elaboration of therapeutic scheme of treatment in general, but to each patient individually is presented. In recipes prepared ex tempore, presented in the book many names of drugs are described which can be included in the recipe and were used in XIX century. Unfortunately, at the present time a lot of ingredients from ancient recipes lost their actuality, because of many factors, scientific progress as well. They can't be found in modern pharmacopeia. Among these substances are: Radicis ratannhiae, Pulveris rad. Myrrhae rubrne, Radicis ossis sepiae, Pulveris carticis chinae, lapidum cancrorum, Pulveris cornu cervi and others. In old manuscripts some ingredients are mentioned which are also actual at present: Radicis calami aromatici, Olei menthae piperitae, Pulveris herbae salviae, Olei thymi, etc.

After scientific analysis of ancient recipes it can be concluded, that practical doctor-dentist Karl Prokip Kaliga during the period of XIX century carried out an active search and scientific investigations of different active working components with the aim to create new medicinal forms and improve existing drugs, making, thus, an important contribution to the development of modern science and techniques.

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PP 31. AKHTALA MUD - A PROSPECTIVE RAW FOR THE DEVELOPMENT OF MEDICINAL AND COSMETIC PRODUCTS

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One of the possible sources of drugs are peloids – medicinal muds. They are used for than one millennium. Almost all kinds of mud have a pronounced therapeutic effect and are used as baths, applications, wraps. It is suggested, that the components of the mud have specific tissue healing properties. Nowadays mud-containing medicines are widely used, e.g. Dead Sea mud [1].

Among the richest sources of natural biologically active substances is a volcanic origin medicinal mud from Akhtala deposit. Akhtala mud differs from others by higher colloid activity, contains iodine, bromine, lithium, iron, manganese, calcium, magnesium, sulfur and many other elements, some of which are not common for sedimentary rocks.

Mud preparations are characterised with relatively rare occurrence of adverse reactions and exacerbation of the disease and thus used for treatment of many diseases, including, but not limited, to traumatic, nervous, musculoskeletal, gynecological, skin disorders, etc., either itself or as a constituent in compresses, lotions, mikroenemas, suppositories, vaginal irrigations. Mud components can also be administered by iontophoresis of native peloid.

On the other side, it should be considered, that at least 200 kg of mud is necessary for a single procedure, that, along with poor regeneration ability, leads to average 20% loss of the amount used and, in turn, promotes rapid depletion of the natural deposit. Moreover, mud stability is easily affected by transportation, influence of environmental factors, inappropriate storage, that leads to the loss of its curative properties. All above mentioned explains why, despite high therapeutic activity of Akhtala mud, it is applied only in local spa practice, and no corresponding products are in the pharmaceutical market.

Currently a complex of medicinal and cosmetic remedies containing bioactive substances from Akhtala mud is under the development at TSMU Institute of Pharmacochemistry. Toothpaste and ointment that exhibited healing and antibacterial activity are ready for clinical trials. Development of several products proposed for application in dermatology and cosmetology (adhesive plasters, scrubs, peels, masks and packaged forms of mud) is in process.

The development of products containing mud BASes will be beneficial from medicinal, economic and ecological viewpoints due to a significant reduction in consumption of natural resources, pushing the products under Akhtala brand name to the market, increase of out-patient treatment with spa-quality products, and further development of peloidotherapy.

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PP 32. SEARCHING AND SYNTHESIS OF NEW CHELATING DERIVATIVES OF THE THIOUREA AS ANTITUMOR REMEDIES

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Contemporary antitumor drugs are substances of different chemical structures and different biological actions. Conventional antitumor chemotherapy has the goal to destroy tumor cells and suppress possible metastases. But during the usage of antitumor drugs numerous side effects of varying intensity take place. Moreover, the side effects are strengthened by usage of combined therapy.

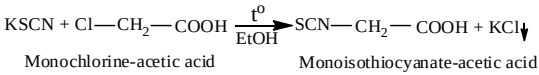
Although special medicines are recently elaborated that improve the tolerability and efficiency of the used antitumor drugs, nevertheless solving the problem is seen in a purposeful synthesis of effective non-toxic compounds of new chemical classes. In particular, it is of interest the synthesis of new drugs that have chemical structure similarities with thiourea and thiosemicarbazide, but are strengthened by various chelating groups, and also by methylhydrazine group.

The synthesis of such compounds having predetermined characteristics of chelants, the knowledge of the properties of complexes they form together with "cancerogenic" cations of some metals, as well as the analysis of a variety of factors that affect the process of complexation, creates the necessary background (data base) for solving one of the most acute problem of our time - the fight against cancer. Our selection of these ligands is additionally motivated by the positive impact of sulfur-containing compounds on the body's immune system.

Taking into the consideration all above-mentioned, the procedures and the synthesis of water-soluble chelants - derivatives of the thiourea are elaborated for the first time. Choice of the derivatives of urea as a basic component had been made due to their high biological activity, antitumor effect of well-known drugs - Hydrea and Metinurum.

For soluble chelants - derivatives of the thiourea – the two-step procedure for synthesis of these compounds is elaborated for the first time.

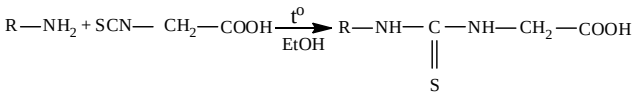
At the first stage of synthesis obtaining monoisothiocyanate-acetic acid in the hot ethanol solution has been envisaged according to the scheme shown below:



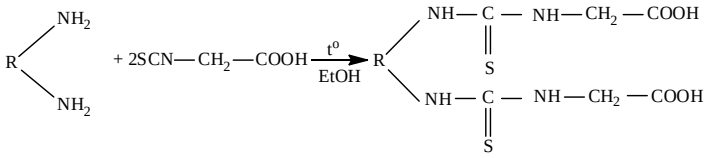
After separation of the potassium chloride sediment the pure ethanol solution of monoisothiocyanate ethanol-acetic acid had been obtained.

At the second stage of the synthesis it is envisaged obtaining in the hot ethanol solution the various derivatives of thiourea according to the schemes shown below:

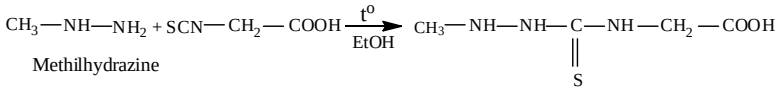
a)



b)



c)



The obtained acids after the careful neutralization by an equimolar amount of alkali are converted into the water-soluble form. All the synthesized chelants have complexing ability with cations of heavy metals and are of considerable practical interest. The studies of physical and chemical and toxicological properties of the obtained substances are conducted.

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PP 33. MODERN APPROACH TO THE QUALITY MANAGEMENT SYSTEM IN PHARMACY

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The quality of products and services has become a sign of a highly developed economy and national wealth. Besides other aspects, the health and well-being of the population is directly proportional to the quality of pharmaceutical products. Thus, it is imperative for the management of the pharmaceutical organization, especially the owners and top managers, to understand the necessity of Quality Management Sysytem (QMS), otherwise no progress will be achieved. Unfortunately, in some cases it's difficult to convince the managers of the importance of implementing QMS. This project illustrates an attempt of QMS implementation in one of Georgian pharmaceutical companies.

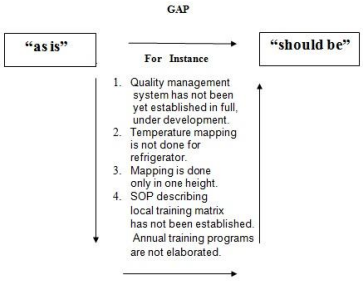

Fig.1. - Model that describes the GAP between "as is" and "should be"

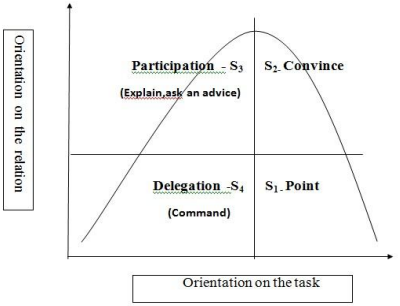

Fig.2. Diagram illustrates the strategy that helps managers lead the staff

First of all, the group of quality assurance managers reviewed the company's quality system and revealed what it has and what should have (Fig.1). The gap between "as is" and "should be" showed, how far is the organization from QMS requirements. The results simply and clearly illustrated the situation at any department of the company, so it was no need to lose time on familiarization of personnel with inconsistencies in their departments. In addition, this model motivates the owners and top managers to correct these inconsistencies, because the model shows them the gap of product and quality product. The biggest problem the group faced while implementing the QMS was to convince the owners in importance of standardization and quality management for pharmaceutical organization. Besides, a majority of customers was interviewed about products and services and all their requirements related to the product/service were fixed. State regulatory requirements for the pharmaceutical organization were summarized as well. All the results influenced the owners, although the most effective way to convince them was a comparative survey of their production and competitors' products argumented by facts. As they understood the role of quality product/service in the development of business, they realize that QMS is the guarantee of successful business and good distribution of drugs to population. It is often heard from pharmaceutical companies - "our goal is to produce high-quality products", but this is a wrong statement - the goal of the pharmaceutical companies, as well as any other company, is making a profit, and first of all profits to its owners. A production of quality products - this is a key responsibility of the company in the way of getting any profit.

Often we face the problems like lack of desire to change anything in the company, because employees are used to work without any system or with incorrect system. They express a negative opinion; sometimes even refuse to work in accordance with the QMS. The cause of this problem is the lack of information about standards, total QMS, etc. Often, workers think that it's just whims of top managers. To exhaust this problem, internal and external trainings and short programs were started. Besides trainings, guidelines for the workers were developed in frame of Point-Convince-Participation-Delegation strategy (Fig.N2). There are two main aspects:

i) orientation on the task – point the task to staff, then convince;

ii) orientation on the relation- let the staff to participate in decision, ask an advice and explain why they should do that task, finally command!

Actually, the trainings and the aforesaid strategy turned out to be one of the best ways to implement QMS in our company.

Each time, it is necessary to find a method that corresponds with maximum of raw data, and then implement it with the amendment to the data. The system will be reliable and sustainable, only if it is firmly entrenched in the mind of every employee of the enterprise. QMS - is a way to structure and discipline the actions of all participants of pharmaceutical organization's processes.

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PP 34. DETERMINATION OF BUPRENORPHINE AND NORBUPRENORPHINE IN URINE BY LC-ESI-MS/MS

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Buprenorphine is a semisynthetic opioid derivative of thebaine. It is a mixed agonist–antagonist opioid receptor modulator that is used to treat opioid addiction in higher dosages, to control moderate acute pain in non-opioid-tolerant individuals in lower dosages and to control moderate chronic pain in even smaller doses [4,5].

Buprenorphine is converted to norbuprenorphine, its major active metabolite. Determination of Buprenorphine and norbuprenorphine in biological samples by forensic toxicology laboratories requires an analytical method capable of reliable detection of these compounds at concentration below 10 ng/ml.

Various methods are used to determine Buprenorphine analysis e.g. immunoassay, GC/MS, which require special sample preparation including derivatization procedure before analysis. Liquid chromatography method is the best choice for determine Buprnorphine and its metabolits [2,3].

A sensitive, simple and rapid high performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS) method was developed and validated for the simultaneous quantification of buprenorphine (BUP) and its N-dealkylated metabolite norbuprenorphine (norBUP) in human urine.

Urine samples were subjected to enzymatic hydrolysis by β-glucuronidase at 60°C for 30 minutes prior to solid phase extraction and LC-MS/MS analysis.

The samples were analysed with the LC-ESI-MS/MS. Instrument was Agilent Technologies 1290 Infinity Agilent Technologies 6460 Triple quad LC/MS. Separation was carried out on a Zorbax Eclipse plus C18 (100 X 2. 1 mm, 1.8µm) column equipped with HPLC guard Zorbax Eclipse plus C18 (5 X 2. 1 mm, 1.8µm) precolumn. The mass spectrometer was operated in the positive electrospray ionization mode. Mobile phase consisted of solvent A: Water with 0.1% Formic acid and solvent B: Acetonitrile with 0.1% Formic acid, flow rate was 0.6 ml/min. Following electrospray ionization, the analytes were quantified on a triple–quadrupole mass spectrometer in multiple-reaction-monitoring (MRM). Fulfilling confirmation criteria with two transitions for each compound with acceptable relative ion intensities. Transitions monitored were 468.3>396.2 and 468.3>414.3 for BUP, 414.3>340.1 and 414.3>326.0 for norBUP.

The analytical procedure was validated in terms of linearity, limits of quantitation (LOQ), intra-batch and inter-batch precision and accuracy, specificity and selectivity. All validation experiments were designed according to the principles outlined in the FDA industry guidance on bioanalytical method[1]. Linearity was achieved from 1.5 to 100 ng/mL for buprenorphine, from 2.0 to 90 ng/mL for norbuprenorphine with r^2>0.99. The LOQ were determined by evaluating signal-to-noise ratios for the two transitions used for each compound. The method was found to be sensitive LOQ ranged from 2 ng/mL to 5 ng/mL for buprenorphine and norbuprenorphine.

The Accuracy and precision at the LOQ were assessed for each compound. Acceptable ranges were considered to be 85-115% recovery and ≤15% coefficient of variation (CV). Accuracy ranged from 92% to 101% for BUP and norBUP. Precision results ranged from 3% to 7% and each compounds had passing results of ≤15% CV for precision. The method was demonstrated with acceptable accuracy, precision and specificity for the detection of buprenorphine and norbuprenorphine in urine samples.

The LC-ESI-MS/MS method has been accredited by Georgian Accreditation Service. The described method have been used in routine analysis for urine for several years.

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PP 35. BENTONITES OF VANI RIDGE AS OINTMENT BASE

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Today the use of Montmorollonite (bentonite clay) in pharmacy and medicine is generally recognized due to its ability to give easily washed-off labile paste when mixed with water, and, most importantly the capability to be mixed with various medicinal substances without affecting their curative properties. Bentonite clays are successfully used in pharmacy, dermatology, gynecology, ophthalmology, proctology and other areas of medicine both itself and as excipients in various soft medicinal forms [1-3]

Recently the bentonite clay from new Vani Ridge deposit was studied at Georgian Technical University of Georgia

Bentonite clay aqueous solution (slip) was obtained by direct flow high performance drum-type dryer, which provides 10% of damp clay intake from career 35% humidity bentonite, which results the sharp increasing of small dispersed particles number.

In a particular stage we insert into the slip a small amount (0.3-0.5%) of surfactant, which greatly influenced on the quality of the clay disparity, due to compensation of excess negative charge of the crystalloid gate of Montmorillonite. Accordingly, after selected additives we received alkaline bentonite with the increased colloidal properties, plasticity and the other positive features. After the comparison of alkaline grounds, it gives us opportunity to receive the high quality small dispersive mass-Vancol (Colloid of the Vani Ridge);

The receiving of dry powder from the Shlicer (humidity – 40%) is a hard consuming and demands the high expense of energy-carrier. That's why we used spray dryer, which provides the receiving of small dispersive powder with 0,1-0,2% humidity; Air temperature in the dryer was 140-150^0, that excluded the necessity of the product sterilization.

Using the afore mentioned simplified technology Vancol - a high quality base for soft medicinal forms was developed.

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PP 36. HPLC SEPARATION OF ENANTIOMERS OF CHIRAL ARYLPROPIONIC ACID DERIVATIVES USING POLYSACCHARIDE-BASED CHIRAL COLUMNS AND NORMAL-PHASE ELUENTS WITH EMPHASIS ON ELUTION ORDER

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The separation of enantiomers of 10 chiral arylpropionic acid derivatives was studied on 6 different polysaccharide-based chiral HPLC columns with various normal-phase eluents. Along with the successful separation of analyte enantiomers, the emphasis of this study was on the effect of the chiral selector and mobile phase composition, as well as of the separation temperature on the elution order of enantiomers. The interesting phenomena of reversal of enantiomer elution order function of the polysaccharide backbone (cellulose or amylose), type of derivative (carbamate or benzoate), nature and position of the substituent(s) in the phenylcarbamate moiety, the polar modifier of the mobile phase (ethanol or 2-propanol), its content in the mobile phase and separation temperature were investigated. In addition, an unusual increase in retention with increasing temperature was observed for some arylpropionic acid derivatives.

