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## Abstracts & Full Papers

ISPBS

# ISPBS-6

## The Sixth International Symposium on Pharmaceutical and Biomedical Sciences


26-28 May, 2022
Gaziantep-TURKEY

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6th ISPBS

The 6th International Symposium on Pharmaceutical and Biomedical Sciences

May 26 - 28, 2022

# ISPBS-6

# The Sixth International Symposium on Pharmaceutical and Biomedical Sciences

# ISPBS – 6

# PROCEEDINGS BOOK

## ABSTRACTS & FULL PAPERS

**May 26th – 28th, 2022**

**Gaziantep University – TURKIYE**

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# ISPBS-6
# ABSTRACTS & PROCEEDINGS BOOK

26-28 May 2022, Gaziantep University-Türkiye
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## Esteemed Colleagues and Dear Friends,

ISPBS-6 (The Sixth International Symposium on Pharmaceutical and Biomedical Sciences) aims to bring together leading academic scientists, researchers and research scholars to exchange and share their experiences and research results on the interdisciplinary aspects of analysis in the pharmaceutical, biomedical, clinical and omics sciences, as well as topics in related scientific area. It also provides a premier interdisciplinary platform for researchers, practitioners and educators to present and discuss the most recent innovations, trends, and concerns as well as practical challenges encountered and solutions adopted in the fields of Pharmaceutical, Biomedical and Biological Sciences.

Having respected scientific board and organizing committee members from all over the world, the ISPBS symposium series is the premier meeting for Pharmaceutical, Biomedical and Biological Sciences. It follows a series of successful symposium organized since 2016, when the first International Symposium on Pharmaceutical and Biomedical Sciences was launched at Kumamoto University-Japan. ISPBS-6 was organized on May 26-28, 2022 in an online manner (via ZOOM application) at Gaziantep University-Türkiye. This symposium was the sixth meeting series of ISPBS, and you can find abstracts of all the scientific works presented in ISPBS-6 in this ABSTRACTS & PROCEEDINGS BOOK.

We would like to thank for their sincere supports of Gaziantep University, Torbalı (Izmir) Chamber of Commerce-Turkey, Gaziantep University, Kumamoto University, Khon Kaen University, Rural Federal University of Rio de Janeiro (UFRRJ)-Brazil, Association of Medicinal and Aromatic Plants of Mediterranean, Association of Pharmaceutical Teachers of India, Cosmetic Producers and Researchers Associations, American Pharmacists Association, Japan Pharmaceutical Association, Phytochemical Society of Europe, Phytochemical Society of Asia, NS Herbals Company and all the other supporters. We would like to thank to all our participants from almost all over the world for their valuable attendance and scientific contribution to ISPBS-6. We are planning to organize the seventh meeting series of ISPBS in 2023 spring and on behalf of the organizing committee, we are looking forward to meeting you at ISPBS-7.

Sincerely,

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# INVITED SPEAKERS

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**KEYNOTE SPEAKER**

# LNP DESIGN AND MANUFACTURING CONSIDERATIONS

**Yvonne Perrie et al.**

*Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK. G4 0RE.*

## Abstract

The efficacy of RNA-based vaccines has been recently demonstrated, leading to the use of mRNA based COVID-19 vaccines delivered using lipid nanoparticles. To investigate the impact of different nanoparticle delivery platforms and administration routes on RNA-vaccine potency, we investigated the immunogenicity of a self-amplifying mRNA encoding the rabies virus glycoprotein encapsulated in different nanoparticle platforms (solid lipid nanoparticles (SLNs), polymeric nanoparticles (PNPs) and lipid nanoparticles (LNPs)). These were administered via three different routes: intramuscular, intradermal and intranasal. Our studies in a mouse model show that the immunogenicity of our four different saRNA vaccine formulations after intramuscular or intradermal administration was initially comparable; however, ionizable LNPs gave higher long-term IgG responses. The clearance of all 4 of the nanoparticle formulations from the intramuscular or intradermal administration site was similar. In contrast, immune responses generated after intranasal were low and coupled with rapid clearance for the administration site, irrespective of the formulation. These results demonstrate that both the administration route and delivery system format dictate self-amplifying RNA vaccine efficacy.

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## KEYNOTE SPEAKER

# REDOX PERSPECTIVE ON CANCER CELL FATE SIGNALING

**Shazib Pervaiz, MBBS, PhD**

*Department of Physiology and NUS Centre for Cancer Research (N2CR), Yong Loo Lin School of Medicine, National University of Singapore (NUS), Singapore; National University Cancer Institute, NUHS, Singapore; ISEP, NUS Graduate School, NUS, Singapore*

**Abstract**

Cellular transformation involves an inbalance between signaling networks that promote cell growth and proliferation and those that restrict abnormal accumulation by turning on exectuion of unwanted cells. This deregulation in growth homeostatsis is exemplified in a host of human cancers by way of amplified growth receptor signaling and/or transcriptional activation of genes associated with the various processes that promote carcinogenesis, such as cell cycle progression, inflammation, apoptosis inhibition and imune evasion. Notably, there is also ample evidence to implicate aberrant redox signaling in the acquisition of cancer hallmarks as well as response to chemotherapy. To that end, mitochondrial metabolism has emerged as a critical gatekeeper that regulates cellular redox status and cell fate signaling. To that end, work from our group has contributed to the redox dichotomy of cell fate signaling in cancer cells, whereby mild oxidative stress promotes cell survival, growth and proliferation while overt oxidative stress creates an environment conducive for death execution. In the efforts to understand the underlying mechanisms of this divergent function of intracellular reactive oxygen species (ROS) in cancer cell fate determination, we have unraveled cellular targets that are amenable to redox regulation/modification(s), both at the transcriptional and post-translational levels. These include the apoptosis inhibitory protein Bcl-2, oncoproteins c-Myc and K-Ras, death receptor inhibitory protein c-FLIP, the putative tumor suppressor phosphatase PP2A and the master transcription factor NF-kB that drives inflammation and other hallmarks associated with cancer. Furthermore, we provide evidence to link drug resistance to a switch to mitochondrial OXPHOS, as well as aberrant redox signaling to mitochondrial morphology changes and mitophagy induction. These signaling networks, their interplay and impact on the biology of cancer cells will be discussed.

**Key Words**: Cell fate, ROS, Bcl-2, c-Myc, NF-kB, PP2A

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**KEYNOTE SPEAKER**

# XANTONE: AN OLD "DOG" THAT LEARNS NEW TRICKS

**Madalena Pinto**[1,2]

[1]*Laboratory of Organic and Pharmaceutical Chemistry (LQOF), Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal*
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## Abstract

Xanthone (9H-Xanthen-9-one) is an "old" compound obtained by synthesis, but with many natural and synthetic derivatives that have in common a dibenzo-γ-pyrone skeleton. These compounds, from terrestrial and marine sources as well as their synthetic analogues, belong to a privileged structure, with wide structural diversity and biological/pharmacological activities, therefore being a source of great interest in Medicinal Chemistry. Based on this "old" scaffold our group obtained a large and diverse library of compounds, namely chiral derivatives, with potential applications as antitumor, antimicrobial, antifouling agents, as well as in cosmetic and analytics. Our strategy is based on molecules of marine origin as raw materials and/or models, improving synthetic methodologies for total synthesis and molecular modifications.

In this presentation, we will chart the evolution of this type of work in our group, with specific focus on the more recent results in the referred areas.

**Key Words**: xanthones, synthesis, antitumor, antibacterial, antifouling, chirality

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**KEYNOTE SPEAKER**

# QUALITY CONTROL OF POLYSACCHARIDES FROM HERBAL MEDICINES

**Li Shaoping, Zhao Jing**

*State Key Laboratory of Quality Research in Chinese Medicine, University of Macau, Macau 999078, China*

**Abstract**

Polysaccharides are biological macromolecules formed by the polymerization of more than 10 monosaccharides through glycosidic bonds. They are widely found in animals, plants and microorganisms. Actually, most traditional Chinese medicines are administered by decoction and oral administration which contains larger proportion of soluble polysaccharides. In last decades, with the development of "glycobiology", studies have found that polysaccharides not only participate in various physiological activities, but also have multiple pharmacological activities. However, their quality control is a challenge due to the comprehensive complexity.

In this presentation, the strategies for quality control of herbal glycans will be introduced, and glyco-analysis including the analysis of oligo- or poly-saccharides such as glycan profiling will be discussed based on our works.

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## KEYNOTE SPEAKER

# SELECTIVE OR DUAL INHIBITORS OF INFLAMMATORY $PGE_2$ AND $LTB_4$ BIOSYNTHESIS BY TARGETING mPGES-1 AND FLAP TO INTERVENE WITH INFLAMMATORY DEREGULATION

**Erden Banoğlu[1*], Burcu Çalışkan[1], Azize Gizem Ergül[1], Tuğçe Gür Maz[1], Abdurahman Olğaç[1], Oliver Werz[2]**

*[1] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, 06580, Ankara, Turkey*
*[2] Department of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, University of Jena, D-07743, Jena, Germany*
*E-mail: banoglu@gazi.edu.tr*

**Abstract**

The arachidonic acid (AA) pathway has an essential role in the biosynthesis of pro-inflammatory prostaglandin (PG)$E_2$ and leukotriene (LT)$B_4$. Released AA can be metabolized via two main pathways, namely cyclooxygenases (COXs) and lipoxygenases (LOs) to produce inflammatory $PGE_2$ and $LTB_4$. Although COX inhibitors blocking PG formation have widely been used to treat pain and inflammation, their clinical use is limited due to severe gastrointestinal side-effects, warranting to identify new therapeutic targets for effective and safer therapy of inflammatory conditions. Recently, two key proteins in COX and LO pathways, i.e., 5-LO-activating protein (FLAP) to produce $LTB_4$ and microsomal prostaglandin $E_2$ synthase-1 (mPGES-1) to produce $PGE_2$, are considered attractive therapeutic targets to selectively control inflammatory deregulation with less side effects. In this presentation, our recent efforts will be summarized in identifying several novel chemotypes as selective or dual inhibitors of FLAP and mPGES-1 to gain insight into the SAR of this family of compounds. Our results will show the ability of these new synthetic derivatives to turn into selective or dual FLAP/mPGES-1 inhibitors with potent in vitro and in vivo anti-inflammatory efficacy on both proteins.

**Key Words**: FLAP, mPGES-1, prostaglandin, leukotriene, inflammation.

**Acknowledgements**
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## INVITED SPEAKER

# SURFACE RECEPTORS, TRANSCRIPTION FACTORS AND INHIBITORY NANOBODY DISCOVERY

**Batu Erman**
*Department of Molecular Biology and Genetics, Boğaziçi University, Istanbul, TURKEY*

**Abstract**

## INVITED SPEAKER

# DEVELOPMENT OF A NOVEL COVID-19 TREATMENT APPROACH

**Derya Unutmaz, M.D.**

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*Department of Immunology, University of Connecticut School of Medicine, Farmington, CT, USA*

**Abstract**

Despite advances in antibody treatments and vaccines, COVID-19 caused by SARS-CoV-2 infection remains a major health problem resulting in excessive morbidity and mortality and the emergence of new variants has reduced the effectiveness of current vaccines. To address this potential problem, we sought to develop a novel treatment approach that can overcome the variations in the Spike protein of the the virus. Accordingly, we engineered primary human T cells to express SARS-CoV-2 Spike protein-specific chimeric antigen receptors (CARs), using extracellular region of ACE2, and demonstrated their highly specific and potent cytotoxicity towards Spike-expressing target cells. To improve on this concept as a potential therapeutic, we then developed a bispecific T cell engager combining ACE2 receptor with an anti-CD3 scFv (ACE2-Bite) that can bind to T cell receptor and the Spike protein expressed on infected target infected cells at the same time. Thus, by bridging the T cells and the target cells, we aimed to activate the T cells. Indeed, similar to CAR-T cell approach, ACE2-Bite activated and endowed cytotoxic T cells to selectively kill Spike-expressing targets. Furthermore, ACE2-Bite neutralized the pseudoviruses of SARS-CoV, SARS-CoV-2 wild-type and variants including Delta and Omicron, as a decoy protein. Remarkably, ACE2-Bite molecule showed a higher binding and neutralization affinity to Delta and Omicron variants compared to SARS-CoV-2 wild-type Spike proteins, suggesting the potential of this approach as a variant-proof, therapeutic strategy for future SARS-CoV-2 variants, employing both humoral and cellular arms of the adaptive immune response."

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**INVITED SPEAKER**

# VALORIZATION OF SOME *INSILICO* METHODS IN AZOLE BASED RESEARCH

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## Abstract

Azoles are a class of molecules possesses numerous biological properties such as antifungal, anticancer, anti-inflammatory etc. In the last two decades, the emergence of *in silico* tools has improved the research related to heathcare studies by providing significant predictions. In the present communication role of insilico techiniqes in the valorization of anmicrobial role of mercaptoimidazoles derivatives were explored with one example. The title compounds were prepared by employing green approach to give imidazole derivatives in excellent yields. Pharmacotherapeutic potential with the possible molecular mechanism of action of the compounds were estimated on the basis of PASS prediction results obtained by PharmaExpert software. The activity profile predicted by PASS was further supported by some theoretical calculations, in vitro experimental evaluation, and then validated via docking studies.

**Key Words**: Azoles, Insilico study, Docking, PASS

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**INVITED SPEAKER**

# MEDICINAL PLANTS AND NATURAL PRODUCTS FROM CAATINGA BIOME WITH ANTI-INFLAMMATORY ACTIVITY: THE CHEMISTRY BEHIND BIOLOGICAL ACTIVITY

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## Abstract

The Caatinga biome (semi-arid vegetation) is a highly threatened biome covering a vast area in Northeastern Brazil and is the source of few studied natural resources. Many medicinal plant species from Caatinga are widely known and used in folk medicine and for commercial manufacturing of phytotherapeutic products. Few ethnobotanical and pharmacological studies have been undertaken in this region, in spite of the great cultural and biological diversity to be found there. The purpose of this lecture is to present results of research carried out at the Federal University of Vale do São Francisco with the species *Hymenaea martiana* and *Passiflora cincinnata*, typical species from the Caatinga biome. The main chemical constituents identified in extracts of these species will be presented. Regarding the pharmacological activity, results will be presented on the antinociceptive and anti-inflammatory activity. The exact mechanism involved in the antinociceptive and anti-inflammatory activities is not completely understood but, at least in part there is the participation of opioid receptors and inhibition of cyclooxygenase enzyme. Docking studies confirm this hypothesis. Pharmacological and chemical studies are continuing in order to characterize the mechanism responsible for these effects.

**Key Words**: Medicinal plants, flavonoids, biological activity, phytochemistry, Caatinga.

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**INVITED SPEAKER**

# RECENT ADVANCES ON THE STABILITY OF DIETARY POLYPHENOLS

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**Abstract**

Dietary polyphenols are one of the most abundant groups of phytochemicals in food. Polyphenols are affected to a variable extent by different thermal and non-thermal processing technologies. Several terms including concentration change, degradation time, reaction kinetics and antioxidant potential have been applied to characterize the stability of polyphenols under certain designed experimental conditions. The stability of polyphenols in food matrixes are significantly affected by as pH value, photo/light, temperature, oxygen availability, metal ions, enzymes, proteins, nitrite salt, and sulfur dioxide, other antioxidants, and interactions with other food constituents. The hydroxylation of polyphenols always reduces their stability, while glycosylation, acylation, and pigmentation improve their stability. During thermal processing, polyphenols in food will be rapidly converted into various derivatives. However, there are few studies on the changes and mechanisms of polyphenols in complex food systems during thermal processing, and there are few reports on thermal degradation products, oxidation products, and enzymatic hydrolysis products of polyphenols in complex food systems. The main unstable products of polyphenols are yielded via dimerization, oxidation, hydroxylation and nucleophilic attack cleavage. The interactions between polyphenols and β-cyclodextrin/protein/polysaccrides via microcapsulation and encapsulation can improve the stability, solubility and bioactivity of polyphenols.

**Keywords:** stability; polyphenols; food matrixes; thermal processing; degradation; oxidation; enzymatic hydrolysis; dimerization; encapsulation; microcapsulation

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**INVITED SPEAKER**

# OLD SOURCES FOR NEW DRUGS IN CONTEMPORARY DRUG DISCOVERY

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## Abstract

The decline or leveling of the output of the R&D programs of the pharmaceutical companies may have begun to turn around when compared to earlier years of the 21st century. Although a responsible for this increase is the immunopharmacology-based treatments, small molecules still play an important role. Medicinal chemistry approaches to find a small molecule lead compound, which shows the desired pharmacological activity, continue to use as sources natural products, synthesis, and existing drugs.
Herein, examples of chemotherapic small molecules lead compounds obtained in our research group will be presented that arise from both natural and synthetic models. Strengths and opportunities in starting from privileged structures, drug repurposing, active metabolites, synthetic intermediates, or natural products as potential sources of new drugs will be highlighted. Case studies will include anticancer and antimicrobial drugs and are expected to contribute to a multidisciplinary vision in drug discovery, with the involvement of several sources.