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PP 37. ENANTIOSEPARATION OF SOME CHIRAL ANTIMYCOTIC DRUGS BY USING POLYSACCHARIDE-BASED CHIRAL STATIONARY PHASES

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In the present work, separation of 8 chiral antimycotic drugs enantiomers was studied on 18 different polysaccharide-based chiral columns with polar-organic and reversed phase eluents in high-performance liquid chromatography.

Antimycotics represent antifungal drugs used for both, prevention and treatment of mycosis. Many of these drugs are chiral and sometimes only one of the enantiomers is responsible for their therapeutic action. Therefore, enantiomeric purity determination of antimycotic drugs is important.

Influence of both, nature of chiral selector and composition of the mobile phase on the separation and elution order of enantiomers was studied. Introducing of ammonium acetate into polar organic solvent (methanol, acetonitrile) improved separation of enantiomers. In contrary to this, addition of formic acid to methanolic mobile phase negatively affected the separation of enantiomers. The mentioned effect was observed on the Cellulose-3 column for the enantiomers of terconazole, sulconazole, miconazole, tioconazole and ketoconazole. The same effect also takes place for some drugs on Cellulose-3 and Cellulose tris (3, 4-dimethylphenylcarbamate)-based columns. Retention times of enantiomers increased with increasing water content in the methanolic mobile phase.

Change of elution order was observed for some drugs. For instance, elution order of terconazole enantiomers was opposite on Cellulose-3 and Cellulose-4 columns. Reversal of elution order takes place also for sulconazole on Cellulose-3 and Cellulose tris(3,5-dichlorpenylcarbamate)-based columns, whilst the same effect is observed for bifonazole on Cellulose-2 and Cellulose tris(2-methylpenylcarbamate)-based columns. Addition of water to acetonitrile up to 20% decreases the retention of analytes and it starts to increase again at higher water content. This phenomenon was not observed when water was added to methanol and the retention increased gradually with increasing content of water in the mobile phase.

PP 38. PHARMACOGNOSTIC STUDY OF SOME LICHEN SPECIES OF AZERBAIJAN.

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Flora of Azerbaijan is reach in lower plants that represented by more than 600 species. [1]

The chemical composition of lichens attracted our interest because of the small amount of the previous research on them, the diversity of lichen species, the species living in extremal conditions and the lack of information on pharmacological properties of lichen acids. [2, 3, 4] Our research covered six most wide spread lichen species of Azerbaijan.

As a result of our study the following compounds were isolated and identificated: parietin and chrisophanol from *Xanthoria parietina*, atranorin and evernic acid from *Evernia prunastri*, physodic and lecanoric acids from *Parmelia caperata*, usnic acid from *Usnea comosa*.We developed the method of isolation of the total of anthraquinones (xantoparin) from the thallus of *X.parietina*. This total contains 5 *compounds with the major parietin and chrisophanol.* The diagnostic anatomical features of the thallus of *X.parietina* were established and the method of its provision was proposed. The maximum of anthraquinones accumulation in the thallus which varies between 0.53 – 0.56%, is reached at the period from April to December. It has been found that a yearly provision of the air-dried *Xanthoria* thallus in Azerbaijan could reach the amount of 15t.The Parisol foam spray containing 0.1% of the total of anthraquinones was prepared [5]. The Parisol has shown to be effective wound healing drug with the effect superior to those of the Buckthorn oil and methyl uracil and can be used in the treatment of skin radial injuries. Parisol is recommended fot the topcal use on the skin and mucous in trophic ulcers, bullous dermatosis and does not cause any side effects.

The new method of isolation (patent) of usnic acid was developed and tested on practice. This method allows increasing the yield and improving the purity of usnic acid [6]. The analytical documentation for the drug, substance (xantoparin) and foam spray Parisol was developed. At the present moment we continue the research on derivatives of compounds isolated from different species of lichen.

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PP 39. EVALUATION OF NOVEL CORE-SHELL POLYSACCHARIDE-BASED CHIRAL COLUMNS FOR SEPARATION OF ENANTIOMERS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Chromatographic adsorbents based on superficially porous (also referred to as fused-core or core-shell) silica are rapidly establishing themselves as the most promising media for achiral separations in high-performance liquid chromatography (HPLC). Some of their advantages are faster mass transfer between the mobile and stationary phase and more uniform particle size distribution leading to more homogeneous packed beds (resulting in higher plate numbers), as well as enabling faster separations without significant loss in column performance. Although a few studies have been published on the use of core-shell silica particles for enantioseparations in HPLC, their potential as supports for the preparation of chiral stationary phases (CSP) has not been adequately studied yet [1-3]. The use of core-shell silica-based stationary phases has been successfully demonstrated in various separation modes (RP, HILIC, etc.) and for both small and large molecules. While fast mass-transfer is expected to have a more significant impact in the analysis of large molecules (e.g. proteins), in practice core-shell silica is used extensively for the analysis of small molecules (such as pharmaceuticals, environmental pollutants, etc.). This success in the analysis of low relative molecular mass species has been attributed primarily to a significant decrease in the value equivalent to a theoretical plate (HETP) brought about by limited eddy dispersion and molecular diffusion contributions for columns made with core-shell particles. In this study the separation performance of chiral stationary phases (CSPs) made of polysaccharide-based chiral selectors coated onto superficially porous (core-shell or fused-core) silica supports were evaluated. High column performance reaching 200 000 plates per meter was observed.

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PP 40. LOOKING FOR HIGHEST POSSIBLE SELECTIVITY OF ENANTIOSEPARATIONS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Separation selectivity makes the major contribution to the peak resolution in high-performance liquid chromatography [1, 2]. Therefore, it is preferable to operate with selectivity in order to improve the final result of chromatographic separation. At the same time improving enantioselectivity means to interfere with the thermodynamics of the separation process that is very challenging approach. In order to achieve the highest enantioselectivity one should optimize the structure of substance, chiral selector, mobile phase and the separation temperature. In the present study 2-(Benzylsulfinyl) N-methyl benzamide, 3-(Benzylsulfinyl) N-methyl benzamide, 4-(Benzylsulfinyl) N-methyl benzamide, 2-(4-nitrobenzylsulfinyl) N-methyl benzamide, 2-(4-trifluoromethylbenzylsulfinyl) N-methyl benzamide, 3-(Benzylsulfinyl) benzamide and 2-(Benzylsulfinyl) benzamide were used as chiral analytes. 4 cellulose-based chiral selectors, in particular, cellulose tris(3,4-dimethylphenylcarbamate), cellulose tris(3,4-dichlorophenylcarbamate), cellulose tris(3-chloro-4-methylphenylcarbamate) and cellulose tris(4-chloro-3-methylphenylcarbamate) were applied. As the mobile phase 2-propanol and the mixture of n-Hexane and 2-propanol were used. The separations were performed at 25^0C, 15^0C and 5^0C.

The highest enantioselectivity ever reported in chiral HPLC (α=780) was obtained under following optimized conditions: Chiral analyte - 2-(benzylsulphinyl) benzamide, chiral selector - cellulose tris(4-chloro-3-methylphenylcarbamate), mobile phase – n-hexane/2-propanol-70/30 volume by volume and the separation temperature was 5ºC.

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PP 41. LIPID PROFILE OF SOME GEORGIAN CULTIVAR SPECIES

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The aim of present study was the investigation of seed oil from several Georgian cultivar species: *Physalis alkenge* var. Franshetii, *Cucurbita maxima* Duch., *Medicago sativa*, *Amaranthus retroflexus* L, and *Chelidonium majus* L. for their fatty acid composition and phospholipid content in order to reveal the prospective raw for food additives and cosmetic preparations.

Identification of free fatty acids was carried out by HPLC analysis on the apparatus PTG-1 with the refractive detector R-401 and μ-bondapak C_{18} reverse phase column. Eluent 1 – methanol-water (1:2); eluent 2 – tetrahydrofuran-acetonitrile-water (5:7:9) + 0.1% acetic acid solution. The results were processed using the "OASIS-74" software. Quantitative determination of phospholipids in polar lipids was determined by spectrophometric assay by as described in [1].

Variations in seed oil content are shown in Tab.1.

Table 1. Oil content in the seeds of some Georgian cultivars.

Plant species	*Physalis alkenge* var. Fr	*Cucurbita maxima* Duch	*Medicago sativa*	*Amaranthus retroflexus* L	*Chelidonium majus* L.
Oil content (%)	10	20	12	6,7	5,5

The predominant fatty acids present were linoleic, oleic, palmitic, and stearic. Significant differences were observed among the cultivars for myristic, palmitic, stearic, oleic, and linoleic acid content of oil. Low lauric and arachidic acid levels were observed (Fig.1).

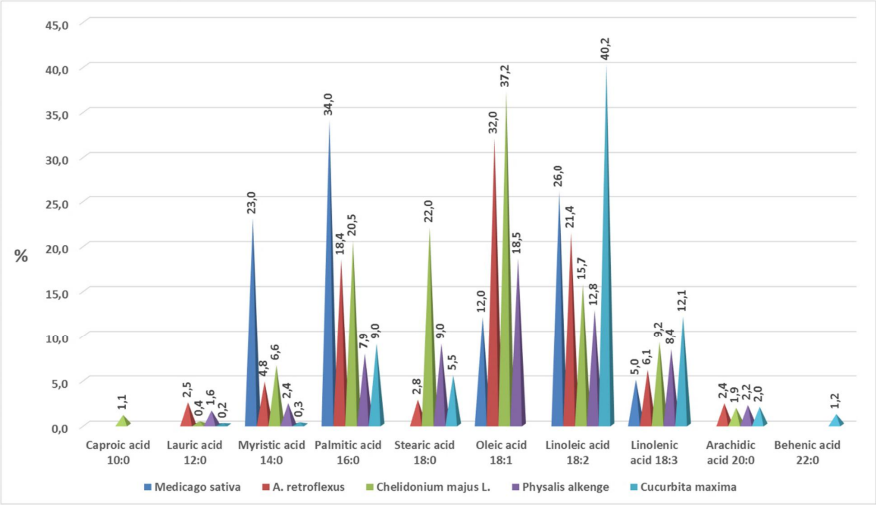

Fig.1. Fatty acid composition of Georgian cultivar plants.

Seven phospholipids: lisophosphatidylcholine, phosphatidylinositol, phosphatilcholine, phosphatidylethanolamine, N-acilisophosphatidylethanolamine, N-acylphosphatidylethanolamine and one unidentified compound were isolated and identified from the polar fractions of *Medicago sativa and Amaranthus retroflexus* L. (Fig.2).

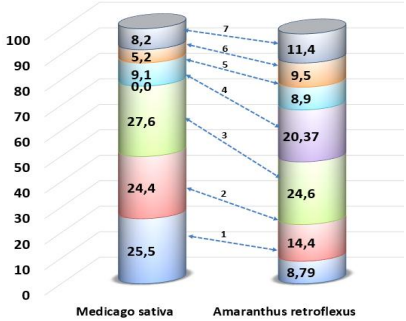

Fig.2. Phospholipid composition : 1-Lysophosphatidylcholine; 2-Phosphatidylinositol; 3-Phosphatidylcholine; 4-Phosphatidylethanolamine; 5-N-acyllysophosphatidylethanolamine; 6-N-acylphosphatidylethanolamine; 7-Unidentified phospholipid

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PP 42. ALKALOIDS OF SOME PLANTS OF HELLEBORACEAE AND RANUNCULACEAE FAMILIES GROWING IN GEORGIA

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Recently, different species of Helleboraceae and Ranunculaceae growing in Georgia: Aconitella Spach., Aconitum L., Caltha L., Consolida (DC) S.F. Gray, Delfinium L., Helleborus L., Clematis L., Thalictrum L., Pulsatilla Hill., Ranunculus L. have been studied for the alkaloid composition. It was determined, that in Helleboraceae species: Aconitella hohenackeri (Boiss.) Sojak, Aconitum nasutum Fish. ex Reichenb., A.orientale Mill., Caltha polipetala Hochst., Consolida orientalis (J. Gray) Schording., C. divaricata (Ledeb) Schroding, Delphinium elisabethae N. Bush, D.speciosum Bieb., D.thamarae Kem.-Nath, Helleborus caucasicus A.Br. and in Ranunculaceae species: Clematis orientalis L., C.vitalba L., Thalictrum alpinium L., T.collinum Wallr., T.buschianum Kem-Nath., T.foetidum L., T.triternatum Rupr., Pulsatilla georgica Rupr., and Ranunculus sceleratus L. alkaloid composition in the overground and aerial parts varies from 0.013% to 1.38% and from 0.17% to 3.5%, correspondingly.

The aim of present investigation was the determination of alkaloid composition in the underground parts of Georgian endemic species Delphinium elisabethae and D. thamarae.

On the basis of alkaloids accumulation dynamics in different vegetation phases, it was suggested that the most reasonable period for harvesting of overground parts of D. elisabethae N. Bush and D. thamarae Kem. is the beginning of vegetation and budding phase, whereas the underground parts should be collected during frutification phase (Tab.1).

Table 1. Dynamics of accumulation of alkaloids in air-dry raw (%)

Phase of vegetation	*Delphinium elisabethae*			*Delphinium thamarae*		
	overground parts	underground parts	seeds	overground parts	underground parts	seeds
Beginning of vegetation	1.37	1,70	-	1,45	1,72	-
Budding	1,83	1,88	-	1,60	1,81	-
Frutification	0,31	1,99	1,96	0,22	2,12	1,81

Alkaloids methyllicaconitine, licoctonine, anthranoyllicoctonine were isolated and identified from *D. elisabethae* and *D. thamarae*.

A base ($C_{20}H_{25}NO_3$) soluble in chloroform and acetone with m.p. 285-287 ^{0}C (acetone) was isolated from the underground parts of *D. thamarae* and named A4. IR spectra (μ, cm^{-1}): 3530-3600 (H and OH group), 1660 (C=C), 750-770 cm^{-1} (extra methyl group). In ^{1}H-NMR ($CDCl_3$, m.d.) the signal of tertiary C-methyl group resonating at 1.12 ppm (3H,s) is detected.

Based on spectral data it is established that alkaloid A_4 contains perhydrophenantren skeleton and belongs to the songorin type diterpenoidal compounds. Comparing its physical, physical-chemical and spectral characteristics with literary data we concluded that A4, discovered in underground parts for the first time, is identical to norzongoramine, which was previously isolated from overground parts of *D. thamarae*.

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PP 43. THE STUDY OF SELF-MEDICATION FOR OCCUPATIONAL DISEASES BY THE RAILWAY STAFF IN GEORGIA

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Georgian Railway, being an integral part of the Silk Road, is the important part of the transport system. Among the current grand projects in the railway system , an importance of health care of the railway staff members is prioritized. The study presents the results of the examination of the medical records of the railway staff. It also identifies the major diseases and medications for treating them. In addition, a survey for the assessment of self-medication risks among railway staff has been conducted.

As it is widely known, self-medication significantly increases within healthcare system. More and more patients independently take decision about management of some illnesses. Despite of minor advantages, self-medication includes many risks, for example: misdiagnosis, use of excessive drug dosage, prolonged duration of use, drug interactions and polypharmacy [1].

Thus, it is a vitally important to clarify better for the patients the nature of potential risks of self medication as well as to encourage them to collaborate more with physicians and pharmacists. Number of the studies conducted in different countries have revealed that the analgesics, anti-inflammatory drugs and antibiotics are commonly used drugs during the self-medication [2-4]. The same can be said on self-meditation practice of in Georgia.

The study was conducted from October 2014 to March 2015. 200 persons, the employees of railway have been interviewed and their medical records studied. It should be also mentioned that the staff members have been selected randomly. The age of the respondents varied from 18 to 45.

After the careful study of the obtained responses as well as medical records of railway staff members (the males- 93%, female – 7%.) 35 % of the respondents had no significant pathology The most common diagnosis were: 14% hypertension, acute bronchitis - 13%, 13% acute respiratory infection , different types of injuries -9.5%, 8% of the urinary system disease, will - sciatica -8% , 7% a nervous condition, cholecystitis - 4%. In the medical records, the medication was sufficiently prescribed in line with the diseases. Respectively, the therapeutic groups and their percentage was adequate to the diagnosis. However, the interviews revealed that instead of the prescribed medicines the patients preferred the self. According to the answers of the respondents frequently used medicines were NSAIDs - 40%, hypotensive drugs - 25% , antibiotics- 20%, and 15% - other medicines.