**Key Words**: privileged structures, existing drugs, metabolites, synthesis, natural products.

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**INVITED SPEAKER**

# WHAT FUTURE FOR THE USE OF MEDICINAL PLANTS AS THERAPEUTIC TREATMENT?

**Pr. Dr. Mohammed Hmamouchi, Ph.D**

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**Abstract**
The medicinal plants have been used since ancient times for the treatment of human ailment without knowing their chemical composition and their active ingredients. Even today we have many examples of inadequately defined medicinal plants and there is still a lot of analytical work to do despite the plant parts are rich in various bioactive compounds. Today pharmaceutical companies constitute an important group of actors focusing on *bioprospecting*, the collection of plants taxonomically identified and screened for medically active components. In these cases main question: What research methodology should be applied to plant extracts for their uses? Since the understanding of formal and informal medical traditions in a modern clinical setting relies on technological advances, the scope of our current project is to contribute to the use of an interdisciplinary scientific approach (such as phytochemical analysis, biological evaluation of animal experimental models, toxicological studies, study of the molecular mechanism and the clinical trials of action of the principles isolated). We will present our approach, findings and then explores the current understanding of the chemical, pharmacological and biochemical properties of the extracts and the main active constituents. Our main focus is exploring new products and engineering its productivity. Studies carried out in our laboratory at the level of phytochemical and pharmacological tests relate to the study of 136 indigenous plant species, 148 extracts, 96 essential oils and 30 identified products. Plants that have been bred and studied are the most obvious choice for developing effective new drugs. We realized careful evaluation of this data to discover and evaluate the specific chemical entities responsible for traditional medicinal uses. We tested different germs and mushrooms for their content of active substances and found interesting extracts. Ours preclinical (in vivo and vitro) investigations have demonstrated antioxidants, hypolipidemic, immunomodulatory, anti-inflammatory, analgesics, antimicrobials, insecticides, antifungal, antibacterial, antidiabetic, and cardiovascular activities. Proposals are also reported.

**Key Words:** ethnobotany, phytopharmacological, chemical composition, bioactive compounds, antioxidants, antibacterial, antifungal, protozoa diseases, molluscicidal, anti-inflammatory, antitumor agents, hypolipidemic, hypercholesterolemia, vasorelaxant effects.

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**INVITED SPEAKER**

# THERAPEUTIC APPROACH TO INBORN ERRORS OF IMMUNITY

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**Abstract**

Inborn Errors of Immunity (IEIs) are a heterogeneous group of inherited disorders, characterized by increased susceptibility to recurrent severe infections, autoimmune diseases, lymphoproliferation and malignancies.

IEIs should not be considered as rare conditions anymore, while the number of diagnosed patients has significantly been growing up during recent years. Nevertheless, because of inadequate medical awareness, including in pediatricians, it is estimated that a significant number of patients with IEIs are not recognized. There are more than 400 different types of IEIs have been identified.

Although our understanding on IEIs is rapidly improving, there is still a delay in diagnosis of patients with IEIs, which leads to an increased rate of morbidity and mortality among the affected individuals. Suspicious to certain IEIs should be made according to their clinical phenotypes. Meanwhile the first step in the diagnostic process starts from a limited set of simple screening tests, which are available in most hospitals. Meanwhile definite diagnosis usually can only be made by genetic diagnosis, where it could change the treatment protocols based on the patients' conditions.

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## INVITED SPEAKER

# EVALUATION OF PROTECTIVE EFFECT ON METABOLIC SYNDROME OF *NYCTANTHES ARBOR-TRISTIS* IN FRUCTOSE-INDUCED HYPERTENSIVE RATS

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**Introduction**: Several epidemiological studies have found a progressive link between dietary fructose consumption and the development of MetS[1]. Flavonoid compounds found in a variety of plants have been demonstrated to have therapeutic benefits in cardiovascular disorders[2]. **Objective:** We investigated the effects of methanolic extract of *Nyctanthes arbor-tristis* (MNAT) 100,200, and 400 mg/kg/day p.o. for 6 weeks on cardiovascular parameters using Power Lab 4SP, in vivo antioxidant activities and biochemical parameters in fructose fed rats. **Methods:** A high fructose diet (fructose 10%, w/v) *ad libitum* for 6 weeks was used to induce hypertension in male Wistar rats (150–200 g)[3]. 60 Albino Wistar rats were randomly divided into a group of six, each group containing 10 animals. Group I received chow pellets and normal drinking water *ad libitum*. Group II received fructose (10%) solution .Group III received fructose (10%) solution and MNAT at a dose of 100mg/kg p.o. Group IV received fructose (10%) solution and MNAT at a dose of 200mg/kg p.o. Group V received fructose (10%) solution and MNAT at a dose of 400mg/kg p.o. Group VI received fructose (10%) solution and Enalapril (10mg/kg p.o) . Physiological parameters, ECG, heart rate, respiratory rate, blood pressure vascular reactivity to various drugs were measured and recorded by the invasive method[4]. The in vivo antioxidant activities of enzyme SOD and CAT, levels of TBARS, along with serum levels of leptin, adiponectin, glucose, triglycerides, cholesterol, uric acid, insulin, sodium, and potassium were measured. Cumulative concentration-response curve (CCRC) of Ang II and ACh were recorded. **Results:** MNAT treatment decreased MABP and altered vascular reactivity to various catecholamines. The activities of SOD and CAT enzymes exhibited a considerable increase and the levels of TBARS in the liver were reduced by MNAT treatment. MNAT has shown decrease in the plasma level of triglycerides, cholesterol, insulin and sodium while increase in plasma adiponectin and potassium levels. The cumulative concentration-response curve of Ang II was shifted towards the right by MNAT treatment using an isolated strip of rat ascending colon. MNAT treatment increased the contractile characteristics of the rat ascending colon in the CCRC of ACh as compared to the fructose-treated group. MNAT treatment reduced fructose-induced tissue damage (as observed in histopath studies) due to the consequence of metabolic syndrome. **Discussion and Conclusion:** MNAT is rich in flavonoids and therefore has powerful antioxidant properties. The findings show that by battling oxidative stress caused by fructose (10%) and reducing Ang II activity, MNAT may be able to prevent the development of high blood pressure and reverses MetS caused by fructose.

**Keywords:** Fructose, metabolic syndrome, hypertension, oxidative stress, *Nyctanthes arbor-tristis*
**Acknowledgement:** This work was supported by All India CTE MODROB [Project number: 9-270/IDC/MODROB/Policy-1/2019-20]

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**INVITED SPEAKER**

# THE ROLE OF THE SERUM EXOSOMAL AND ENDOMETRIAL MICRORNAS IN RECURRENT IMPLANTATION FAILURE

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**Abstract**

Recurrent implantation failure (RIF) is diagnosed when good-quality embryos repeatedly fail to implant after transfer in several in vitro fertilization (IVF) treatment cycles. RIF is a major problem encountered in IVF. Important factor in the development and function of RIF disorder is epigenetic regulation of gene expression, in which one of the most noteworthly molecules are microRNAs (miRNAs). It has been identified that endometrium specific microRNAs have different expression levels in endometrial tissues and maternal serum during endometrial cycle. There are several additional molecules which have been suggested to play a role in endometrial receptivity and implantation including different genes and miRNAs. However, little is known about the molecular events that provide receptivity before implantation and the mechanisms mediating early dialogue between the embryo and the endometrium Our group were to analyzed microRNA expression levels in recurrent implantation failure patients and healthy controls endometrial samples for enlightening the aetiopathogenesis of the disease.

Quantitative miRNAs expression level can be measured in biological sample by different methods which the most important method is quantitative real-time PCR (qRT-PCR) technique. This technology measures quantitatively expression of targeted miRNA in biological sample with high sensitivity.

In this study there are twenty RIF samples and ten normal fertility samples which are collected as two peripheral venipunctures (5 mL) and two endometrial biopsies; one in the proliferative phase (CD 7-10) and one in the implantation phase (CD 20-24). In first step RNA will be isolated from endometrium tissue and sera exosomes, next step is quantitative analyses of targeted miRNAs (hsa-miR-31, hsa-miR-30b, hsa-miR-145 and hsa-miR-23b) in samples and the last step is bioinformatic analysis of quantitative RT-PCR results.

The significant feature of this study is analysis of miRNA expression level in two different types of samples in same cases. The first aim in the study is revealing the factors involved in the biological process of disease by targeted expression of miRNAs. Second aim is to create a basis for developing a new theory for the potential treatment of RIF patients and the last aim of study is that significantly different expressed miRNAs can be used for non-invasive molecular biomarkers in early diagnosis of RIF disorder.

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**INVITED SPEAKER**

# ELECTROACTIVE BIOMATERIALS FOR SKELETAL MUSCLE TISSUE ENGINEERING APPLICATIONS

**Pathomthat Srisuk[1*], Vitor M. Correlo[2,3] and Rui L. Reis[2,3]**

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**Abstract**

Congenital defects, acute injuries from accidents or athletic activities, and neurological illnesses all degrade skeletal muscle functionality. Muscle anomalies can impair the body's overall physiological function, which in turn affects psychological reactions. Despite the innate ability of skeletal muscle tissues to self-repair, this ability is insufficient to restore functioning in the event of serious tissue injury or loss. The allocation of autologous muscle through surgical therapy is one of the most used clinical treatment techniques, however, clinical results are not very satisfactory. Novel electroactive spongy-like hydrogels were developed by combining gellan gum with two different conductive synthetic polymers: polypyrrole (PPy) and polyaniline (PANi). Gellan gum (GG) is a linear anionic polysaccharide of natural origin that has been extensively studied for a variety of biomedical applications, especially when processed as spongy-like hydrogels, it retains the structural properties of hydrogels that are relevant for tissue engineering applications. Thus, the rationale behind adding synthetic conducting polymers, PPy and PANi, was to improve the electroconductive properties of the final constructs, maintaining simultaneously the improved mechanical features and intrinsic cell-adhesive ability of GG spongy-like hydrogels. The physical, chemical, and electrical properties were analyzed, and bioactivity, as well as biocompatibility, was assessed both *in vitro* and *in vivo*. *In vitro* experiments revealed that both PPy-GG and PANi-GG electroactive spongy-like hydrogels showed high porosity and interconnected pores, resulting in enhanced cellular response. Moreover, a negligible inflammatory response was observed during *in vivo* analysis. The results demonstrate that the electroactive PPy-GG and PANi-GG spongy-like hydrogels meet all the functional requirements for mimicking the ECM microenvironment of muscle tissue, being interesting candidates to be used in skeletal muscle tissue regeneration strategies.

**Key Words:** skeletal tissue engineering, electroactive biomaterials, gellan gum, spongy-like hydrogels

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**INVITED SPEAKER**

# NATURAL COMPOUNDS FROM CITRUS FRUITS AS BIOACTIVE HYPOCHOLESTEROLEMIC COMPOUNDS AN *IN VITRO* STUDY

**Stefania Sut[1], Irene Ferrarese[1], Maria Giovanna Lupo[1], Ilaria Rossi[1], Nicola Ferri[1], Stefano Dall'Acqua[1]**

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**Abstract**

Bergamot (*Citrus bergamia*) is a common fruit in southern Italy with traditional uses for fever, sore throat, mouth, skin, respiratory and urinary system infections [1], [2]. Bergamot extracts in the last years have been frequently included in food supplements with claimed activity as cholesterol controlling agents. Some flavonoids are considered as the active compounds for the cholesterol lowering properties of the extracts [4]. Up to now the importance of bergamot constituents as hypocholesterolemic agents is still to be fully elucidated, and more research is needed to lighten possible molecular targets and mode of actions useful to assess doses and to establish its safety. With the same idea we selected the *Citrus tangelo,* Mapo as source of hypocholesterolemic compounds. Thus, the aim of this work was to study the constituents of *C. bergamia* and *C. tangelo* as hypocholesterolemic agents. Extract and isolated compounds were tested in cultured human hepatoma cell line Huh7 for their potential modulating properties of both LDL receptor (LDLR) and proprotein convertase subtilisin/kexin type 9 (PCSK9) expression.

The phytochemical composition of *C. bergamia* and *C. tangelo* extracts were assessed by LC-DAD-MS or GC-MS, and thirteen constituents were isolated from bergamot and six from Mapo using semipreparative HPLC. Structure was elucidated with MS, 1D and 2D NMR experiments. Compounds were tested, and significant effect was observed for flavonoids, especially melitidin, narirutin and neohesperidin from bergamot, that were able to induce the expression of both LDLR and PCSK9 in a similar manner of simvastatin. These results allowed us to ascribe at least in part the claimed bioactivity *C. bergamia* to some of its flavonoids. Thus, the identification of the active compound of bergamot represents one linkage of the molecular targets, LDLR and PCSK9, and the hypocholesterolemic effect of the plant.

**Key Words**: hypocholesterolemic agents, flavonoids, limonoids, citrus, HPLC, NMR, MS.

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**ORAL PRESENTATION**

# INHIBITION OF STRUCTURAL ALTERATION OF BIOMOLECULES DURING HYPERGLYCEMIA BY NATURAL PRODUCTS *IN VITRO*

**Dinesh Kumar, Prairna Balyan, Additiya Paramanya, Ahmad Ali***

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**Abstract**

The toxicity of glucose during hyperglycemia is due to its interaction with other biomolecules especially proteins and nucleic acids via a process known as glycation. As a result of this interaction a group of advanced glycation end products accumulate in the body which cause damage to the structure of biomolecules. These structrral alterations are implicated in many pathophysiological conditions. In recent times the focus has been onto develop natural products or their derivatives as antiglycating agents. The present study explores the role of some well-known natural products like ferulic acid, thymoquinone and phycocyanin in the inhibition of glycation-induced structural alteration of biomolecuels. The in vitro glycation system consisted of a sugar and a protein incubated for 28 days at 37 °C. The amount of glycation products generated were measured in the presence and absence of natural products by established methods like NBT, DNPH, and total AGEs by fluorometry. Glycation-induced aggregation and structural alteration were analysed using Thioflavin T method, CD and electrophoretic methods. The analysis of results in indicate the potential role of these natural products in the prevention of accumulation of glycation products at both early and advanced stages. Similarly, there was significant reduction in glycation-induced aggregation of proteins in the presene of natural products. Glycation causes noticeable changes in the structure of biomolecules whiuch was either reversed or inhibited by most of the natural products used in the study. Thymoquinone was found to be the most effective against glycation and its induced processes like aggregation, glycoxidation and sturcural alteration of biomolecules. It can be concluded that these natural compounds have potent antiglycating capacity alongwith their other known and reported properties like antioxidation.

**Key Words**: Aggregation, Ferulic acid, Glycation, Natural products, Phycocyaninc, Thymoquinone

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**ORAL PRESENTATION**

# INVESTIGATION OF THE EFFECT OF PROTEASOME INHIBITOR BORTEZOMIB ON CELLULAR SENESCENCE IN THE PARENTAL AND BORTEZOMIB RESISTANT PC3 PROSTATE CANCER CELL LINE

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**Objective/Purpose:** The Ubiquitin Proteasome Pathway (UPP) is a multienzyme and a multi catalytic pathway involved in the turnover of over 80% of intracellular proteins. UPP is involved in cellular signaling, transcription, cell cycle, apoptosis, immune regulation, tumorigenesis, and epigenetic mechanisms [1]. Proteasome inhibitors are a new class of chemotherapeutic agents. The first proteasome inhibitor introduced to clinics was bortezomib, by the approval of the U.S. Food and Drug Administration (FDA) in 2003. The therapeutic application of proteasome inhibitors in senescence is much less explored. Recent studies show that cellular senescence is not a passive antiproliferative program but a key cellular program that continually limits the proliferation of damaged cells [2]. Senescence is considered anti-tumorigenic as it inhibits cell division and triggers immune clearance of pre-malignant cells. However, in the Senescence-associated secretory phenotype (SASP), bioactive proteins are widely secreted from senescent cells, and they also cause tumor development by stimulating neighboring non-senescent cells. SASP cells are also thought to be responsible for chronic inflammation in the ageing process [3]. Our study aimed to examine the senescence states of parental and bortezomib resistant PC3 prostate cancer cell lines in the absence and presence of bortezomib treatment. **Material and Methods:** To detect senescence, we analyzed the expression of CDK inhibitor p16 INK4a protein expression by Western blot. Then, the expression of SASP phenotype factors have been detected by the Human Cytokine Array and the expression of MMP-1 (one of the SASP factors) was been analyzed by Western blot. Lastly, we analyzed β-galactosidase activity at pH 6.0, which is one of the well-known markers of senescence. **Results:** It was observed that expression of MMP-1 was decreased in both PC3-P and PC3-R cells after 100 nM bortezomib treatment for 24 hours and 48 hours compared to the control groups. Moreover, it was shown that p16 INK4a decreased in both cell lines compared to the control group after the application of 100 nM bortezomib for 48 hours. Investigation of β-galactosidase activity showed that this activity decreased as a result of bortezomib treatment in PC3-P cells. **Conclusions**: Our results indicate that bortezomib has a senescence-reducing effect and it may be a senolytic drug candidate.
**Key Words**: Senescence, proteasome inhibitors, bortezomib, prostate cancer, senolytic drugs

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**ORAL PRESENTATION**

# ANTI-QUORUM SENSING AGENTS IN THE FIGHT WITH SARS-CoV-2 INFECTION

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**Abstract**

**Objective:** Quorum sensing (QS) is a bacterial cell-cell communication process in which cells regulate the transcription of the specific genes responsible for antibiotics production, biofilm formation, cell division, etc. QS inhibition is evaluated as a remarkable point to develop anti-infective treatments as blocking of QS would decline the pathogen virulence, making them more susceptible to therapy [1]. SARS-CoV-2 or COVID-19 known as coronavirus has recently occurred a serious threat to human health. Due to the uncertainty of effective treatments for this virus, there is an urgent need for anti-infective agents [2]. In this study, thus, we focus on revealing effective anti-QS agents to fight SARS-CoV-2.