Medical cards and survey analysis results shows that the drug use in railway employees of Georgia is quite irrational. There were revealed major diseases, medicines subscribed by physicians and medicines used for self-medication.

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PP 44. MINERAL RESOURCES (PELOIDS AND CLAYS) OF ADJARA REGION

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Peloids and clays are used for treatment and prevention purposes since the ancient times /1/. The works of the Georgian scientists are dedicated to the use of clays in medical practice /2/. As for the history of the treatment with peloid (mud) in Georgia, it is directly associated with Akhtala healing mud, the first information of which can be found in 18th century, in the geographical description of Vakhushti Batonishvili.

Adjara is one of the regions of Georgia, rich in clays and peloids, which can be found on the seacoasts, as well as in the mountains /3,4/. All these resources were known to the local population since the ancient times and were used for the treatment of various diseases. The study of the mineral resources of Adjara region will promote the development of the resort and tourist activities of the region. The aim of our research was to study the physical properties and to determine the chemical composition of Sphagnum peat peloid (Ispani); to study the antibacterial properties of clays and sulphide silt peloids.

The objects were the samples taken on the territory of Adjara: the peat peloid of different ages of Ispani, Chakhati and Kvirike sulphide silt peloids, Beshumi and Chirukhi clays.

The research methods applied were: the instrumental methods – for studying the physical properties and the chemical composition of Sphagnum peat peloid of Ispani; the Disc Diffusion Method (DDM) and Agar Well Diffusion method – for the antibacterial activity evaluation of sulphide silt peloids and clays.

As a result of the conducted studies there have been determined the physical properties and the chemical composition of micro- and macro elements of Sphagnum peat peloid of Ispani. The essential micro and macro elements for the human body have been established: magnesium, silicon, calcium, iron, etc., which represent the biologically valuable components.

There have been revealed, that Chakhati and Kvirike sulphide silt peloids have antibacterial effect predominantly on gram-negative bacteria (Escherichia coli and Proteus species), the influence on the growth of gram-positive bacteria (Staphylococcus aureus) was relatively weak. There has been also noted the certain inhibitory effect of Beshumi clay on the growth of gram-positive bacteria (Staphylococcus aureus).

Based on the conducted studies, there has been established, that the study objects represent perspective substances for further research in order to develop the curative and preventive products.

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PP 45. HPLC SEPARATION OF ENANTIOMERS OF SOME CHIRAL CARBOXYLIC ACID DERIVATIVES USING POLYSACCHARIDE-BASED CHIRAL COLUMNS AND POLAR ORGANIC MOBILE PHASES

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In the present study the separation of enantiomers of chiral carboxylic acid derivatives was attempted on different polysaccharide-based chiral HPLC columns with polar organic eluents. Along with the successful separation of analyte enantiomers the emphasis of this study was on differences in enantiomer elution order between various columns and mobile phases. In addition, the effect of column temperature on the retention and separation of enantiomers was studied [1].

The separation of enantiomers of 14 chiral carboxylic acid derivatives was studied on 6 different polysaccharide-based chiral columns in high-performance liquid chromatography with methanol, ethanol and acetonitrile as mobile phases with emphasis on the elution order of enantiomers.

Some interesting examples of enantiomer elution order reversal were observed function of the nature and composition of chiral selector and mobile phase. For instance, the enantiomer elution order for carprofen, ketorolac, naproxen, proglumide and surprofen reversed with changing the chemical structure of the chiral selector. Also, the enantiomer elution order for carprofen, ketorolac and naproxen changed by varying the composition of the mobile phase. In addition, the interesting effect of column temperature on the retention and separation of some analytes was observed. For instance, the enantiomers of surprofen were only partially resolved at lower temperatures but baseline resolved at higher temperature.

As this study illustrates, the affinity of enantiomers of chiral carboxylic acid derivatives towards polysaccharide-based chiral stationary phases is dependent on the nature of the chiral selector and on the mobile phase composition. Sometimes only subtle changes in the structure of a chiral selector or mobile phase composition may lead to a reversal in the affinity for a particular pair of enantiomers. Temperature can be considered as a very useful parameter for improving the separation. Further studies of these phenomena may provide useful information for understanding the chiral recognition mechanisms with polysaccharide-based chiral stationary phases.

This study was financially supported in part by the Rustaveli Georgian National Science Foundation (RGNSF) grant No 31/90 for fundamental research.

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PP 46. SYNTHESIS OF SOME 2,3-DIHYDROXY-3-(3,4-DIHYDROXYPHENYL)-PROPIONIC ACID DERIVATIVES

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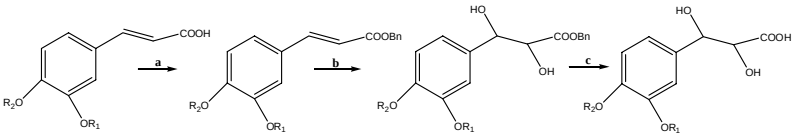
Recently we have reported about the synthesis and antioxidant and anticancer activity of both racemic and pure enantiomeric forms of 2,3-dihydroxy-3-(3,4-dihydroxyphenyl)-propionic acid [1,2]. Novel racemic glyceric acid derivative (10) and its pure enantiomers exhibited expressed antioxidant activity against reactive oxygen species, such as hypochlorite or DPPH. The inhibitory effect of racemic product (10) and corresponding enantiomers appeared three-fold higher than that of trans-caffeic acid.

In order to study the effects of substituents on the antioxidant activity of phenolic compounds we have synthesized some of 2,3-dihydroxy-3-(3,4-dihydroxyphenyl)-propionic acid derivatives (11-12) on the basis of caffeic (1), isoferulic (2) and ferulic (3) acids (Scheme 1).



1-3 **4-6** **7-9** **10-12**

1 R_1= H R_2=H
2 R_1= H R_2=CH_3
3 R_1=CH_3 R_2=H

4 R_1= Bn R_2=Bn
5 R_1= Bn R_2=CH_3
6 R_1=CH_3 R_2=Bn

7 R_1= Bn R_2=Bn
8 R_1= Bn R_2=CH_3
9 R_1=CH_3 R_2=Bn

10 R_1= H R_2=H
11 R_1= H R_2=CH_3
12 R_1=CH_3 R_2=H

a) BnBr, K_2CO_3, $(CH_3)_2CO$ **b)** K_2OsO_4x $2H_2O$, NMO, $CH_3CN/(CH_3)_2CO/ H_2O$ (3:3:1) **c)** Pd/C, THF/EtOH

Protection of starting acids (1-3) was performed with benzylbromide in acetone. The protected acids (4-6) were dihydroxylated according to Sharpless dihydroxylation procedure using a K_2OsO_4 and N-methylmorpholine oxide in acidic area [1]. Removing of protected groups from compounds (7-9) by catalytic hydrogenation on Pd/C gave desired dihydroxylated acids (10-12). The structures of synthesized compounds (4-12) were established on the basis of NMR and IR spectroscopy data.

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PP 47. THE DEVELOPMENT OF OINTMENT WITH COMBINED EFFECT FOR TREATMENT OF FESTERING WOUNDS

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New scientific approaches in pharmaceutical technology along with the necessity of creating ointments with combined action, that ensures drug efficacy and increases its multilateral effect on main symptoms of the disease, emerged during elaboration of new preparations.

Accomplishment of complex action (affecting both I and II phase) in wound healing is pretty actual nowadays, as along with high specific (antibacterial, anti-inflammatory, healing) effect allows to achive pain-relieving, styptic effect and in case of long-term use avoid irritation.

Based on the above and considering the process and phases of the wound healing and mechanism of action of preparations, existing on the market, the Department of Pharmaceutical Technology of Tbilisi State Medical University developed an ointment consisting of chloramphenicol, sulfadimethoxine, aloe dry extract, nettle and calendula thick extract, water, PEO-400, PEO-1500, and propylene glycol. Selection of ointment ingredients was stipulated by their pharmacological action and compatibility with each other.

Flow characteristics of the ointment, absorption activity of the base (by methylene blue absorption capacity), activity of the base (by weight method), dynamics of release of active substances from the ointment (by diffusion method on agar plate), quantitative determination of chloramphenicol and sulfadimethoxine (by dialysis method) were studied.

When selecting the base, it was considered that ointments for treatment of septic wounds should have certain “similarity” to wound tissues, good moisturizing properties, ability to distribute ingredients (including channels and cavities of the wound) evenly, and simultaneously perform wound cleansing.

Usage of propylene glycol gel in ointments is revolutionary in established methods of treatment of septic wounds, which is preconditioned by dehydrating action of polyethylene oxide on tissues, that approximately 20 fold exceeds that of sodium chloride. However, negative side of polyethylene oxides is extreme desiccation of the tissue, suppression of granulation and creation of the so-called “crust”, which hampers healing process. Propylene glycol used in the basis due to fast penetration creates a balance between the basis of the ointment and the tissue and activates the process of granulation.

The developed ointment differs from ones existing on pharmaceutical market by combination of natural and synthesized ingredients, which increases its efficacy and in case of long-term use ensures its safety.

PP 48. ENANTIOSEPARATION OF SOME CHIRAL BETA-BLOCKER DRUGS BY USING POLYSACCHARIDE-BASED CHIRAL STATIONARY PHASES IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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In the present work separation of enantiomers of some chiral beta-blocker drugs on polysaccharide-based chiral stationary phases was studied by using various mobile phases. Beta – blockers represent chiral compounds with basic nature, which are widely used in the medical practice to cure cardiac insufficiency. In many cases only one enantiomer of chiral b-blocker drug is responsible for its desired pharmacological effect, while the other one may be inactive, exhibit different activity or be even toxic. Therefore, enantiomeric purity determination of chiral beta-blocker drugs is very important.

Separation of the enantiomers of 28 chiral beta – blockers was studied on polysaccharide-based chiral stationary phases by hexane - isopropanol eluents with different ratio in the presence of additive of diethylamine. The emphasis was placed on some interesting examples of enantiomer eluent order reversal observed depending on the chemistry of the chiral selector, separation temperature, major component, as well as the minor additive to the mobile phase. The reversal of elution order of enantiomers was observed in some cases. For instance, the elution order of sotalol was opposite on Cellulose-1 and Amylose-2 columns. The similar result was observed for penbutalol and timolol on Cellulose-1 and Cellulose-2 columns, which differ from each other by the nature and location of substituents. The change of composition of the mobile phase also resulted in the reversal of the elution order. The elution order of bupranolol reverted by decreasing the content of 2-propanol in the mobile phase.

PP 49. ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF HERBAL FORMULATION PS-551

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Species of genus Fatsia have been used as herbal medicines in Japan and in Taiwan in treating various diseases, such as coughing, ankylosing spondylitis, osteoarthritis, rheumatism, rheumatoid arthritis and tendinitis, and in blood circulation improvement [1, 2].

The analgesic and anti-inflammatory effects of PS-551, *developed on the basis of* extract of the *Fatsia japonica* leaves and its finished herbal formulation (1% solution for injections) were investigated in various experimental models in mice and rats respectively. Analgesic activity in mice was evaluated using tail-flick model. Anti-inflammatory activity in rats was studied using formalin induced rat hind paw edema model. Statistical analysis was performed using Student's T-test. $P < 0.05$ was considered statistically significant.

Hot plate test

24 h prior the experiment animals were placed on a hot plate maintained at a temperature of 50 ± 0.5ºC. The latency to jump from the hot plate was noted as basal reaction time. The cut off time was considered as 30 s [3]. The next day mice were randomized in groups of five each and treated with saline (control), PS-551 substance (50 mg/kg, i.p.) and finished form (1 ml i.p.). The reaction time was noted at 0, 15, 30, 45 and 60 min after the treatment. Both preparations significantly ($P < 0.05$) elevated the reaction time as compared to control group, but PS-551 substance exhibited the highest nociception inhibition at 30 min (Fig.1).

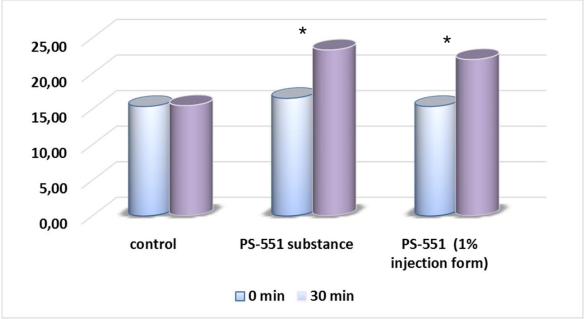

Fig.1. Analgesic effect of PS-551. Y-axis – reaction time. * - $P < 0.05$

Formalin induced rat paw edema

Rats in groups of six each were treated with PS-551 substance (50, mg/kg, i.p.) and PS-551 (1 ml) finished form one hour prior to formalin injection. 100 µl of 2.5% w/v solution of formalin was injected into the subplantar tissue of left hind paw of each rat. The right hind paw was injected with 0.1 ml of saline. Swelling of formalin-injected paw was measured at 0, 24 and 48 h using digital micrometer. The % decrease in paw volume was calculated as described in [4]. The PS-551 as well as its finished form significantly ($P<0.05$) inhibited the development of formalin induced rat paw edema.

PS-551 substance (50 mg/kg) at 24 h showed maximal inhibition of paw edema when compared to the control group. The dynamics of edema decrease is shown in Fig 2.

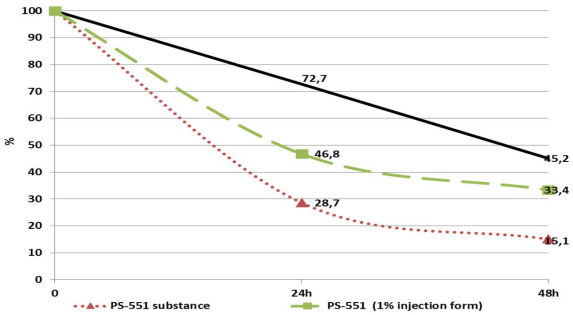

Fig.2. Anti-exudative effect of PS-551. X-axis – time, Y-axis – change in paw volume (% from the initial)

The hot plate method is suitable for the evaluation of centrally but not peripherally acting analgesics and the involvement of endogenous substances such as PGs in this model is minimized. On the other hand, formalin induced edema originates from acute inflammation accompanied by participation of kinins and leukocytes with their pro-inflammatory factors including PGs. The observed analgesic and antiinflammatory effect of PS-551 and its finished formulation suggests the action via central inhibitory mechanism as well as inhibition of prostaglandin synthesis. Thus, PS-551 may appear beneficial for the management of pain and inflammatory disorders.

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PP 50. STUDY ON SECONDARY METABOLITE CONTENT OF *HELLEBORUS CAUCASICUS* FLOWERS

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Helleborus caucasicus is an evergreen, perennial flowering plant from Ranunculaceae family. There are very few reports on the chemical composition of *Helleborus* flowers. So far only quercetin 7-glucoside-3-(caffeoyl) sophoroside was isolated from *Helleborus foetidus* petals [1]; recently cyanidin glycosides and flavonoids have been identified and quantified in sepals of *Helleborus niger* [2].

The leaves of *Helleborus caucasicus* along with the furostanol glycosides, bufadienolides and ecdysterone [3] turned out to be a rich source of natural polyphenols co-occurring with gama-pyron derivative and sterol. Flavonoid glycosides from the leaves have revealed pronounced antioxidant activity. This circumstance prompted us to investigate *H. caucasicus* flowers due to their long lasting flowering period.

This is the first report on the secondary metabolite content of *Helleborus caucasicus* flowers.

Plant material was extracted with 80% MeOH once at room temperature and two times for 30 min. at 45°C. Extracts were pooled and concentrated under reduced pressure. Crude residue was dissolved in water and successively portioned with ethylacetate/*n*-BuOH. The obtained fractions further were separated by preparative column chromatography (10 mm × 200 mm, LiChroprep Si 60, 25-40 µm; Merck), using solvent system chloroform/methanol/water (26:14:3). This approach led to the isolation of 12 individual compounds belonging to the phenolic and steroid class. The structures of isolated compounds were established by extensive 1D and 2D NMR spectroscopy, and MS analyses.