**Method:** Recently published articles in the Web of Science database concerning the anti-QS activity were investigated for SARS-CoV-2 infection. Searches were performed using keywords of "quorum sensing", "anti-quorum sensing", "COVID-19" and "SARS-CoV-2" in the titles, and/or the abstracts.

**Results:** In this study, the agents with QS inhibition potential were evaluated for their ability to combat SARS-CoV-2 infection. According to the search from the Web of Science database, phenolic compounds such as curcumin, resveratrol, quercetin, apigenin, some *Eucalyptus* species, *Citrus* flavonoids, thymol and thyme essential oils could play a significant role in the fight with COVID-19.

**Conclusions:** Anti-QS agents are therapeutic compounds that might be of great importance in the fight against viral infections and, could also help prevention of COVID-19 infection. Therefore, these agents should be investigated in more detail with their anti-viral aspects and studied at the molecular level as a therapy option for the treatments of viral infections such as COVID-19.

**Key Words**: Anti-quorum sensing, SARS-CoV-2, COVID-19, curcumin, resveratrol, limonene.

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**ORAL PRESENTATION**

# CATECHOLAMINERGIC NEURONS IN THE DORSAL AND VENTRAL HIPPOCAMPUS ARE INVOLVED IN THE RAT'S SOCIAL MEMORY

**Ahmed Algali[1], Hilal Akyel[1], Burcu Nur Akguner[1], Elif Cinar[2], Gul Yalcin-Cakmakli[3], Bulent Elibol[3], Banu Cahide Tel[1]**

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**Abstract**

**Introduction**: Changes in catecholamines in different brain regions have been linked to a variety of neurodevelopmental and neurodegenerative diseases that affect sociability and social memory. In this study, we investigated whether the catecholaminergic neurons in the dorsal and ventral hippocampus contribute to the social memory in rats.

**Method**: Female Wistar rats weighing 220-280g (for all groups n=8, except for sham n=3) were injected bilaterally with 6- OHDA (6 ug/hemisphere) in either the ventral or dorsal hippocampus. Following a 10-day recovery period, behavioral tests were conducted including open-field locomotor activity (OF), buried-food seeking (BFS), 3-chamber social memory (3-CSM), and 2-trail direct interaction (2-TDI). Ethical approval was obtained from the Ethics Committee of Hacettepe University (No. 2022/01-09).

**Results:** In OF, the depletion of catecholamines in the ventral hippocampus increased the locomotor activity ($p<0.05$), whereas in the dorsal hippocampus it did not affect the locomotor activity. Regarding BFS, there is no difference between the groups in the latency to find the pellet. In comparison to the naive group, catecholamine depletion in either the dorsal or ventral hippocampus was sufficient to impair social memory in the 3-CSM. Furthermore, catecholamine depletion in the ventral hippocampus impaired sociability in 3-CSM. The dorsal group demonstrated impaired social memory but not sociability in the 2-TDI. However, the ventral group, showed an impaired sociability shown as a decrease in interaction time with both familiar and novel rats($p<0.05$).

**In conclusion**, the catecholaminergic neurons in the dorsal and ventral hippocampus play a key role in modulating the social memory in rats. Moreover, the ventral hippocampus catecholaminergic neurons may play more important role than the dorsal one in modulating sociability and locomotor activity in rats. These findings could open the door for further investigation of their role in neurodevelopmental disorders such as autism spectrum disorder (ASD).

**Key Words**: Catecholaminergic neurons, hippocampus, social memory, rat, 6-OHDA

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**ORAL PRESENTATION**

# POTENT HETERO-OLIGOARYL LIGANDS FOR CANCER-RELEVANT G-QUADRUPLEX DNA

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**Abstract**

Oligomeric compounds, consisting of consecutive N,O-rich heteroaromatic rings, introduce useful and tunable properties as versatile ligands for biomolecular recognition. In this study, we have employed a synthesis relying on Van Leusen oxazole formation, in conjunction with C–H activation of the formed oxazoles and subsequent C–C cross-coupling to 2-bromopyridines in order to assemble a focused library of variable-length, 'head-to-tail'-connected, pyridyl-oxazole oligomers. Through investigation of the interaction of these ligands (5-mer, 6-mer, 7-mer) with cancer-relevant G-quadruplex structures (human telomeric/22AG and c-Myc oncogene promoter/Myc2345-Pu22), the asymmetric pyridyl-oxazole motif has proved to be a prominent recognition element for G-quadruplexes. Fluorescence titrations reveal excellent binding affinities of the 7-mer and 6-mer for a $Na^+$-induced antiparallel 22AG G-quadruplex ($K_D = 0.6 \times 10^{-7}$ $M^{-1}$ and $0.8 \times 10^{-7}$ $M^{-1}$, respectively), and satisfactory affinities for the 22AG/$K^+$ and Myc2345-Pu22/$K^+$ G-quadruplexes. All ligands tested exhibit ability for stabilizing G-quadruplexes, along with substantial selectivity for G-quadruplex versus duplex (ds26) DNA, as evidenced by competitive Förster resonance energy transfer (FRET) melting assays. Additionally, the 7-mer and 6-mer are capable of promoting a switch-like topological transition of 22AG/$K^+$ G-quadruplex.

**Key Words:** pyridyl-oxazoles, N,O-oligoaryl ligands, G-quadruplexes, conformational transition, C–H activation.

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## ORAL PRESENTATION

# ORAL CARE COMPOSITOIONS WITH ANTIMICROBIAL AND ANTIBIOFILM-FORMING PROPERTIES BASED ON MEDICINAL HERBS

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Awareness of the problem of formation and circulation of microorganisms resistant to antimicrobial preparations is unceasingly growing. In this condition, the development of hygiene products with antimicrobial properties based on natural, especially herbal substances encourages particular attention. For a long time, medicinal herbs have been used in folk and traditional medicine due to a broad spectrum of their biological activity. The application of compositions based on medicinal herbs with antimicrobial, antioxidant and anti-inflammatory activity is especially important for prevention and comprehensive care of the oral cavity affected by inflammatory periodontal diseases. The multifactorial progression of such diseases that includes the infection factor and disorders of the antioxidant and immune status of periodontal tissues and mucous membrane, explains the relevance and long-term viability of development of herbal-based compositions with complete activity. It is important that inflammatory periodontal processes are accompanied by growth and ratio distortion of both periodontopathogenic microorganisms and representatives of the facultative microbiota characterized by high level of antibiotic resistence and biofilm-forming ability.

We have conducted a comprehensive screening of antimicrobial and antibiofilm-forming properties of herbal extracts (18 species) and essential oils (15 species) originating from the Carpathian region. The susceptibility of microorganisms to plant-based preparations was identified by agar diffusion method and determination of minimum inhibitory concentrations (Balouiri M et al., 2016). The antibiofilm activity was tested in 96-well microtitration plates spectrophotometrically (Greiner-BioOne, Austria) according to (O'Toole G, 2011).

*Test cultures*. The following were used for the purpose of the study: reference museum cultures ATCC (American Type Culture Collection, USA) *Candida albicans* ATCC 885-653; *Staphylococcus aureus* ATCC 25923*; Escherichia coli* ATCC 25922*, Enterococcus faecalis* ATCC 29212, *Streptococcus pyogenes* ATCC 19615, *Pseudomonas aeruginosa* ATCC 27853, and clinical cultures isolated from the oral cavity of patients suffering from inflammatory periodontal diseases: microscopic fungi of *Candida* (*C. albicans),* and bacterial isolates *S. aureus, E. coli, S. pyogenes, E.faecalis, H. alvei*, and *K. rhinoscleromatis.*

Based on the obtained results, medicinal herbs with proven antimicrobial, antibiofilm-forming and antioxidant effect upon antibiotic resistant biofilm-forming microorganisms isolated from periodontal diseases were chosen. The study proved high activity level of the following extracts: *Vaccinium vitis-idaea L.* (leaves), *Potentilla erecta L.* (rhizome), *Equisetum arvense L.* (shoots), and of the following essential oils: *Thymus vulgaris* L., *Origanum vulgare* L. and *Mentha pipperita* L. The chosen plants were combined into compositions to ensure additive activity – in addition to their antimicrobial activity, they are able to prevent the formation and to destruct biofilm. The herbal components in the composition of oral hygiene products provide for antimicrobial, deodorizing and antibiofilm-forming effect. The obtained compositions are promising as constituents of oral hygiene products targeted at patients suffering from periodontal diseases in professional combination treatment protocols.