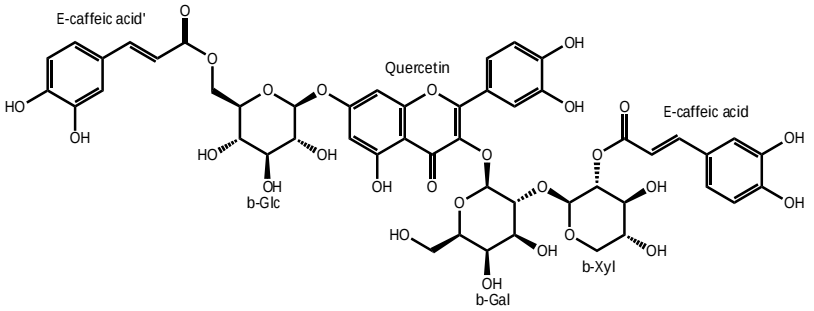

Fig.1. Chemical structure of novel flavonoide: [Quercetin 3-O-(2-E-caffeoyl)-β-Xyl-(1→2)-β-Gal 7-O-(6-E-caffeoyl)-β-Glc]

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PP 51. CHEMICAL COMPOSITION OF *PERIPLOCA GRAECA* L.

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Periploca graeca is an ornamental plant in the Asclepiadaceae family native to Southern Europe and the Middle East. It is wide-spread in West Georgia and in Khartli region.

In the Institute of Pharmacochemistry of the Georgian Academy of Sciences an original method of obtaining periplocin from the bark of *Periploca graeca* of the Georgian flora was elaborated and periplocin in two medicinal forms, in ampoules and tinctures was worked out. The both preparations were produced by the Georgian pharmaceutical industry. Periplocin as an official cardiac remedy was included in IXth edition of the State Pharmacopoiea [1,2].

Continuing studies on *Periploca*, the barks were further researched for their chemical composition.

Plant material was threefold extracted with 70% MeOH, once at room temperature and two times at 45°C. The collected alcohol-aqueous extract was concentrated and successively partitioned between $CHCl_3$-MeOH (1:2; 2:3), ethylacetate and n-BuOH. The obtained fractions were subjected to the column chromatography on Sephadex LH-20. Subfractions further were separated by RP-HPLC (Waters XTerra Prep MSC18 column, 300x7.8 mm i.d.) at flow rate 2.0 ml/min using different mixtures of MeOH:H_2O in isocratic conditions. This approach led to the isolation of a cardenolide aglicone and its glycoside, along with other metabolites belonging to the class of phenolic, triterpenic and phenolic acid glycosides. The structures of individual compounds were established by 1D and 2D NMR spectroscopy.

Acknowledgements: The work was supported by the Shota Rustaveli National Science Foundation (Contract No. YS/62/8-404/13). The authors declare no competing financial interest.

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PP 52. 5α-PREGNENOLONE OXIMES CHEMICAL MODIFICATION WITH N-PROTECTED AMINO ACIDS

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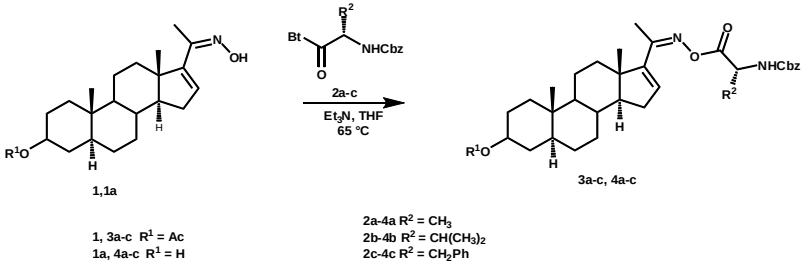
The importance of amino acids, peptides and steroids to life is well known. Combinations between amino acids and steroids give another class of compounds that might prove interesting. Steroidal oximes have been selectively acylated with Ac_2O in the presence of alcohols [1] or by the use of coupling agents [2]. However, there are relatively few literature examples of reactions of steroidal oximes with protected amino acids.

Our main purpose was to prepare a series of peptide derivatives of oximes of 5α-pregnenolone using N-protected amino acids and to investigate their physical, chemical and spectral properties.

The *O*-acylation of biologically active 3β-acetoxy- 1 and of 3β-hydroxy-20-hydroximino-5α-pregnenolones 1a [3] was achieved by coupling with *N*-protected (α-aminoacyl)benzotriazoles 2a–c in THF at 65°C in the presence of Et_3N to afford the desired products 3a-c and 4a-c in yields of 44 – 57% after recrystallization. In the case of compound 1a the acylations proceeded chemoselectively towards the oxime in the presence of the free hydroxy group.



To conclude, N-acylbenztriazoles of protected amino acids have been utilised in the successful acylation of steroidal oximes.The structures of synthesized compounds 3a-c, 4a-c were established by ^1H, ^{13}C NMR spectral data and elemental analysis.

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PP 53. SOME OF THE PHYSICO-CHEMICAL PROPERTIES OF BROMELAIN

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Bromelain, a mixture of enzymes from pineapple (*Ananas comosus*) fruits or stems, contains a number of cysteine proteinases and biologically active agents (vitamin C, organic acids, sugars, pectins, polysaccharides etc.) [1].

Bromelain is considered very promising for the treatment of inflammation and cancer due to fibrinolytic, antithrombotic, antiedematous,and anti-inflammatory activities. It also contributes to antibiotic penetration to tissues and promotes tumor cell apoptosis [2-5]. In our opinion,the demand for bromelain-containing preparations for their application as not only a food additive but also a therapeutic drugs, especially in surgery and oncology,will increase rapidly in nearest future.

Current study represents the data obtained during the standardization of bromelain using the method adopted for Caripazym - enzyme complex from dried latex of papaya fruit, developed at the Laboratory of Medicinal Enzymology of TSMU Institute of Pharmacochemistry [6]. Optimal pH values were established in the enzymatic hydrolysis of casein assay, as well as the influence of concentration of cysteine and bromelain and reaction time on the rate of hydrolysis.

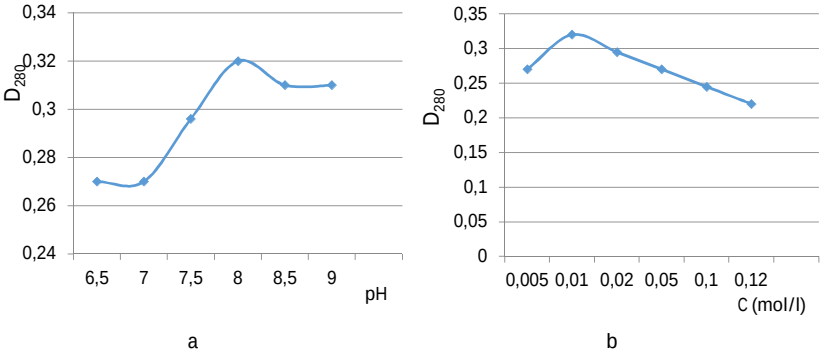

Fig. 1. Effect of pH (a) and cysteine (b) on the proteolytic activity of bromelain.

It was found that notable increase of bromelain activity begins from the pH 7, reaches its maximum at pH 8, then decreases slightly and remained unchanged till pH 9. The optimal concentration of cysteine was 0.01 mol / l (with the substrate in the reaction area - 0.004 mol / l). A further increase in the concentration causes a marked decrease in the rate of lysis.

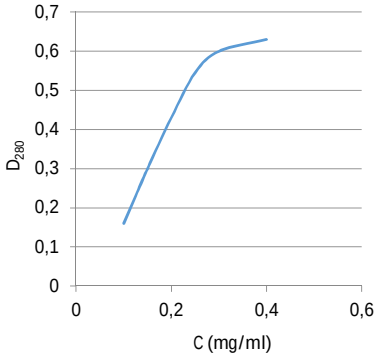

Fig. 2. The dependence of rate of lysis of casein on the concentration of bromelain.

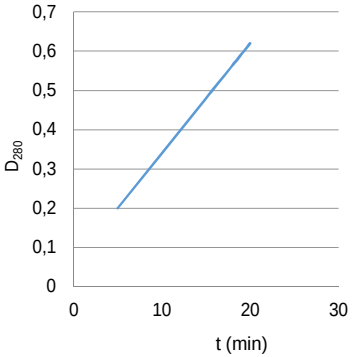

Fig. 3.The rate of casein lysis

In the reaction area at a concentration of the substrate (casein) 0.75% the rate of lysis increases linearly within D_{280} 0,2-0,5.

The rate of lysis of casein (1% solution in the reaction area) remains linear in a studied time interval from 5 to 20 minutes.

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PP 54. FLAVONOID GLYCOSIDES FROM LEAVS OF *TRIBULUS TERRESTRIS*

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Tribulus terrestris L. – ground burnut, Fam. Zygophyllaceae is a widespread plain annual plant with creeping radiating stems. The ground burnut has been used in oriental folk medicine for treatment of various diseases from time immemorial.

Preparation *Tribusponin* for treatment and prevention of atherosclerosis and a non-hormonal anabolic agent was developed on the basis of steroid glycosides of *T. terrestris*, at I.Kutateladze Institute of Pharmacochemistry of the Georgian Academy of Sciences [2]. Pharmacological tests [3] and clinical trials showed that *Tribusponin* promotes a decrease in the blood cholesterol content and an increase in the lecithin cholesterol factor, reduces the index of phospholipids and lipoproteids, decreases lipopexia in the aorta, myocardium and liver, controls blood tension, and exhibits vasodilatative and anticoagulant properties. Another preparation called *Atherosponin* with the action similar to *Tribusponin* is recommended as a biologically active food additive.

Tribulus terrestris is well known with the content of flavonoids. There is found that the flavonoid constituents of *Tribulus terrestris* are quercetin, kaempferol and isorhamnetin, with quercetin as a dominant one [4,5].

Phytochemical investigation of *Tribulus terrestris* leaves allowed us to isolate three flavonoid glycosides: quercetin 3-O-α-L-rhamnopyranosyl-(1→6)-O-β-D-glucopyranoside, quercetin 3-O-α-L-rhamnopyranosyl-(1→6)-O-β-D-galactopyranoside, and quercetin 3-O-α-L-rhamnopyranosyl-(1→6)-O-β-D-glucopyranosyl 7-O-β-D-glucopyranoside. The latter was never reported in literature before.

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PP 55. PHARMACEUTICAL CARE AS A PART OF PHARMACY PRACTICE

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The recent years' concept of patient-oriented health care system considers the development of pharmaceutical services and their implementation into the system along with the optimization of financial expenses, reduction of drug side effects, and enhanced quality of life for both patient and the entire society. Correspondingly, the pharmacists' responsibility for patients increases. The notion "pharmaceutical care" suggests the qualified assistance for the patient, that is associated not only with advisory functions, when a selection of nonprescription drug is needed, but also consulting patient about dosage regimens, possible interactions with other drugs and food, as well as redirecting to the physician, if necessary.

Proper communication between the pharmacist, patient and other health care professionals should be set at the very beginning of treatment, in order to allow a pharmacist to gather, summarize and analyze information. When assisting a patient, the pharmacist should take into account all the factors about both the patient and possible side effects of the drug for the minimization of further treatment-related problems. At present, pharmacists are trained to obtain appropriate skills required for giving individual patient-specific drug recommendations, consulting about effective and safe drug usage, and warning about self-treatment risks.

Due to the above mentioned, we aimed to evaluate how the "pharmaceutical care" is performed in Tbilisi pharmacies, what kind of problems pharmacists are faced to when communicating with patients and physicians, how can the pharmacist fit a leading role in patient-oriented and sustainable public health activities. We performed the analysis of the statistical data from the anonymous questionnaires delivered to 30 pharmacists and 30 physicians. The survey revealed the following information: answering the question: "How often patients ask the pharmacist for advice when choosing nonprescription drugs?" 95% of respondents indicated "almost always". The most common requests were related to the selection of generic drugs.

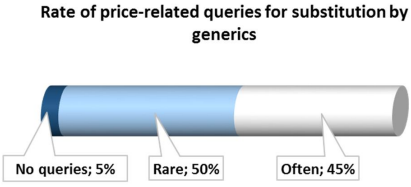

Fig.1.

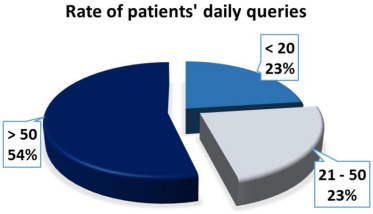

Fig.2.

The survey revealed that the majority of patients' purchases is shifted towards generics due to high prices of brand drugs (Fig.1). Answering the question on how often they are asked for consultation on choice of a drug, 54% of pharmacists responded that, perhaps, in 50-70 cases daily (Fig 2). On a question how often the patients are redirected to the physician, the 50% rate of such cases was detected.

At the same time when doctors were asked to estimate a pharmacist' additional roles, particularly the consultations on selection of nonprescription medicines, 65% of doctors believe that this function belongs exclusive to the physician and only 35% of responders agreed to share this function with the pharmacist. Based on this data, we can conclude that despite some resistance, the pharmacists hold quite favorable position to ensure the effective and safe use of medicines, but they have to take more responsibility for the drug therapy management. Moreover, in order to avoid misunderstanding between physicians and pharmacists, it is necessary to upgrade the qualification of pharmacists in clinical issues.

PP 56. SYSTEM ANALYSIS AND APPLICATION OF PHARMACEUTICAL-INFORMATIONAL PRODUCT

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Pharmaceutical-informational (herein pharmainformational) product or is relatively new term in the relevant field. It can be considered as a derivative of pharmaceutical information, has its specific name, a customer and the executor.

Pharmaceutical Information itself is quite a loose concept that suggests data collection on drug circulation, processing and transmission of its medical and pharmaceutical features in order to create "bank of information" where various regulations, planning and accounting documents, statistical and clinical-epidemiological reports, as well as technical guidelines are accumulated [1]. Pharmainformational product, retrieved from a database, represents a flow of documented information, suitable for primary and secondary (intermediate) use. It is supposed that a customer (student, graduate, resident, researcher, etc.) will operatively receive the product that precisely meets customer's demand. The quality of the information will be guaranteed [1-3].

The goal of the study was to analyze consumer attitude and opinion on current pharmainformational products in order to reveal possible mistakes in pharmacotherapy as well as its irrational cases.

Thus, the objective was a systematic analysis of the obtained results and delineation of related relevant information. Use of this method is convenient, since it considers the broad infrastructure of deskwork as a unity of constituent elements and interlinks between them. Interrelations with the environment is actualized via "Input and output" subsystem that fixes citizens' behavior peculiarities. Thus, the final and basic product represents a generalized model of real situation [4, 5] .

The comprehensive questionnaire consisting of 100 open and closed tests was drawn up. The tests were grouped in 5 sets each of 20. Respondents were chosen from drugstores of Mtskheta-Tbilisi region.

Results and Discussion: delivery of both prescription and over-the-counter drugs it was fixed a new trend related with likelihood errors and dissatisfaction with drug safety, neglect or lack of awareness of the correct usage, loss-loosening of the therapeutic effect, side effects, information on toxicity and contraindications. The majority of respondents considered quality, efficiency, and safety of drug as a measure for prevention of undesirable effects. The appropriate service was associated with honest advertising along with high interprofessional skills of personnel.

The results of aforementioned study pushed us towards the creation of "mini pharmainformational bank" within the department and its further implementation in corresponding syllabi. Certain categories of pharmainformational products such as a drug-alcohol, drug-brand, drug-taking, drug-manufacturer, drug-food, drug-storage, drug-incompatibility are proposed. Arrangement of easy-to-found alphabetical search system (stage I of algorithm elaborating) will contribute to the availability of information on medicines.

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PP 57. PREVENTION OF NSAID- AND STRESS-INDUCED ULCERATION WITH IVY EXTRACT

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Peptic gastric ulcer (PGU) is considered among dominant causes of morbidity and mortality, especially in developing countries and still remains amongst global health problems. Despite undoubtful efficacy of prescription antiulcer drugs such as H2 blockers, M1 blockers, proton pump inhibitors and antacids, the use of medicinal plants is yet quite general in many countries and regions of the planet [1]. Among them the ivies are known as plants possessing anticough, diuretic and bronchospasmolitic activities. Anti-inflammatory, antibacterial, bronchospasmolytic, antimicrobial, antifungal, anti-helminthic, memory improvement, antiprotozoal and contraceptive activities of triterpene saponins from *Hedera* species was reported as well as antiviral and antioxidant activitiy of ivy's crude extract [2,3]. Recently the ulcer preventive efficacy of polar and non-polar extracts from different ivy species against ethanol-induced ulcers was established [4].