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**ORAL PRESENTATION**

# CARDIOPROTECTIVE POTENTIAL OF FRUITS OF PISTACIA PALAESTINA BOISS EXTRACT ON ISOPROTERENOL-INDUCED CARDIAC INJURY IN RATS

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**Abstract**
**Objectives:** The treatment options for decreasing the damage produced by myocardial ischemia are limited and not devoid of adverse effects. When the plants that can be used in cardiovascular diseases are examined, it is known that Pistacia species, known as peanut, are used as cardiotonic and invigorating. Pistacia species has recently attracted attention in alternative therapies as an important source of phenolic compounds, terpenoids, monoterpenes, flavonoids, alkaloids, saponins, fatty acids and sterols. The study aimed to investigate the protective effects of Pistacia palaestina Boiss (P. palaestina) fruits extract which is thought to have antioxidant and anti-inflammatory properties, in isoproterenol (ISO)-induced MI. **Methods:** Male Sprague-Dawley rats were divided into control, ISO-control, P. palaestina fruits (250 and 500 mg/kg, respectively), P. palaestina fruits 250+ISO, P. palaestina fruits 500+ISO groups. ISO was administered at 120 mg/kg at two consecutive days and P. palaestina fruits 250 and 500 mg/kg/day were administrated for 16 days. At the end of the 18th day, the rats were sacrificed. Tissue samples were stored at −80°C in a deep freeze until analysis. **Results:** There was a significant increase in TBARS level in ISO-control group. A significant decrease in SOD, CAT, GSH, GPx was seen with ISO-induced MI. P. palaestinafruits pretreatment (250 and 500 mg/kg, respectively) significantly ameliorated TBARS activity, Troponin t, CK-MB, TNF-α, IL-1β, IL-6 levels. **Conclusions:** Our results suggest that P. palaestina fruit extracts significantly protect against cardiac injury and ISO-induced MI.
**Key Words**: P. palaestina fruit extracts, isoproterenol, cardiac ischemia, oxidative stress, inflammation

**Acknowledgements**
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**ORAL PRESENTATION**

# METABOLIC PROFILE AND ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF *LYTHRUM SALICARIA* L.

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## Abstract

**Objectives:** Metabolomics is defined as a comprehensive quantitative and qualitative analysis of large scale of metabolites [1]. Alzheimer's disease is a neurodegenerative disease and compounds/extracts with acetylcholinesterase inhibitory activity are potential drug candidates for Alzheimer's [2,3]. In this study acetylcholinesterase inhibitory activity and metabolic profile of *Lythrum salicaria* L. (Lythraceae) were investigated. **Materials and Methods:** Aerial parts of *Lythrum salicaria* were extracted by methanol to determine metabolic profile by using GC-MS and LC-QTOF-MS. Also, an enzymatic assay was performed for acetylcholinesterase inhibitory activity on the same extract. **Results:** 278 known and 1106 unknown metabolites were detected by using gas chromatography-mass spectrometry (GC-MS) while 261 known 39398 unknown metabolites by using liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS). The methanolic extract of *Lythrum salicaria* was exhibited acetylcholinesterase inhibitory activity with the $IC_{50}$ value of 129.9 µg/mL. **Conclusions:** Methanolic extract of aerial parts of *Lythrum salicaria* showed significant acetylcholinesterase inhibitory activity. Fatty acids and conjugates; amino acids, peptides, and analogues; carbohydrates and carbohydrate conjugates were detected by the GC-MS analyses while flavonoid and anthocyanidin glycosides were the major groups detected by the LC-QTOF-MS analyses.

**Key Words**: *Lythrum salicaria*, Acetylcholinesterase inhibitory activity, Metabolomics.

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## ORAL PRESENTATION

# *PISTACIA PALAESTINA* BOISS LEAF EXTRACT IS CARDIOPROTECTIVE IN ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION BY SUPPRESSING TNF-α, IL-1, IL-6 SIGNALING PATHWAYS, INFLAMMATION, AND OXIDATIVE STRESS

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**Abstract**

**Objectives:** Myocardial infarction (MI) is one of the leading causes of death worldwide. The increase in inflammation triggered by the disruption of the oxidant/antioxidant balance during MI reduces cell viability and heart functions. The study aimed to investigate the protective effects of Pistacia palaestina Boiss leaves extract (*P. palaestina* leaves), which is thought to have antioxidant and anti-inflammatory properties, in isoproterenol (ISO)-induced MI.

**Methods:** Forty-eight Spraque Dawley rats were divided into 6 groups in the study (n=8). Control: were administrated saline, ISO group: were administrated ISO (120 mg/kg, ıp on the 17th and 18th days of the experiment), P. palaestina leaves 250 control group: were administrated P. palaestina leaves (250 mg/kg/day orally for 16 days), P. palaestina leaves 500 control group: were administrated P. palaestina leaves (500 mg/kg/day orally for 16 days), P. palaestina leaves 250+ISO group, and P. palaestina leaves 500+ISO group. Thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) activity, catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) levels were measured in heart tissue. Troponin t, CK-MB, necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), IL-6, and IL-10 levels were determined in serum by the Elisa method.

**Results:** While lipid peroxidation indicator TBARS activity increased in the ISO group, antioxidant enzyme levels and GSH activity decreased. Troponin t, CK-MB, TNF-α, IL-1β, and IL-6 levels, which are cardiac serum biomarkers, increased while anti-inflammatory IL-10 levels decreased. Low and high dose P. palaestina leaves treatments significantly decreased TBARS activity, Troponin t, CK-MB, TNF-α, IL-1β, IL-6 levels, improved antioxidant enzyme levels, and GSH activity.

**Conclusions:** *P. palaestina* ameliorated cardiac biomarkers in ISO-induced MI by suppressing oxidative stress, inflammation, and apoptosis. *P. palaestina* leaves may play an important cardioprotective role in the treatment of MI with their antioxidant and anti-inflammatory effects.

**Key Words**: *Pistacia palaestina* Boiss leaves, Myocardial infarction, Oxidative stress, Inflammation

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**ORAL PRESENTATION**

# TRAM-34 PREVENTS FRUCTOSE-INDUCED HYPERTENSIVE RESPONSE IN RATS

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## Abstract

It is suggested that high fructose intake increases blood pressure through brain microglia activation and neuroinflammation which trigger the sympathetic system in rats. Since intermediate-conductance calcium-activated potassium channels (KCa3.1) play a critical role in microglial activity, we hypothesized that the KCa3.1 inhibitor TRAM-34 could prevent blood pressure increase in fructose-induced hypertensive rats. However, the blocking effect of TRAM-34 on endothelial KCa3.1 may mask this effect by decreasing endothelium-dependent-hyperpolarizing (EDH-type) relaxations.

To test this, rats were assigned into 4 groups (n=7). The control (CON) had ad libitum access to water, whereas the fructose group (FRU) was given water with 10% fructose for three weeks. TRAM-34 (40 mg/kg) was administered i.p. twice daily thorough three weeks (FRU+TRAM). Another group of rats received minocycline (45 mg/kg) via oral gavage once daily (FRU+MINO) as a positive control for microglia inhibition. Systolic blood pressure (SBP) and heart rate (HR) were measured with tail-cuff in all groups. At the end of the experiments, rats were euthanized and mesenteric arteries were isolated for determining the acetylcholine-induced EDH-type relaxations in all groups (Tukey 2-way ANOVA).

The SBP increased after 3 weeks of fructose intake in FRU (141.7±3.4 mmHg) compared to CON (118±1.9 mmHg) ($p<0.05$). Both minocycline (119.4±2.2 mmHg) and TRAM-34 (111.4±2.9 mmHg) significantly prevented this increase. Fructose intake also accelerated HR (393.6±8.8 beat/min) compared to the CON (358±8.3 beat/min) ($p<0.05$). TRAM-34 (351±13.4 beat/min), as well as minocycline (338.4±14.8 beat/min), reversed this effect ($p<0.05$). No significant change in EDH-type relaxations was observed in any of the groups compared to CON.

Our data suggest that TRAM-34 is as effective as minocycline in preventing fructose-induced hypertension without interfering with EDH-type vasodilation. Although TRAM 34 is known to reach the brain, further experiments are required to reveal the precise mechanism of action of TRAM 34 on microglial activity.

**Key Words**: Hypertension, fructose, TRAM-34, minocycline, rat.

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**ORAL PRESENTATION**

# THE CURRENT TRENDS AND TREATMENT GUIDELINES OF GENITAL LICHEN SCLEROSUS

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**Abstract**
Lichen sclerosus is a common chronic inflammatory skin disorder that most often affects genital and perianal areas. All age may be affected with LS, but it is more common in perimenopausal andmenopausal women. Pruritus is the main symptom seen in more then 90% of the women while pain and problems with erection in males. Initial sign may be non-specific like slight erythema, fissures, oedema. Later there can be hyperkeratosis, atrophy, sclerosis, scaring. The diagnosis is made clinically in most cases. The indication for biopsy is when diagnosis in uncertain or whenplanocelular carcinoma is suspected. The aim of treatment is to improve the quality of life, and improve symptoms and signs and to prevent scaring and development of the cancer.

Recent studies did not confirm the association with autoimmune diseases like thyroid disease, vitiligo, autoimmune bowel disease, rheumatoid arthritis, etc. Koebner phenomenon (mechanicalfactors like friction due to tight clothing, occlusion, surgical trauma, scars, etc.) might play an important role in triggering and maintaining LS. Recent research has identified altered enzyme expression in vulvar LS resulting from an epigenetic change and pointing to a possible epigenetic background for pathogenesis. Potent and super-potent topical corticosteroids are the gold standard for obtaining remission in genital LS. Studies have confirmed their safety and highly effectiveness in both children and adults. Patients are reviewed every 6 months until they have been in a stable remission for 2 years. Long-term treatment should be adjusted according to the severity of disease.

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**ORAL PRESENTATION**

# AN OVERVIEW OF THE SIGNIFICANCE OF CHIRALITY IN DRUG MOLECULES IN TERMS OF EFFICACY AND TOXICITY VARIATIONS

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## Abstract

**Background and Objective:** In drug molecules, stereoisomeric structures are formed by the different orientations of the atoms around the chiral center. These structures, which are mirror images of each other but can not be superimposed like our right and left hands, are called enantiomers. The physical and chemical properties of enantiomers are identical and they don't differ in the interaction with the achiral systems. Their differences emerge when they interact with the chiral systems. The biological targets such as receptors, nucleic acids and enzymes to which drugs have to bind are chiral. Therefore, like the incompatibility of our right hand with the glove producted for our left hand, either one of the enantiomers may not interact with the biological systems that are compatible with the other one or may bind to completely different systems. As a result of this, significant differences may occur in terms of pharmacokinetic, pharmacodynamic, therapeutic and toxicological aspects of enantiomers. The intoxication cases in Pakistan (2012) and Paraguay (2013) resulting from the syrups containing dextromethorpan contaminated with levomethorpan were one of the most striking examples showing the significance of chirality in drug molecules in the recent past. Considering this, this study aims to overview of the different pharmacokinetic, pharmacodynamic, therapeutic and toxicological effects of the enantiomers of chiral drugs and chiral switch approaches. **Method:** The information in this review was gathered from the published articles and WHO's Drug Alerts. **Results:** After the policy statement of FDA (1992), the importance of identifying enantiomers of chiral drugs has increased. Since then, chiral switch approaches have been widely adopted and many enantiopure drugs have been marketed. Currently, 12% of the chiral drugs are marketed as enantiopure. **Conclusion:** The efficacy of the enantiomers of chiral drugs should be evaluated separately and usage of either as enantiopure or as racemate should be decided considering the benefit-harm-cost balance.

**Key Words**: Chiral drugs, Enantiopure drugs, Chiral switch, Racemate

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**ORAL PRESENTATION**

# DESIGN, SYNTHESIS AND α-GLUCOSIDASE INHIBITORY ACTIVITY OF SOME QUINAZOLIN-4(3*H*)-ONE & 4-AMINO BENZENESULFONAMIDE HYBRID COMPOUNDS

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## Abstract

**Background and Objective:** Diabetes is a chronic metabolic disease that has a high prevalence rate and can cause fatal complications. Therefore, it's necessary to treat diabetes effectively. Diabetes treatment protocol aims to reduce high blood glucose levels in patients and α-glucosidase inhibitors play an important role in managing the disease. The efficacities of the drugs currently used as α-glucosidase inhibitors are limited and high-cost synthesis procedures are needed for producing them. So, there is an urgent need for new α-glucosidase inhibitor drugs which are more efficient and can be obtained with low-cost synthesis procedures. For this purpose, some novel quinazolin-4(3*H*)-one & 4-aminobenzenesulfonamide hybrid compounds were synthesized and evaluated for their α-glucosidase inhibitory activities in this study. **Methods:** The title compounds were synthesized by coupling of 2-chloroquinazolin-4(3*H*)-one and appropriate 4-amino-*N*-(substitutedphenyl) benzenesulfonamide intermediates, each obtained with three-steps reactions. Their structures were confirmed by spectral analysis and α-glucosidase inhibition assays were performed by spectrophotometrical method using a microplate reader. Results were expressed % inhibition of α-glucosidase inhibitory activity at 100 µM concentration of tested compounds and the reference drug acarbose **Results:** According to the biological activity results, all the synthesized compounds (1-4) showed α-glucosidase inhibition equal to or higher than the reference drug acarbose at 100 µM concentration. **Conclusions:** Preliminary activity screening results indicated that quinazolin-4(3*H*)-one & 4-aminobenzenesulfonamide hybrid molecules could be promising compounds for further studies in the development of new α-glucosidase inhibitors.

**Key Words**: Synthesis, Quinazolin-4(3*H*)-one, 4-Aminobenzenesulfonamide, α-Glucosidase Inhibitors

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**ORAL PRESENTATION**

# A NEW HPLC METHOD WITH UV DETECTION FOR THE DETERMINATION OF CARNOSOL IN HUMAN PLASMA AND APPLICATION TO A PHARMACOKINETIC STUDY

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## Abstract

In this study, to present a simple and sensitive, HPLC-UV method, which was developed to determine carnosol in human samples. Chromarographic separation was achieved with C18 column (150 mm × 4.6 mm × 5 µm), at 25 ºC with gradient elution of the mobile phase consisting of methanol-water (2% o-phosphoric acid) at flow rate 1.2 mL/min. The analyte was detected at 230 nm by UV detector. The retention time of carnosol is 3.40±0.01 min. This currently developed method was validated according to ICH criteria by evaluating the specificity, linearity, precision, accuracy and robustness. The method was determined to be linear in a concentration range of 1–20 ng/mL with the correlation coefficient of 0.9942. The proposed method was applied successfully to the analysis of carnosol in spiked human plasma with good recovery as 96.4 % and the precision of the method was determined by intra day and interday assays with the highest RSD % values 5.71. The method successfully applied to a pharmacokinetic study with determination of $C_{max}$, $t_{max}$, $t_{1/2}$ and AUC, by administration of carnosol to a healthy volunteer.

**Key Words**: Carnosol, HPLC-UV, Validation, Pharmacokinetics

**Acknowledgements**
This study is financially supported by the Scientific Research Projects Units of Bezmialem Vakıf University (Project No:20200203).

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**ORAL PRESENTATION**

# EVALUATION OF ANTIOXIDANT AND ENZYME INHIBITORY ACTIVITY OF THREE DIFFERENT TEAS

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## Abstract

Tea has been one of the widely consumed beverages all over the world for thousands of years. In this study, three different types of tea (black, green, and white tea) obtained from the *Camellia sinensis* plant were investigated in terms of antioxidant and enzyme inhibition activities. Total phenol and flavonoids were investigated by Folin-Ciocalteu and aluminium chloride colorimetric method respectively. The antioxidant activity was assessed with DPPH and ABTS radical scavenging assay. Extracts prepared from three different types of tea were investigated by the 96-well plate method for their inhibitory effect against important enzymes in the treatment of human pathologies such as: diabetes ($\alpha$-amylase and $\alpha$-glucosidase), neurodegenerative disorders (acetylcholinesterase and butyrylcholinesterase) and hyperpigmentation (tyrosinase). According to results, thegreen tea extract showed strong DPPH radical scavenging and tyrosinase inhibitory activity than the black and white tea extracts. The green tea extract contains higher amount of phenolic compounds (185.98±0.48 mgGAE/g) while black tea extract contains highest total flavonoid contents (80.23±6.51 mgQE/g). Green tea extract was found to have the highest inhibition effect on acetylcholinesterase and butyrylcholinesterase enzymes used in Alzheimer's disease therapeutic strategy. The results suggests that differet tea types ara a valuable source of polyphenolic compounds and functional dietary supplements and green tea has a potential use in antioxidant and anti-alzherimer drug formulations as well as food supplements.

**Key Words**: Tea, *Camelia sinensis*, antioxidant activity, enzyme inhibitory

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ORAL PRESENTATION

# INVESTIGATION OF ANTIOXIDANT, TYROSINASE INHIBITORY AND DNA INTERACTION PROPERTIES OF LEAF EXTRACTS FROM *FICUS CARICA*

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## Abstract

Türkiye is the world's leading producer of figs, the typical fruit of the Mediterranean coast. Referring to *Ficus carica* (Moraceae), the fig has been widely cultivated since ancient times due to the nutritional value of its fruits [1]. Its fruit, root, and leaves are traditionally used to treat a variety of ailments, including diarrhea, sore throats, coughs, inflammatory, cardiovascular, and ulcerative diseases [2]. In this study, total phenolic content, tyrosinase inhibitory, and DNA interaction effects of *F. carica* leaf extracts were investigated.

*F. carica* leaves were extracted with 70% methanol at 40°C under reflux. The extract was respectively fractionated with *n*-hexane, dichloromethane, and *n*-butanol to obtain extracts of different polarities. Total phenolic content, DPPH radical scavenging, anti-tyrosinase actions of all extracts were investigated using spectrophotometric methods [3]. Moreover, DNA-damage protective properties of extracts against Fenton's reagent, UV radiation, and singlet oxygen were investigated using electrophoretic methods [3].