The aim of present research was further investigation of anti-ulcer activity of Hedera leaves polar extract (HLPE) using NSAID- and stress-induced ulcer models in rats. Correspondingly, Indometacin- and cold-restraint stress- induced ulcer models were chosen for the experiment [5,6].

In brief, 24 albino rats were randomly distributed in four groups of animals, each consisting of six rats. The animals were maintained in common conditions (12 h light/dark cycle at a constant temperature 22±2 ◦C with free access to feed and water). 48 h prior to the experiment the access to food was restricted, and animals were relocated in cages with raised floors of wide wire mesh to prevent coprophagy. During the fasting period, rats received a nutritive solution of 8% sucrose in 0.2% NaCl to avoid excessive dehydration. On day 3 the ulcers were induced either with indomethacin (30 mg/kg, i.p.) or by placing immobilized animal in cold chamber at 4–6 ◦C for 2 h. HLPE (300 mg/kg i.p.) was administered 1 hour prior the ulcerogenic factor. Animals of corresponding control groups were given 1 ml of saline. Animals were euthanized by CO_2 inhalation 1 hour after the indomethacin injection or cold-restraint sessation. The stomachs were immediately removed, opened along the great curvature, rinsed and digitally photographed. Ulceric lesions were measured using Image-J software and macroscopic ulcer index (MUI) – the sum of the length (mm) of all lesions was calculated for each stomach. The efficacy of HLPE expressed as percentage of ulcer inhibition (%I) was estimated on the basis of the MUI and calculated using the following formula:

$$\%I = \frac{MUI_C - MUI_t}{MUI_t} \times 100$$

where MUIC and MUI_t are macroscopic ulcer indexes in control and test groups, respectively. Statistical analysis was done by one-way ANOVA followed by Student's t-test. p values less than 0.05 were considered significant.

The research protocols of all animal experiments were approved by TSMU Animal Welfare and Use Monitoring Committee and performed in accordance with the principles of [7].

HLPE appeared potent to significantly reduce ulcerogenic effects of both cold-restraint stress and indomethacin in rats, however the gastroprotective effect of HLPE in indomethacin-induced ulcer model was almost twofold higher, than in cold-restraint stress induced model: ulcer inhibition %I was 80% ($P < 0.001$) and 38 % ($P < 0.01$) , respectively (Fig.1 and Fig.2)

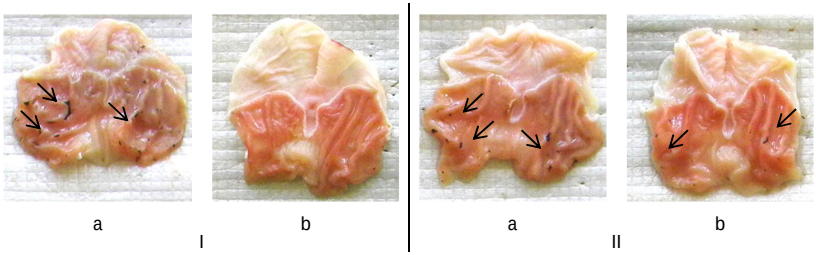

Fig.1. Anti-ulcer effect of HLPE in indomethacin- (I) and cold-restrained stress-induced (II) ulcer model in rats. a – control (untreated), b – test (HLPE 300 mg/kg i.p); → ulcer lesions

Group	MUI
I. Stress	17,65±2,14
II. Stress+ HLPE	12,25±1,72
III. Indomethacin	22,16±2,63
IV.Indomethacin+ HLPE	4,42±0,71

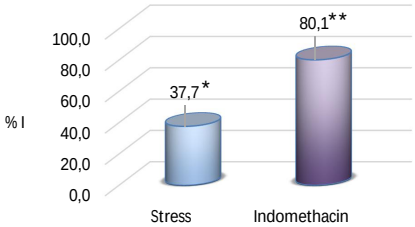

Fig.2. Gastroprotective effect of HLPE in stress- and indomethacin-induced ulcers. Data are represented as mean ± S.E.M (n = 6). *P < 0.01 and **P < 0.001 as compared to corresponding control.

To our knowledge, this study provides first evidence of gastroprotective effect of HLPE against NSAID- and stress-induced ulcers.

The authors gratefully acknowledge financial support from Shota Rustaveli National Science Foundation of Georgia (research grant D-13/17).

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PP 58. SELECTION OF THE OPTIMAL METHOD OF BAS EXTRACTION FROM MARIGOLD FLOWERS

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Rich content of various groups of biologically active substances (BAS) stipulates a broad spectrum of pharmacological action of calendula flowers.

BAS of lipophilic nature are usually extracted with vegetable and mineral oils, non-polar organic solvents and condensed gases, whereas water-alcohol mixtures are used for the extraction of hydrophilic compounds. At the same time after the processing of valuable marigold raw the remains still contain sufficient amounts of BAS [1,2].

Considering the aforesaid, the aim of present work was the development of rational resource-saving technology, which would provide a full transition of the active ingredients of the raw - air-dried calendula flowers drug with carotenoids content (in terms of β-carotene) -722 mg%, flavonoids (in terms of quercetin) - 2.71%, and a residual moisture content of not more than 7%.

The following methods of extraction were compared during the experiment - Soxhlet extraction, extraction with two-phase solvent system, a relatively new method for the extraction with molten suppository base and extraction with liquefied gas [3]. Raw/solvent ratio, extraction time and temperature of the mixture were adjusted experimentally. Determination of carotenoids and flavonoids were determined by differential spectrophotometry. The parameters of linearity, repeatability and accuracy of quantification methods were determined. Total content of carotenoids and flavonoids were considered as criteria of exraction exhaustiveness (Tab).

Extraction method	Solvent	Raw / solvent ratio	Temp (^{0}C)	Time (min)	Yield of carotinoids %	Yield of flavonoids %
Soxhlet extraction	Hexane	1-3	75,0±5,0	48 h	69,44	3,27
2-phase system	Ethanol 70%	1-5	20,0±0,5	45,0	-	78,36
	Vegetable oil	1-5	70,0±0,5	90,0	75,00	12,61
Suppositories' base	Vitepsol W35	1-1	50,0±2,0	120,0	43,65	15,33
	Cacao butter	1-1	40,0±0,5	120,0	29,82	7,58
Condensed gas	Freon 12	1-2	60,0±0,5	120,0	4,23	-

A 2-phase solvent system appeared optimal allowing to reach 75% yield of carotenoids in the oil extracts and 78.36% yield of total flavonoids in hydroalcoholic extract.

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PP 59. EVALUATION OF NOVEL CORE-SHELL POLYSACCHARIDE-BASED CHIRAL COLUMNS FOR SEPARATION OF ENANTIOMERS OF BENZOIN IN HIGH-PERFORMANCE LIQUID CHROMATOGRPAHY

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The dominant trend in liquid chromatography column technology has been the development of progressively more efficient media in order to increase laboratory productivity while maintaining chromatographic performance. To this end, the use of higher efficiency particles has the promise of allowing analysts reduced run times while maintaining, or even improving, chromatographic resolution. Core-shell particles provide an elegant solution to the problem of maximising column performance without generating excessive back-pressure. They consist of an impermeable inner core surrounded by a layer of fully-porous silica and thus are morphologically quite distinct from conventional fully-porous silica particles. It has been widely reported that 2.8 or 3.6 micrometer core-shell particles can provide performance that is on-par or even exceeds that of fully-porous sub-2µm media. The pressure generated by HPLC/UHPLC columns is inversely related to the particle size of the media used to pack the column. Thus, columns packed with sub-3µm coreshell particles are able to achieve sub-2µm efficiencies at operating pressures that are much lower than sub-2µm packed columns, and hence it is possible for chromatographers to achieve levels of performance close to that of sub-2µm packed columns without the need of a UHPLC system. However, although core-shell particles do possess the potential of delivering UHPLC-like performance on conventional HPLC systems, the actual capacity of the end-user to fully realise that potential is highly dependent upon the nature of the HPLC systems that they are utilising. In the present work application of polysaccharide-based chiral stationary phases made by using core-shell silica particles for separation of enantiomers of benzoin are reported in high-performance liquid chromatography. High plate numbers per unit of time, as well as ultrafast enantioseparation in few seconds are reported.

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PP 60. HOMEOPATHIC REMEDIES AT THE GEORGIAN PHARMACEUTICAL MARKET

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The modern pharmaceutical market represents a complex object from a scientific, as well as practical points of view. Of special interest is the formation of pharmacy institutions with a diverse and effective assortment, including homeopathic remedies (HR) - medicines made either from a substance or a mixture of the substances of natural (mineral, vegetable, animal) origin [1,2].

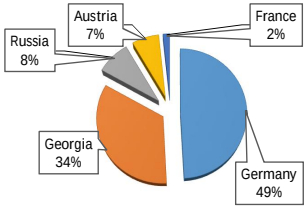
In Germany – the motherland of homeopathy - 80% of the population entrusts their health to homeopathic drugs. The efficacy of homeopathic treatment has been proven through many clinical and laboratory tests and practices, and the development of new HR is under way [2-5].

According to the data submitted by the LEPL State Regulation Agency for Medical Activities, 59 HR are registered on the Georgian pharmaceutical market in different dosage forms [6]. These are: drops 42.37% (25 names); pills 33.9% (20 names); ampoules 13.6% (8 names); powder 5.08% (3 names); capsules, granules, suppositories, ointment, each 3.39% (two names each); sirup, spray, each 1.69% (with one name each). The biggest share falls on drops and pills. 56 HR (95%) belong to over the counter remedies, the rest (5%) are prescription drugs.

Providers of these drugs are: Germany 49.15% (29 names), Georgia 33.9% (20 names), Russia 8.47% (5 names), Austria 6.78% (4 names) and France 1.69% (1 name). The largest share comes on import (66.1%) (Table 1).

Table 1. HR spectra in Georgian Pharmaceutical Market.

Manufacturer	no HR
Sanum Kehlbeck GmbH& Co. KG	1
Weleda AG	9
Biologische Heilmittel Heel Gmbh	15
Bionorica SE	1
Medice Arzneimittel Pütter GmbH & Co. KG	1
Hevert-Arzneimittel GmbH & Co.KG	2
IBERI LTD	11
BIOPHARMI L LTD	7
DAVATI LTD	2
NPF Materiamedica Holding LTD	5
Richard Bittner AG	4
SEVENE PHARMA	1



44 (74.9%) of registered HR are of herbal origin. Among 394 herbs, which are used to receive HR, 45 are officinal ones and 26 (57,78%) are representatives of Georgian Flora. These are*: Acorus calamus* L., *Arctostaphylos uva-ursi* L. Spreng., *Artemisia absinthium* L., *Betula pendula* Roth, *Capsella bursa-pastoris* L., *Chelidonium majus* L., *Convallaria majalis* L. *Datura stramonium* L., *Equisetum arvense* L., *Frangula alnus* Mill., *Gnaphalium uliginosum* L., *Hyoscyamus niger L., Hypericum perforatum L., Inula helenium* L., *Menyanthes trifoliata* L., *Origanum vulgare* L., *Plantago major* L., *Polygonum hydropiper*

L., *Rubia tinctorum* L., *Sambucus nigra* L., *Tanacetum vulgare* L., *Taraxacum officinale* Wigg., *Tilia cordata* Mill., *Vaccinium myrtillus* L., *Valeriana officinalis* L., *Viburnum opulus* L. [7,8].

These herbs along with widely cultivated sage - *Salvia officinalis* L., as well as witch-hazel - *Hamamelis virginiana* L., introduced in Georgia can be considered as a raw for local HR [9].

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PP 61. PLANTS OF THE FAMILY LEGUMINOSAE OF GEORGIAN FLORA AS POTENTIAL SOURCES OF BIOLOGICALLY ACTIVE FLAVONOIDS

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Identification of plant resources and biologically active substances with potential medicinal application is one of the main directions in the phytochemical research.

Among representatives of Georgian flora studied at the Institute of Pharmacochemistry the species belonging to *Leguminosae* family are distinguished from the others by high content of biologically active compounds. The results of study of flavonoids from some genera *Cercis, Galega, Hedysarum, Melilotus, Phaseolus, Onobrychis* and *Oxytropis* are presented in table 1.

Table 1. Flavonoids from some *Leguminosae* species

№	Plant	Flavonoid aglycons	Isolated compounds
1	*Cercis canadensis L.*	Myricetin	Myricitrin (Myricetin-3-O-rhamnoside)
2	*Cercis siliquastrum L.*	Myricetin	Miricitrin (Myricetin-3-O-rhamnoside)
3	*Galega orientalis L.*	Kaempferol Cyanidin	Kaempferol, Cyanidin Afzelin (Kaempferol-3-O-rhamnoside)
4	*Hedysarum caucasicum M.B.*	1,3,6,7-tetrahydroxy-xantone	Mangiferin, Isomangiferin
5	*Hedysarum sericeum M.B.*	1,3,6,7-tetrahydroxy-xantone	Mangiferin, Isomangiferin
6	*Melilotus officinalis L.*	Kaempferol	Robinin (Kaempferol -3-0-robinobiosyl, 7-0-α-L-rhamnopyranoside) Kaempferol-3-0-Galp-Glcp-Arap-Rhamnoside
7	*Phaseolus vulgaris L.*	Quercetin	Quercetin, Rutin (Quercetin-3-0-β-D-rutinoside)
8	*Onobrychis angustifolia Chinth.*	Isorhamnetin Quercetin Apigenin	Isorhamnetin Isorhamnetin-3-0-β-D-Galactofuranoside Rutin (Quercetin-3-0-β-D-rutinoside) Quercetin-3-0-β-D-Galactopyranoside Vitexin (Apigenin-8-C-glucoside)
9	*Onobrychis iberica Grossh.*	Quercetin Kaempferol	Rutin (Quercetin-3-0-β-D-rutinoside) Quercitrin (Quercetin-3-0-β-D-Glucopyranoside) Kaempferol-3-0-α-D-Glucofuranoside Kaempferol Astragalin (Kaempferol -3-0- β-D-Glucopyranoside) Nicotiflorin (Kaempferol-3-0- rutinoside)
10	*Oxytropis pallassii Pers.*	Kaempferol	Robinin (Kaempferol -3-0-robinobiosyl, 7-0-α-L-rhamnopyranoside)

The isolation of the individual components was performed by *CC* and *HPLC*. The identification of isolated compounds and their chemical derivatives was carried out based on their physical-chemical properties, as well as IR, UV, ^{1}H- and ^{13}C-NMR spectroscopy data.

The genus *Onobrychis* attracts the attention due to the dominant content of quercetin-, kaempferol- and isorhamnetin derivatives. Crude flavonoids from *Onobrychis angustifolia* show the ability to bind radioactive ^{90}Sr and practically do not upset the balance of ^{45}Ca.

The genus *Hedysarum* stands out from others by xanthones content [1]. Xanthones exhibit various biological activities including strengthening of capillaries, antibacterial, antifungal, antiinflammatory, antioxidant, antiplasmodial, cytotoxic, and potential cancer chemopreventive activities. Moreover, some of the them have been found to affect specific enzyme activities, such as aromatase, HIV-1 protease, inhibitor of κappa B kinase, quinone reductase, sphingomyelinase, topoisomerase and several protein kinases, and they also modulate histamine H1 and 5-hydroxytryptamine 2A receptor binding [2].

Species of genera *Oxytropis, Melilotus* and *Galega* synthesized mainly kaempferol derivatives. The occurrence of hypoazotemic and diuretic flavonoid - robinin in *Oxytropis pallassii* and *Melilotus officinalis* makes them an important source of this glycoside [3]. Robinin previously has been isolated from *Astragalus falcatus*. It was also found in other species of *Astragalus* [4]. According to the state-of-art data [5] it turns out that *Oxytropis pallassii* on the basis of morphological signs is referred as *Astragalus lanatus Pall*. According to the results of our research this opinion seems fair and is supported by the content of robinin that is a characteristic feature for the *Astragalus*.

The sum of phenolic compounds from aerial parts of *Galega orientalis* has a hypoglycemic activity and often presents as an ingredient of the antidiabetic herbal tea [6]. Phenolic compounds of *Phaseolus vulgaris* also show hypoglycaemic effect. These plants can be recommended for making of antidiabetic food supplements.

Myricitrin was isolated from *Cercis siliquastrum* leaves (yielding up to 2% of air-dried raw). It has a choleretic effect; moreover it inhibits a nitric oxide and protein kinase C and exhibits antipsychotic-like and anxiolytic-like effects in *in vivo* experiments [6].

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PP 62. SEPARATION OF ENANTIOMERS OF CHIRAL SULFOXIDES WITH METHYL-AND CHLORO-METHYL-SUBSTITUED TRIS-PHENYLCARBAMATE OF CELLULOSE AS CHIRAL SELECTORS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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A screening of newly synthesized chiral sulfoxides on their enantioseparation with 15 different cellulose phenylcarbamate-based chiral selectors was performed in high-performance liquid chromatography. Most of these 15 chiral selectors were also non-commercial products and synthesized in the frame of this experiment. By systematic variation of the chemistry and structure of chiral selectors and selectands those structural features were prevailed which are most critical for selector-selectand binding and chiral recognition ability. In this presentation we discuss only columns with methyl- and chloro-methyl substituted-phenylcarbamates. The experiments were performed by using HPLC. With following mobile phases: methanol, ethanol, 2-propanol and the mixture of n-hexane and 2-propanol. Chiral recognition ability is dramatically affected by the fine modification of the structure of a chiral selector or selectand [1].

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PP 63. OVERVIEW OF REGULATION FOR MEDICINES AND PHARMACEUTICAL ACTIVITIES IN GEORGIA

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Georgian legislation regarding medicines and pharmaceutical activities covers the Constitution of Georgia, International Treaties and Agreements, Law of Georgia on Medicines and Pharmaceutical Activities, statutory acts.

In 2009, significant changes were introduced in the Law of Georgia on Medicines and Pharmaceutical Activities aimed at growing competition at the market.

Removal of legislative barriers was applied into 2 main directions:

- To simplfy import of pharmaceutical products through introduction of new regimes and simplification of registration of pharmaceutical products;

- To simplify commencement of pharmaceutical activities.

Barriers established for import of medicines were eliminated as a result of changes (access to Quality Certificates of a Manufacturer) and gave companies more vast opportunities in terms of import. Legislative changes regulated the problems related to import of medicines and therefore, import of pharmaceutical products increased as illustrated below:

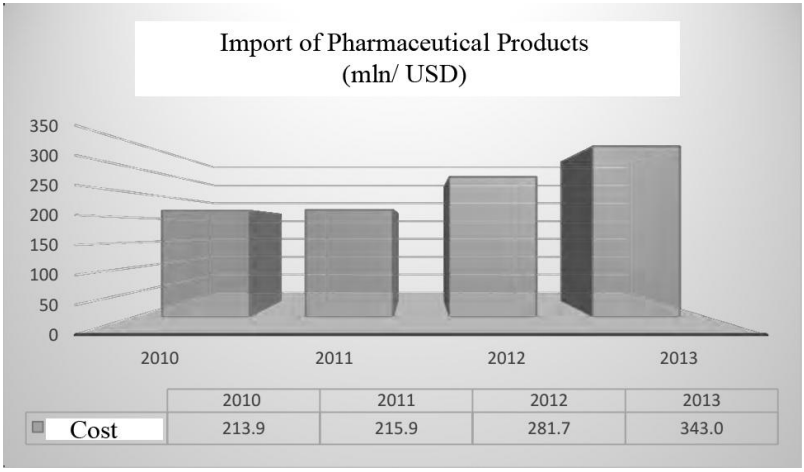
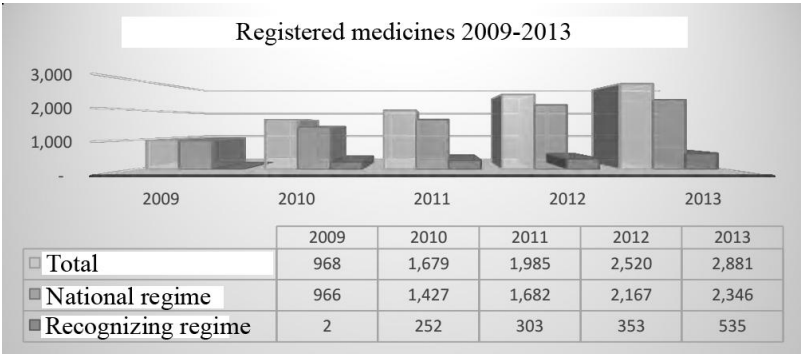


Diagram 1. Import of pharmaceutical import during 2010-2013 years (mln USD).

Import possibilities were increased by allowance of recognition regime and parallel import of medicines.

In addition, changes to laws allowed parallel import and enabled suppliers to import such products already registered by an initial importer.



	2009	2010	2011	2012	2013
Total	968	1,679	1,985	2,520	2,881
National regime	966	1,427	1,682	2,167	2,346
Recognizing regime	2	252	303	353	535

Fig.2 Registered medicines in Georgia 2009-2013.

Besides improvement of import possibilities, commencement of pharmaceutical business became easier. Pursuant to the Law of Georgia on Licenses and Permits[1], permits shall be obtained for manufacture of a pharmaceutical product, clinical study, authorized pharmacy, export and import of a pharmaceutical product subject to special control. The Agency makes optional control of permit conditions.

Purpose of current laws governing medicines and pharmaceutical activities is to facilitate access of consumers to reliable pharmaceutical product and to ensure the same the laws prescribe legal grounds for regulation of circulation of pharmaceutical products and rights and obligations of natural persons and legal entities involved in pharmaceutical business.

References

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PP 64. PERMITS FOR PHARMACEUTICAL ACTIVITIES IN GEORGIA

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Agency for Regulation of Pharmaceutical Activities grants permits pursuant to the Law of Georgia on Licenses and Permits for manufacture of a pharmaceutical product, clinical study, authorized pharmacy, export and import of a pharmaceutical product subject to special control.

Pharmaceutical activity – means batch manufacture of a pharmaceutical product in a manufacturing plant in full compliance with appropriate standards.

Currently, permits are granted for 75 pharmaceutical manufacturers.

Clinical study – following development, a pharmaceutical product undergoes clinical study in patients. Such practice requires a permit of a regulating agency. In order to obtain a permit for linical study, a concerned person should submit results of pre-clinical study.

Currently, 45 clinical studies are underway in Georgia.

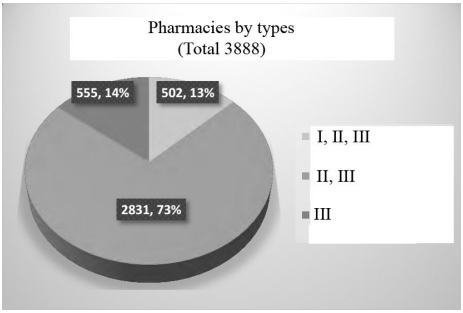
Export-import of medicines subject to special control – export-import of medicine with consideration of its specific nature is subject to issuance of permit.

Permit for authorized pharmacy – authorized pharmacy is subject to permit control and it is permitted to sell pharmaceutical products attributed to I, II and III groups, also preparation of a pharmaceutical product with officinal or magisterial prescription.

Besides authorized pharmacy, there are specialized pharmacy and retail outlets.

Specialized trade outlet mat sell pharmaceutical products attributed to II and III groups (products issued with and without prescription). Such practice requires no permit.

Retail outlet may sell products attributed to III group (issued without prescription) and such practice requires no permit.



References

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[2] Law of Georgia on Licenses and permits, 2005

PP 65. THE STUDY OF MORPHOLOGICAL AND ANATOMICAL SIGNS OF JERUSALEM SAGE (PHLOMIS PUNGENS WILLD.) RAW MATERIAL

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At present, the demand for medicinal plant raw materials in the world market is increasing annually. Therefore, it is necessary to identify new areas of growth and to determine the potential resource of medicinal plants.

With the identification of new areas of the species used it is important to conduct a comparative pharmacognostical study and set a compliance with the pharmacopoeial monograph. Naturally, the new regions are located in different bio-ecological conditions and there may be a possible shift of chemical composition as well as the changes in diagnostic features of the anatomical structures.

We have identified the regions of massive growing of *Phlomis pungens* Willd. on the territory of Azerbaijan and set the goal to study this plant in phamacognostical aspect. There have been studied essential oils, flavonoids, amino acids and microelement composition and other substances [1,2] .

The literature provides the information on diagnostic characteristics of *P.pungens* Willd. [3, 4] Such diagnostic elements as tortuosity of epidermal cells, various forms of hair, stomatal apparatus are described. However, they are repeated and are not characteristic to the species, but are specific for members of the Lamiaceae family.

Leaves, stems, seeds and inflorescence represent the raw materials of the plant. We have set the task to study the inflorescence to identify additional and specific features of *P.pungens* Willd.

The seeds have quadripartite crosswise-arranged deepenings. The upper surface of the seeds is smooth, glossy; the base part is covered with trichomes.

Bracts are elongated lanceolate, surface densely covered with multicellular branched hairs. The end of the bracts are covered with straight simple hairs.

Calyxes of flowers are whole; the tips of the calyxes are ending by stretched thin formations. Calyxes in inner upper side are covered with multicellular simple hairs with the outer-branched hairs.

Coronets are covered at the top of the inner side with simple hairs and in lower part, they are branched. The outer side of coronets is covered only with branched hairs.

Petioles are covered with a continuous layer of branched hairs. The stalk has the usual layer of tissues.

A dense layer of hairs covers the upper side of the leave, whereas the edges of the leave blade are covered with the glands. The bottom of the plate is rich with hairs, and, especially, with the main conductive veins.

As a result, the specific features of the anatomical structure of raw materials of *P.pungens* Willd are revealed. On the inner and outer surface of the sepals and the corolla we observed the different structure of trichomes.

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PP 66. THE CHEMICAL COMPOSITION AND MICROSTRUCTURAL FEATURES OF THE ROOTS ONONIS ARVENSIS L.

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Genus *Ononis L*. includes up to 75 species growing in the Mediterranean, northern and central parts of Europe, Asia. Three species are widespread in Georgia. The roots of *Ononis* species have been used as a diuretic and lithotripsic in medicine for a long time [1]. Their main active ingredients are phenolic acids, flavonoids, triterpene alcohols, isoflavones. *Ononis* species from Georgian flora have not been investigated from this point of view.

Isoflavones - formononetin, ononin, daidzein and daidzin, chlorogenic acid and triterpene alcohol α-onocerin were isolated and identified by us from the aerial parts and roots [2, 3]. The roots contain α-onocerin in a significant amount (0.3-0.5% yield). The purified sum of substances (yield 11%) from the roots shows diuretic effect *in vivo*. Based on these results the roots of *Ononis arvensis* growing in Georgia can be recommended as a diuretic remedy. Therefore, the microstructural analysis of the roots was carried out (Fig. 1).

The root system is characterized by prominent cork tissue. The structure of phellogen (cork cambium) is large and consists with 6, 7 and sometimes 8 layers of orderly manner adjacent thin walled tangential elongated cells. Phelloderm is formed by phellogen and is represented by thin walled, slightly angular, unequal size cells. The bottom layer of periderme (phelloderme) borders to the parenchyma of secondary root cortex. The cells with asymmetrical angular walls and with various shapes and sizes are marked in it structure. Sclerenchyma cells are scattered in the bark parenchyma. The cells are filled with starch grains and also the single crystals of calcium oxalate are detected as druses and rhomb-shaped crystals.

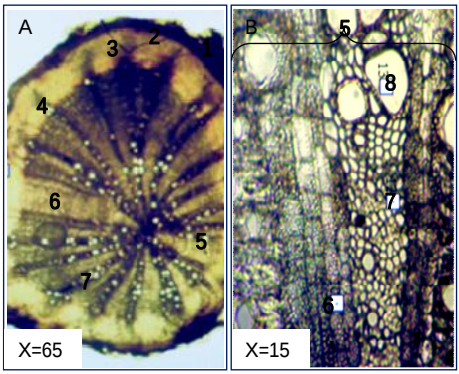

Fig.1. Microstructural features of *Ononis arvensis* root: A. Detail of a cross section of the root; B. Structural elements of the central cylinder1.cork; 2. periderm; 3. parenchyma of cortex; 4. phloem; 5. secondary conduction system; 6. radial rays; 7. secondary timber; 8. timber vessels

The complicated radial rays and secondary conductive tissue are located in the central cylinder of the root. Deep irregular bands of the conduction system are also visible. Primary timber and phloem are

arranged radially in the area of the pith elements. Libriform is clearly differentiated in the secondary timber, conducting elements reticulate and spiral-annulated timber vessels. The volume of radial rays of the main root is heterogeneous. There are marked 4, 18 and 22 rows of radial rays. The cells are filled with spare starch grains.

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PP 67. DEVELOPMENT OF THE METHOD OF ANALYSIS OF QUETIAPINE

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Introduction - Development of the rapid, sensitive and selective methods of determination of various subjects of intoxication is the main goal of the Toxicological chemistry and Forensic medicine. Our analyt of research was quetiapine, (QTP, Fig. 1) an antipsychotic drug used for the treatment of Schizophrenia and other psychotic syndromes. It produces less exptrapyramidal side effects, hyperprolactinmia, and agranulocitosys than other neuroleptics [1, 2].

In order to study the pharmacokinetics and toxicokinetics in patients treated with therapeutic doses or in the case of overdose was chosen the method of LC–MS-DAD determination, which is the most utilized technique in forensic and pharmacokinetic laboratories, nowadays [3].

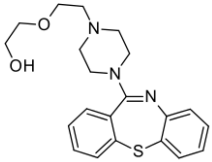

Fig.1. Chemical structure of Quetiapine (2-(2-(4-dibenzo[b,f][1,4]thiazepine- 11-yl- 1-piperazinyl) ethoxy)ethanol)

Materials and Methods: reference standard of quetiapine and internal standard risperidone (RIP) were obtained by *Sigma Aldrich*, all solvents and reagents were HPLC grade.

Standard solutions – An QTP and RIP stock solution (1.0 mg/mL) in methanol was diluted with methanol to produce an intermediate stock solution (500 μg/mL). This was further diluted to prepare standard and (independently) QC solutions. An IS stock solution (100 μg/mL) was also prepared in methanol and diluted to give an IS working solution (10.0 μg/mL). All solutions were stored at 4°C when not in use. QTP calibration standards (CS-1 to CS-10, 0.10, 0.15, 0.20, 0.50, 1.50, 3.00, 10.0, 25.0, 40.0 and 50.0μg/mL) (n=5) were stored at -20°C pending analysis.

Chromatographic conditions: the LC system was AGILENT TECHNOLOGIES 1290 Infinity AGILENT TECHNOLOGIES 6460 Triple quad LC/MS. Separation was performed by isocratic elution on Zorbax Eclipse plus C18 (250×4.5 mm, 5.0 μm) column, equipped with pre-column: UHPLC GUARD Zorbax Eclipse plus C18 (5×2.1 mm, 1.8 μm); column temperature was - 35°C. Mobile phase consisted of 0.1 % water solution of formic acid (H_2O):0.1 % acetonitrile solution of formic acid = 70 : 30 (v/v); flow rate - 1 mL/min and run time – 7 min.

MS-DAD detection conditions: ionization and detection of analyte and IS was performed on a triple quadrupole mass spectrometer equipped with Turbo Ionsprays operating in the positive ion mode in the range 80-500 m/z (Fig. 2), with following diode detection at 280 nm.

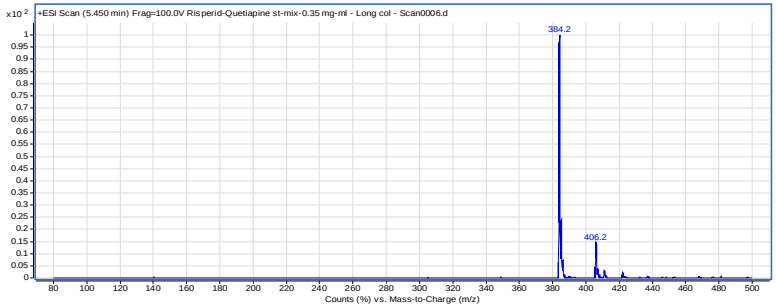

Fig.2. Mass spectra of Quetiapine

Results and discussion: the described condition causes good resolution, without extensive peaks tailing, with retention times – 5.2 min (QTP) (Fig.2). A linear calibration curve shows a R^2 value of 0.9988 for QTP. The assay is linear in the range 0.5-40.0 μg/mL. It is admirable, the short run time – 7 min. These gives the opportunity to laboratory obtains the respective results in very short time.

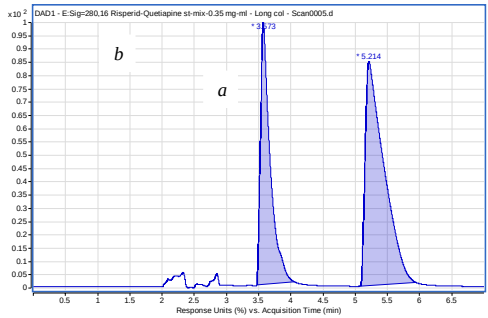

Fig.3. Chromatogram of Quetiapine (a) and Risperidone (b)

Conclusion: the sensitive and selective LC-MS-DAD method of analysis for quetiapine determination was developed successfully. The developed method will contribute to the future study of the product in biological materials, both *in vitro* and *in vivo* conditions.

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PP 68. THE STUDY OF THE ANTIBACTERIAL ACTIVITY OF THE EXTRACT OF DEPOSIT PRYBYCH SAPROPEL

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In recent years, the attention of specialists is attracted to application of various natural substances, including sapropel, as therapeutic agents. Despite the advances in modern pharmacy, existing means of drug therapy do not solve many problems that may occur during treatment. This item contributes to the search for new drugs of natural origin that would have the efficiency, quality and safety not inferior to traditional medicines; moreover, they should have no contraindications and cause no complications.

The purpose of the research comprises the study of antibacterial properties of the sapropel extract from Prybych deposits, located in Shatsk district of Volyn region.

Antibacterial activity of the sapropel extract, which was obtained by treatment of dewatered sapropel with 0.1 N solution of potassium hydroxide in an aqueous medium at a ratio of sapropel and water (1:10), pH value less than 10. Simultaneous homogenization of the mixture at 90 °C for 2 h. is followed by neutralization with 10 % hydrochloric acid solution up to pH 6,8-7,0 after that evaporate in the vacuum evaporating apparatus at temperature 50-60 °C. Research was conducted at the Laboratory of microbial biochemistry and microbial growth media at the Government agency "I.I. Mechnikov Institute of Microbiology and Immunology NAMS of Ukraine" by an agar diffusion method.

According to the WHO recommendations for the evaluation of antimicrobial activity the following test strains were used: Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Basillus subtilis ATCC 6633, Proteus vulgaris ATCC 4636, Candida albicans ATCC 885/653.

In assessing of antimicrobial agents were considered the following criteria: absence of microbial growth inhibition zones around the wells or occurrence of inhibition zones with diameter up to 10 mm indicate that the organism is insensitive to the drug; zone diameter 10-15 mm - low sensitivity to culture; 15-25 mm regarded as an indicator of the sensitivity of the microorganism; growth zone with a diameter above 25 mm indicates the high sensitivity of microorganisms to the studied drugs. All the results of research were processed using a Statistica 5.0 software. Statistical analysis of the research results was carried out using Student's t-test and Wilcoxon-Mann-Whitney test.

Results of antibacterial activity study of the test sample are presented in Table 1.

Table 1. The antibacterial activity of the sample

Sapropel extract	The diameters of the growth inhibition zones, in mm (n = 3)					
	S. aureus ATCC 25923	*E. coli ATCC 25922*	*P. vulgaris ATCC 4636*	*P.s aeruginosa ATCC 27853*	*B.s subtilis ATCC 6633*	*C. aibicans ATCC 653/885*
1	14, 15, 16	16, 16, 16	12, 13, 13	14, 14, 13	18, 19, 19	18, 17, 17

The investigated sample of a preparation has antibacterial activity against Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Basillus subtilis ATCC 6633, Candida aibicans ATCC 653/885; and a weak antibacterial activity against Proteus vulgaris ATCC 4636 and Pseudomonas aeruginosa ATCC 27853.

PP 69. BIOLOGICALLY ACTIVE ALKALOIDS OF *VERATRUM LOBELIANUM* BERNH

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Plant *Veratrum lobelianum* Bernh. (fam. *Melanthiaceae*), widespread in alpine and subalpine zones. *V.lobelianum* is rich with steroidal alkaloids possessing hypotensive, antimicrobial, anti-inflammatory and other activities.

In the underground parts the leading alkaloid is biologically active iervin. Except of iervin, psevdoiervin, veralozin, veralozidin, veralozinin, veralodin, veratroilzigadenin, O-acetyliervin and 12α, 13β- dihydroiervin are isolated from the plant. Among them O-acetyliervin and 12α, 13β-Dihydroiervin (Fig.1) appeared novel compounds [1].

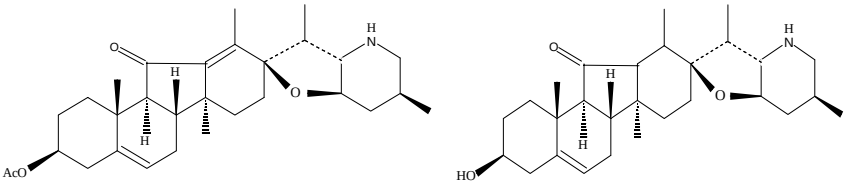

Fig.1. Structures of O-acetyliervin (a) and 12α, 13β- dihydroiervin (b)

It is established that the acumulation of the sum of steroidal alkaloids in *V.lobelianum* depends on ecological and geographical factors, in particular, the higher from sea level is the growth area the higher alkaloids content is. The analysis of iervin accumulation dynamics in aerial parts of V.lobelianum revealed the presence of alkaloid practically on every stage of active vegetation, and the maximum content is observed in 20-30 cm height plant. By the studying of alkaloid content variation in stems and leaves separately, it was established that iervin is more intensively accumulated in the leaves in the early stages of plant development. The alkaloids content is much higher in stems than in leaves in early flowering phase, while in fructification stage iervin content changes vice-versa. Hence the correlation between the vegetation period and accumulation of individual alkaloids in the underground and aerial parts of *V.lobelianum* is established.

Biological activity of iervin was studied in the Department of Biological Research of I. Kutateladze Institute of Pharmacochemistry. It was found that alkaloid iervin exhibits stimulating activity in serotonin-sensitive isolated organs test system. Iervin reveals serotonin-like effect on fibroblasts and activates their proliferation by interaction with serotonin receptors [2].

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PP 70 THIN-LAYER CHROMATOGRAPHY INVESTUGATION OF OLANSAPINE AND RISPERIDONE

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Olanzapine is a derivative of 1,5-benzodiazepine. It belongs to atypical antipsychotics for treatment of acute psychosis, schizophrenia and moderate to severe manias [1, 4]. It has affinity to serotonin, muscarinic, histamine-H1, adrenergic(α1) receptors in the central nervous system. Olanzapine overdose is associated with a reduced and fluctuating consciousness up to coma in severe cases [7].

Risperidone is also atypical antipsychotic drug used for schizophrenia treatment (including adolescent schizophrenia), schizoaffective disorder, the mixed and manic states associated with bipolar disorder, and irritability in people with autism [3, 8]. It is derivative of benzoisoxazole. An overdose amount varies according to a body weight, duration of intake, and interaction with other drugs . The most common signs of a risperidone overdose include drowsiness, lethargy, hypotension, shaky, convulsions, tachycardia. Even lethal cases were reported [2,5,6].

In recent years, intoxication due to drug overdose, shifts up rapidly in poisoning statistics. Thus, fast, simple and cost-effective analytical method could facilitate the work of forensic expert in identifying certain acute poisoning endangered preparations.

The aim of research was the development and validation of a thin-layer chromatographic (TLC) method suitable for the separation of olanzapine and risperidone mixture and their identification in forensic analysis through:

- evaluation of various solvents, in order to find suitable mixture for TLC, and developing a various reagents for identification and separation of olanzapine and risperidone;
- selection and validation of appropriate reproducible and sensitive method, resistant to labile conditions.

Methodology: Sorbfil and Merck plates were used for chromatographic separation. 1, 5, 10 μl of 100 μg/ml olanzapine and resperidone solutions in methanol were dropped on start line of the plates. Spots of investigated substances on chromatograms were treated by spraying with solutions of Fast blue B, ninhydrine, mercury nitrite, acidified potassium permanganate, and the Dragendorff, Marquis, Mandelin reagents and developed under UV light at 254 nm.

The following chemically pure grade solvents in different combinations and ratios were tested for chromatographic systems: methanol, ethyl acetate, toluene, hexane, chloroform, glacial acetic acid, acetone, dimethyl formamide, 1,4-dioxane, 25 % ammonium hydroxide, and diethyl amine.

Results: The most suitable systems for olanzapine and risperidone identification appeared (each has two different solvent ratios):

- methanol – 25 % ammonium hydroxide (48:2 and 49,5:0,5);
- ethyl acetate – acetone – 25 % ammonium hydroxide (25:24:1 and 22: 27:1);
- ethyl acetate – methanol – 25 % ammonium hydroxide (24:24:2 and 22:26:2);
- toluene – 1,4-dioxane – dimethyl formamide (10:38:2 and 12:36:2).

The Dragendorff reagent (in Mounier modification) develop both investigated substances. Olanzapine may be developed additionally by acidified potassium permanganate solution.

The solvent system toluene – 1,4-dioxane – dimethyl formamide (10:38:2) appeared the most acceptable. The R_f values (n = 10) of olanzapine is 0.62 and risperidone – 0.74 (on Sorbfil plates); 0.68 and 0.78 – on Merck plates respectively. Detection limits of olanzapine and risperidone were 0.02 μg and 0.05 μg, respectively.

2 ml Increase or decrease of both toluene and 1,4-dioxane significantly affected the R_f values of tested compounds. In system toluene – 1,4-dioxane – dimethyl formamide (11:37:2) R_f of olanzapine is 0.61 and risperidone – 0.75 (on Sorbfil plates); and 0.66 and 0.76 – on Merck plates respectively. In system with another solvents ratio – 12:36:2, R_f of olanzapine is 0.61 and risperidone – 0.74 (on Sorbfil plates); and 0.65 and 0.75 – on Merck plates respectively.

Calculation of relative standard deviation (SSN percent) showed that the repeatability error of results is less than 3 percent. Thus, the proposed solvents system is suitable for the desired separation and identification of analyzed compounds.

Conclusion: Thus, the described TLC technique for the separation and identification of olanzapine and risperidone mixture meets the desired requirements (reproducibility, sensitivity and stability) and can be proposed for application in forensic analysis

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PP 71. HPLC DETERMINATION OF POLYPHENOLS IN WINES AND PARTS OF CLUSTER OF AUTOCHTHONOUS GEORGIAN WHITE GRAPES

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A new reversed-phase high-performance liquid chromatography (HPLC) method was developed for separationand quantitative determination of phenolic compound sinvarious parts of white grapes and wines. Studied wines were produced based on traditional Georgian (Kakhuri) and European technologies. The phenolic compounds: gallic acid, protocatechuic acid, (+)-catechin, vanillic acid, coffeic acid, syringic acid, (-)-epicatechin, ferulic acid, dihydroquercetin, rutin, o-coumaric acid, resveratrol, quercetin, p-oxybenzoic acid are determined quantitatively (mg/l) in various parts of white grapes and wines. Seperation was performed on a new Poroshell-type C-18 reversed-phase column and water-acetonitrile gradient. The detection was performed by using UV-VIS detector.

Table 1. Phenolic compounds in grape bunch and wine of vine variety Tsolikouri

№	Phenolic compounds	Retention time (min)	Parts of grape bunch (mg/g dry weight)			Wine Mg/L
			Stem	Skin	Seeds	
1	Gallic acid	5.057	0.096±0.007	nd	0.535±0.194	23.287±7.59
2	Protocatechuic acid	8.855	0.071±0.008	0.037±0.003	0.212±0.012	10.33±5.34
3	(+)-Catechin	12.965	0.021±0.025	traces	3.131±0.057	23.987±9.04
4	Vannilic acid	1.682	traces	traces	traces	traces
5	Caffeic acid	15.750	0.026±0.006	0.005±0.013	nd	0.616±0.169
6	Syringic acid	17.514	nd	nd	0.313±0.187	1.591±0.066
7	(-)-Epicatechin	19.454	0.007±0.005	0.014±0.011	0.211±0.274	1.130±0.601
8	Ferulic acid	27.108	nd	nd	0.02±0.003	traces
9	Dihydroquercetin	29.285	traces	nd	nd	0.556±0.007
10	Rutin	36.433	0.112±0.028	0.149±0.134	nd	9.103±3.26
11	*o*-Coumaric acid	39.474	traces	traces	traces	traces
12	Resveratrol	47.683	nd	nd	nd	nd
13	Quercetin	52.653	0.064±0.027	nd	0.102±0.059	1.629±0.24
14	*p*-Hydroxybenzoic acid	69.759	nd	0.063±0.009	nd	nd

Abbreviation: nd - not detected;

PP 72. ALKALOIDS OF *BUXUS COLCHICA* POJARK. AND THEIR BIOLOGICAL ACTIVITY

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Boxwood - *Buxus colchica* Pojark (Buxaceae) - an evergreen shrub widely distributed in Georgia. In folk medicine *Buxus* species find extensive use in treatment of various diseases, such as rheumatism, skin disorders, venereal diseases. Buxus alkaloids were tested for acetylcholinesterase and butyrylcholinesterase inhibitory activity, because buxaminol – E, a steroidal alkaloid from *B. sempervirens*, was reported as a good enzyme inhibitor [1, 2].

Phytochemical study of *B.colchica* leaves and stems resulted in the isolation of steroidal alkaloids. The structures of the compounds were determined using spectral methods.

Individual alkaloids were isolated chromatographically on columns with aluminum oxide using the methods of solubility dependent distribution in organic solvents in accordance with the degree of polarity and alkalinity in citric-phosphoric buffers. 8 individual compounds were isolated and structurally indetified: among them known buxamin-E, cyclobuxin-D, pseudocyclobuxin-D, L-cyclobuxin-C, derivatives of cycloprotobuxin - A and buxaminol - G. According to UV -, IR -, MS-, ^{1}H, ^{13}C and NMR spectral data, DEPT, homonuclear (^{1}H-^{1}H, COSY) correlation spectra two alkaloids: 3β-dimetylamin-20α-methylamin-4β,4α,14α-trimethyl-9β,19α - cycloprotobuxin-C and (-)N-3-O-16-hydroxy-3α,20α-monomethylamino-4-methylen,14α-methyl-9β19α – cyclo-pseudobuxin-D appeared to be novel ones. Besides, the N-oxide derivative has been found among *Buxus* steroidal alkaloids for the first time.

For a quantitative determination of pharmacologically active sums, standardization of *Buxus* leaves and stems and compilation of guidelines it was necessary to work out the reproducible techniques of the quantitative analysis. The latter was done relying on acid-base titration methods recommended by State Pharmacopoeia. Extraction of alkaloids from raw material, solubility of buxamine in a glacial acetic acid and titration the sums of alkaloids with 0,01N solution of $HClO_4$ were studied. The proposed technique was applied for studying accumulation dynamics of buxamine and ester-soluble sum of alkaloids in vegetative bodies of Buxus during the different phenophases. The standardization of aforesaid sums of alkaloids and raw material - *Buxus* leaves and stems was carried out.

The anatomy of vegetative bodies of *Buxus* overground parts, necessary for identification of raw material and obligatory for reference documentation, was described for the first time at the Department of Pharmacobotany.

Pharmacological study of hydrochlorides of buxamine, ester-, chloroform- and water soluble fractions was carried out at the Department of Pharmacology of I.Kutateladze Institute of Pharmacochemistry. Buxamine and ether-soluble fractions of alkaloids completely prevents $BaCl_2$-induced constriction of the isolated fundus strips of rats. Buxus alkaloids have relatively low toxicity and exhibit expressed spasmolytic activity. The ether fraction appeared tenfold active, than the chloroform one. The aqueous extract exhibited minor antihistaminic activity [2].

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PP 73. DETERMINATION OF POLYPHENOLS IN BETULA RADDEANA GROWING IN GEORGIA BY HPLC-DAD-ESI-MS/MS

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The genus *Betula* of the family Betulaceae, has a wide distribution in the northern hemisphere from Canada to Japan. Five *Betula* species, namely *B. litwinowii* Doluch., *B. medwediewii* Regel, *B. pendula* Roth., *B. raddeana* Trautv. and *B. megrelica Sosn.* are naturally growing in eastern and northern Georgia, at high altitudes [1].

Diverse phytochemical investigations of Betula species have shown that they contain mainly phenolics, flavonoids, tannins, saponins, glycosides, sterols and terpene derivatives [4,6]. Leaves and other parts of different birch species (Betula spp., Betulaceae), and products of birch such as buds, bark, essential oil, juice, wood, tar, etc. are used mainly for treating urinary tract disorders, severe infections and inflammations [2,3].

The purpose of this research was to identify phenolic compounds, quantify main flavonoids and phenolic acids that was found to be characteristic for *B. raddeana* Trautv.

The leaves of *Betula raddeana* Trautv. were collected in Georgia in June 2012.

The leaves of *B. raddeana* Trautv. were dried and ground into powder. Two grams of powdered leaves were mixed with 50 ml methanol and phenolic acids and flavonoids were ultrasonically extracted at room temperature for 40 min and eluated by using a Bond elut C18 500 mg cartridge. Finally, the filtrate was collected, and filtered through a 0.45 µm nylon filter. A 5 µl volume extract was injected into the HPLC column for analysis by LC-DAD-MS/MS.

Quantification of phenolics was performed by using an Agilent Technologies 1290 Infinity LC system consisting a DAD and coupled to a Agilent Technologies 6460 Triple quadrupole LC/MS.The column was a 250 mm X 4.5 mm 5 µm particle size Zorbax Eclipse C18, maintained at 35^{0}C and protected with a UHPLC guard Zorbax Eclipse column of the same material in the gradient of 0.1% aqueous formic acid (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The column temperature was 35 °C, eluent rate 1.0 ml/min, injection volume 5 µl.

Polyphenols were identified by comparing their MS/MS fragmentation spectra either with the fragmentation spectra of the respective commercial standards or with literature data [5] and quantitated using chromatographic peak heights at the wavelengths at 280 (chlorogenic acid), 305 (ferulic acid glycoside), 330 (apigenin glycosides) and 370 (quercetin and luteolin glycosides) nm. Electrospray mass spectra data were recorded on a negative ionisation mode for a mass range *m/z* 100 to *m/z* 1000. The conditions of MS^2 detection were: m/z interval 50-1000; target mass, 400; number of fragmented ions, two; interval: 0.5 s; scan speed: 1500 amu/s; nebulizing gas nitrogen from generator, flow: 1.5 L/min; Heat block: 300 °C, DL temperature: 300 °C; DL voltage: –34 V; probe voltage: 4.5 kV; Qarray voltage: 1.0 V; RF voltage: 90 V; detection gain: 1.0 kV.

A sensitive, accurate and specific method coupling HPLC with DAD and electrospray ionization mass spectrometry was developed for the separation and identification of phenolic acids, flavonoid glycosides and aglycones in the methanolic extract of *B. raddeana* Trautv.

After LC-DAD and MRM analysis Apigenin, rutine, kaempferol , myricetin, hyperozide, quercetin, Quercetin-3-glucoside (isoquercitrin), myricetin-3-O-galactoside, myricetin glucuronide, quercetin-3-O-rhamnoside (quercitrin), p-coumaric-, chlorogenic-, Protocatechuic-, ellagic-, ferulic-, gallic, vanillic-, caffeic acides were determined in *B. raddeana* Trautv.

Based on the retention behavior as well as absorption and mass spectra, 8 phenolic acids and 10 flavonoids were identified and quantified in with the former ranging from 85.7 μg/g to 949.1 μg/g and the latter from 78.3 μg/g to 1061.7 μg/g. The glycosides of myricetin, quercetin and kaempferol were found to be the main flavonoids in the birch leaves studied. Chlorogenic acid was used for quantitation of phenolic acids, whereas hyperozide and myricetin glucuronide were found suitable for quantitation of flavonoids. The developed method showed high reproducibility, as evident from the relative standard deviation values for intra-day and inter-day variability being 1.8–6.7% for phenolic acids and 2.2–6.4% and 2.4–7.1% for flavonoids, respectively.

The proposed HPLC–ESI-MS/MS method for the determination of several flavonoids has quite good linearity, accuracy, precision and low limit of detection. All results demonstrate this method is suitable for the quality control of *B. raddeana* Trautv. leaves.

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PP 74. PRESENTATION OF THE TSMU I.KUTATELADZE INSTITUTE OF PHARMACOCHEMISTRY EXPERIMENTAL PRODUCTION BASE PROJECT

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Special approaches in the field of drug circulation are determined by outstanding social priority of pharmaceutical product. First of all, basic principles of quality assurance, production and quality control should be considered. Quality assurance is a broad notion and covers all individual and general issues that affect the product quality. Quality assurance includes the implementation of Good Manufacturing Practice (GMP) and other factors. In turn, GMP is considered as a part of production and quality control system that ensures the compliance of product with given specification requirements.

Quality systems should include:

development of pharmaceutical products that should be accomplished in accordance with the principles of Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP);

clearly defined components of the manufacturing process; they should be systematically reviewed, revised and renovated upon accumulated experience;

mandatory validation for any necessary change at any stage of the manufacturing process;

entire GMP platform suggests the presence of: educated and skilled personnel, appropriate buildings, facilities (including storehouse and transport), equipment and services; necessary raw materials and reagents, approved procedures and instructions, validated analytical methods.

Taking into account the political preferences of Georgia and its aspirations towards the European Community, and rich professional experience and scientific potential of TSMU I.Kutateladze Institute of Pharmacochemistry, the rebuilding project has been elaborated to bring to conformity with GLP/GMP. The implementation of the project will allow developing and manufacturing of high-quality medical products that meet modern international standards.

The quality assurance along with the development and implementation of appropriate system, as well as drug manufacturing depends on various factors: the number of skilled personnel that can do all the work, design of manufacturing and auxiliary areas consistent with the equipment. In turn, design, construction and location of buildings and equipment should be adapted in order that minimizes the risk of errors and ensures the effective placement, cleaning and operation, in order to avoid completely not only cross-contamination from dust and dirt, but any negative impact that may affect the quality of product.

The project foresees all operations related with product manufacturing, such as the reception and quarantine of raw materials, sampling, storage, labeling, packaging and distribution to be conformed with principles of GMP.

PP 75. SEPARATION OF ENANTIOMERS OF CHIRAL SULFOXIDES WITH CHLORO-SUBSTITUTED TRIS-PHENYLCARBAMATES OF CELLULOSE AS CHIRAL SELECTORS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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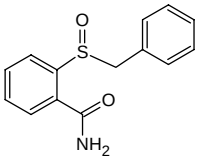
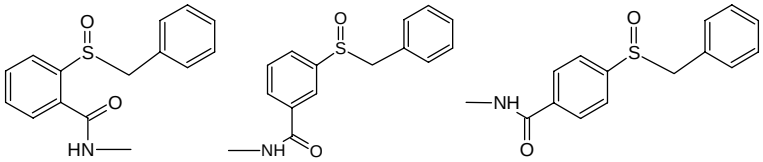
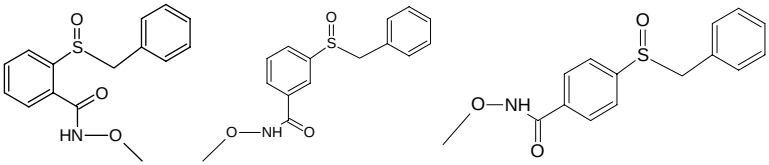
The goal of the present project was to screen newly synthesized chiral sulfoxides on their enantioseparationwith 16 different cellulose phenylcarbamate-based chiral selectors in high-performance liquid chromatography. Most of these 16 chiral selectors were also non-commercial products and synthesized in the frame of the present project. By systematic variation of the chemistry and structure of chiral selectors and selectands those structural features must be prevailed which are most critical for selector-selectand binding and chiral recognition ability.In this presentation the columns with chloro-substituted-phenylcarbamatesare discussed. The separation of enantiomers were performed using mobile phases such as methanol, ethanol, 2-propanol and the mixture of n-Hexane and 2-propanol. We used chiral selectors: Cellulosetris(2-chlorophenylcarbamate) , Cellulosetris(3-chlorophenylcarbamate), Cellulose tris(4-chlorophenylcarbamate), Cellulose tris(2,3-dichlorophenylcarbamate), Cellulose tris(2,4-dichlorophenylcarbamate), Cellulose tris(2,5-dichlorophenylcarbamate), Cellulose tris(2,6-dichlorophenylcarbamate), Cellulose tris(3,4-dichlorophenylcarbamate) and Cellulose tris(3,5-dichlorophenylcarbamate).



Scheme 1. 2-(Benzylsulfinyl) benzamide, the most interesting chiral sulfoxide.



Scheme 2. Difference in structure of chiral sulfoxides: 2-(Benzylsulfinyl) N-methyl benzamide, 3-(Benzylsulfinyl) N-methyl benzamide, 4-(Benzylsulfinyl) N-methyl benzamide.



Scheme 3. Various chemical structure of benzoates. Methyl-2-(Benzylsulfinyl) Benzoate, Methyl -3-(Benzylsulfinyl) benzoate, Methyl -4-(Benzylsulfinyl) benzoate.

PP 76. HPLC DETERMINATION OF POLYPHENOLS IN WINES AND PARTS OF CLUSTER OF AUTOCHTHONOUS GEORGIAN RED GRAPES

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A new reversed-phase high-performance liquid chromatography (HPLC) method was developed for separationand quantitative determination of phenolic compound sinvarious parts of red grapes and wines. Studied wines were produced based on traditional Georgian (Kakhuri) and European technologies. The phenolic compounds: gallic acid, protocatechuic acid, (+)-catechin, vanillic acid, coffeic acid, syringic acid, (-)-epicatechin, ferulic acid, dihydroquercetin, rutin, o-coumaric acid, resveratrol, quercetin, p-oxybenzoic acid are determined quantitatively (mg/l) invarious parts of red grapes and wines.Seperationwas performed on a new Poroshell-type C-18 reversed-phase column and water-acetonitrile gradient. The detection was performed by using UV-VIS detector.

№	Phenolic compounds	Retention time (min)	Parts of grape bunch (mg/g dry weight)			Wine mg/L
			Stem	Skin	Seeds	
1	Gallic acid	5.057	0,259	0,0587	0,4753	12,53
2	Protocatechuic acid	8.855	nd	0,0725	0,0578	5,56
3	(+)-Catechin	12.965	0,2601	0,1370	0,1155	26,58
4	Vannilic acid	1.682	nd	nd	0,3265	nd
5	Caffeic acid	15.750	nd	0,2418	nd	1,29
6	Syringic acid	17.514	0,1092	1,9307	0,1503	3,02
7	(-)-Epicatechin	19.454	nd	1,362	nd	0,56
8	Ferulic acid	27.108	nd	nd	nd	nd
9	Dihydroquercetin	29.285	nd	0,0488	nd	0,76
10	Rutin	36.433	nd	nd	nd	nd
11	*o*-Coumaric acid	39.474	nd	0,0280	nd	nd
12	Resveratrol	47.683	0,0347	0,0432	nd	0,5
13	Quercetin	52.653	0,1169	0,8642	nd	5,67
14	*p*-Hydroxybenzoic acid	69.759	nd	nd	nd	nd

Table 1. Phenolic compounds in grape bunch and wine of vine variety Ocxanuri Safere

Abbreviation: nd, not detected;

PP 77. ENANTIOSEPARATION OF CHIRAL DIHYDROPYRIDINE DERIVATIVES WITH POLYSACCHARIDE-BASED CHIRAL STATIONARY PHASES AND NORMAL-PHASE ELUENTS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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The separation of enantiomers of 10 chiral dihydropyridine derivatives was studied on 6 different polysaccharide-based chiral HPLC columns with various normal-phase eluents. Along with the successful separation of analyte enantiomers, the emphasis of this study was on the effect of the chiral selector and mobile phase composition on the elution order of enantiomers. The interesting phenomena of reversal of enantiomer elution order function of the polysaccharide backbone (cellulose or amylose), type of derivative (carbamate or benzoate), nature and position of the substituent(s) in the phenylcarbamate moiety, the polar modifier of the mobile phase (ethanol or 2-propanol) were observed.

References

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PP 78. PARTICIPATION OF α_2-ADRENERGIC AND I_1-IMIDAZOLINE RECEPTORS IN HYPOTENSIVE EFFECT OF NEWLY SYNTHESIZED IMIDAZOLINE COMPOUNDS

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The synthesis and hypotensive properties of centrally acting imidazoline agents: 1-[(imidazo-lidin-2-yl)imino]-1*H*-indazole (marsanidine, AK-24) and 7-chloro-1-[(4,5-dihydro-1*H*-imidazol-2-yl)methyl]-1*H*-indazole (TCS-80) were described and tested in rats [1, 2]. Recently we have synthesized two novel marsanidine analogues that display hypotensive activity in rats: 7-chloro-1-[(imidazolidin-2-yl)imino]-1*H*-indazole (AK-71) and 7-chloro-1-[(4,5-dihydro-1*H*-imidazol-2-yl)methyl]-1*H*-indole (TCS-213). The observed decrease in blood pressure after imidazolines administration might be mediated through activation of the central α_2-adrenergic and/or I_1-imidazoline receptors and subsequent decrease of sympathetic nerve activity. Therefore, the present studies were performed to determine whether α_2-adrenergic and/or I_1-imidazoline receptors are involved in hypotensive effect of tested imidazoline compounds: marsanidine, TCS-80, AK-71 and TSC-213 in rats.

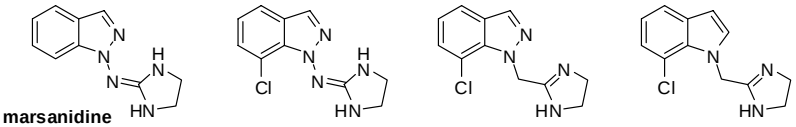

Fig.1 Imidazoline compounds – marsanidine analogues

Compounds were infused *iv* to anesthetized Wistar rats in the dose 100 μg/kg b.w. The *iv* injections of selective α_{2A}-adrenoceptor antagonist RX821002 (RX) or nonselective α_2-adrenergic / I_1-imidazoline receptor antagonist Efaroxan (EFA) were given 5 min before tested compound administration. Mean arterial blood pressure (MAP) and heart rate (HR) were monitored directly and constantly throughout the experiment.

Table 1. Influence of RX821002 (10 μg/kg) and Efaroxan (100μg/kg) administration on maximal hypotensive effect (ΔMAP) *[mmHg]* of tested compounds

	n	Compound	*n*	Compound + RX	*n*	Compound + EFA
AK-24	*5*	-32.3 ± 5.0 &	*5*	-19.1 ± 2.0*	*4*	-18.3 ± 1.1*
AK-71	*5*	-49.6 ± 3.8 &$	*5*	-38.9 ± 2.0*	*6*	-30.9 ±4.0*@
TCS-80	*5*	-43.1 ± 5.1 &$	*5*	-26.0 ± 2.4*	*5*	-16.4 ± 1.9*#
TCS-213	*5*	-61.2 ± 5.3 $	*4*	-37.6 ± 2.4*	*5*	-36.2 ± 3.7*@

p<0.05: * vs compound; # vs compound+RX; & vs TCS-213; $ vs AK-24; @ vs TCS-80+EFA;

Table 2. Influence of RX821002 (10 μg/kg) and Efaroxan (100μg/kg) administration on maximal chronotropic effect (ΔHR) *[bpm]* of tested compounds

	n	Compound	*n*	Compound + RX	*n*	Compound + EFA
AK-24	*5*	-116 ± 9	*5*	-89 ± 10	*4*	-91 ± 14
AK-71	*5*	-154 ± 16	*5*	-99 ± 8*	*6*	-90 ±4*
TCS-80	*5*	-112 ± 11	*5*	-65 ± 10*	*5*	-43 ± 6*
TCS-213	*5*	-138 ± 8	*4*	-88 ± 8 *	*5*	-70 ± 12*

p<0.05: * vs compound; n – number of experiments

Our study confirm that the hypotensive and chronotropic activities of marsanidine (AK-24) and its analogues AK-71, TCS-80 and TCS-213 are mediated by both the α_2-adrenergic and I_1-imidazoline receptors. Moreover, these experiments showed that the nonselective Efaroxan exerts more potent effect on circulatory activities of TCS-80 compound than the selective α_2-adrenoceptor antagonist RX821002.

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NOTEBOOK