It was determined that *n*-butanol extract had the highest total phenolic content with 72.58 ± 4.52 mg GAE/g dry weight. The n-butanol extract which was found to show the highest tyrosinase inhibitory actions among the extracts showed 37.01% ± 1.15% and 82.57 ± 0.88% radical scavenging activity at 80 and 200 µg/mL, respectively. In electrophoretic studies, Form I percentage of DNA control was 90.90%. It was determined that the density of Form I in all bands did not change remarkably and was around 90-95%. In this case, it was determined that the extracts did not damage plasmid pBR322 DNA at studied concentrations. Finally, the *n*-butanol extract had the highest protective effects against Fenton's reagent, UV radiation, and singlet oxygen.

In the light of these results, it can be argued that *F. carica* leaves can be evaluated for the development of products with the potential to be used in the treatment of many diseases.

**Key Words**: *F. carica*, antioxidant, tyrosinase, Fenton's reagent, DNA-damage.

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**ORAL PRESENTATION**

# EVALUATION OF SYSTEMIC INFLAMMATORY AND OXIDATIVE STRESS STATUS IN NATURALLY OVERWEIGHT DOGS

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## Abstract

In this study, it was aimed to evaluate the parameters related to systemic inflammation, oxidative stress and liver in naturally overweight dogs.

A total of 20 dogs were used in the study, including 10 naturally overweight (BCS >6-9) owner dogs and 10 owner dogs with breed and age-specific ideal BCS score (BCS <6). After systematic clinical examination of the dogs, CBC, CRP, total protein, albumin, ALT, AST, triglyceride, cholesterol, HDL, total antioxidant, total oxidant and paraoxanase-1 levels were determined in the blood samples taken into tubes with and without anticoagulant.

Compared to the values found in dogs with ideal weight, it was determined that the monocytes ($p= 0.039$), AST ($p=0.02$), CRP ($p< 0.001$) values measured in naturally overweight dogs were statistically significantly higher, but HDL ($p= 0.045$) and PON-1 ($p< 0.001$) values were found to be statistically significantly low.

It was concluded that AST, which is one of the liver enzymes values, and monocytes, CRP, HDL and PON-1 levels, which are among the systemic inflammation and oxidative stress parameters, are very valuable indicators in terms of evaluating the burden on the organ systems of the systemic pathophysiological changes in the body in naturally overweight dogs.

**Keywords:** Overweight dogs, Systemic inflammation, Oxidative stress, Liver-related parameters

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**ORAL PRESENTATION**

# SYNTHESIS OF NEW PARTIAL BIOACTIVE MOTES IN SERIES OF IMIDAZO [1, 2-a] PYRIDINE

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## Abstract

The heterocyclic chemistry is one of the most important fields of organic chemistry for their different medicinal and remedial parcels [1-7]. Natural goods and medicals have both used the imidazo [1,2-a] pyridine [8]. arylated imidazo [1,2-a] pyridines are particularly useful as medicine [9]. Antibacterial [10], anti-viral [11], antiprotozoal [12], respiratory virus fusion inhibitor [13], and anticancer activities [14] have been reported for imidazo [1,2-a] pyridine derivatives. Our work is part of a study launched in our laboratory aimed at forming novel motes in collections of imidazo [1,2-a] pyridine through the Mannich condition. Some of Mannich bases in the imidazo pyridine collections are included through motion of secondary amines on starting product and aldehyd. The result compounds were anatomized with infrared, ultraviolet, nuclear photo proton glamorous and nuclear photo carbon 13 glamorous spectroscopic.

**Key words**: Imidazopyridine, C and N aminomethylation, amines secondary, bioactive molecules, bases of Mannich.

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**ORAL PRESENTATION**

# INVESTIGATION OF OLEUROPEIN CONTENTS OF OLEA EUROPAEA FOOD SUPPLEMENTS BY HPLC AND THEIR EVALUATION IN TERMS OF COMPLIANCE WITH EUROPEAN PHARMACOPOEIA

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**Abstract**

*Olea europaea*, known as the olive tree, has been used in traditional medicine for centuries. The species is registered in the European Pharmacopoeia, Martindale, Commission E Monographs, PDR Plant Monographs and FFD Monographs. Olive leaf contains an important secoiridoid namely oleuropein, which is responsible for numerous biological effects of the plant including antioxidant, antimicrobial, anti-inflammatory, antiatherogenic, anticarcinogenic, and antiviral activities.

In this study, six different *O. europaea* samples sold as food supplements obtained from local pharmacies and herbal drug stores were purchased and examined for their conformity in terms of oleuropein content of the drug to scientific definition. 9 different extract were used and oleuropein percentages were investigated by HPLC. Although the percentage of oleuropein in sample 1 and 2 was stated as 20% on the product packaging, as a result of HPLC analysis, oleuropein amounts were found to be 8.687% and 11.857% respectively. According to the PDR Monographs, the amount of oleuropein in the leaves of the samples 3, 4, 5, 6 and 9 was found to be under the limit values and the amounts of oleuropein in the extract of the samples no. 3, 5, 6 and 8 are below the limit values according to the Turkish Pharmacopoeia II European Pharmacopoeia Adaptation. Among investigated extratcs only sample 7's content of oleuropein was within the appropriate range and almost no oleuropein was found in sample 9. The fact that oleuropein content percentages do not match to scientific monographs reveals the necessity of standardization for these products used mostly to improve health conditions

**Key Words**: *O. europaea*, leaf extracts, HPLC, standardization, oleuropein

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**ORAL PRESENTATION**

# A MATHEMATICAL QSAR MODEL TO PREDICT THE SAFE USE OF ANTIHISTAMINES DURING PREGNANCY

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## Abstract

Antihistamines are a pharmacological group frequently prescribed during pregnancy, as allergic reactions are common during pregnancy. In order to use a drug during pregnancy, it must be included in the US Food and Drug Administration (FDA) pregnancy category, in groups A and B. On the other hand, C, D, and X group drugs should not be used during pregnancy due to the risk of developmental toxicity. We constructed a mathematical model to predict the safe use of antihistamines during pregnancy. Since current antihistamines are only in groups B and C as FDA pregnancy categories, our model made predictions over these two groups. If the drug is in group B or C, it gives us information about whether the drug can be used or not. In our model, we included all antihistamines with a determined pregnancy category on the market. The polynomial interpolation developmental model is constructed based on the two descriptor values of the antihistamines data, AlogP and MW. With this new model, we achieved a very high estimation success of 85%. Our work is highly innovative among predictive toxicology studies, as we focused on a specific drug group, such as antihistamines. Our study supports non-animal-based studies and contributes to the literature for new drug development studies using such methods.

**Key Words**: mathematical toxicology; polynomial interpolation; QSAR; developmental toxicology; antihistamine; FDA pregnancy category

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**ORAL PRESENTATION**

# AMINOQUINAZOLINE-BASED EGFR-TK INHIBITOR TARGETED TO MITOCHONDRIA UPON CONJUGATION WITH RU(II) FLUORESCENT PROBE

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**Abstract**

The epidermal growth factor receptor (EGFR) is a key target in cancer, since it has been implicated in severe irregularities in critical cellular processes, including progression of cell cycle, proliferation, differentiation, and death or survival. Mutant EGFR variants, either transmembrane or translocated to the mitochondria and/or the nucleus, frequently develop resistance to EGFR inhibitors. The ability to image and quantify EGFR, in a non-invasive manner, provides new possibilities for detection, monitoring, and treatment of EGFR-related malignancies. This study aimed to generate a new theranostic agent, which combines fluorescence imaging properties with EGFR inhibition. This was achieved by means of conjugating a ((4-bromophenyl)amino)quinazoline inhibitor of mutant EGFR-TK, selected from a focused aminoquinazoline library, with a Ru(II)-based fluorescent probe. A triethyleneglycol-based diamino linker featuring (+)-ionizable sites was employed to link the two functional moieties, affording the desired conjugate. This bis-quinazoline-Ru-conjugate, which retains an essential inhibitory activity, was found by fluorescence imaging to be effectively entering Uppsala 87 (grade IV malignant glioma) cells. The fluorescence imaging study and a time-resolved fluorescence resonance energy transfer (FRET) study indicated a specific subcellular localization of the conjugate, coinciding with that of a mitochondria-targeted dye, suggesting mitochondrial distribution of the conjugate. This creates enhanced potential for association with mitochondria-translocated forms of EGFR. Mitochondrial localization was further verified by the specific concentration of the conjugate in a mitochondrial isolation assay.

**Key Words:** ruthenium conjugate, aminoquinazoline, EGFR-TK, fluorescent probe, mitocan, TEG linker

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**ORAL PRESENTATION**

# THE RELAXANT MECHANISMS OF *PRANGOS UECHTRITZII* ROOTS IN MOUSE CORPUS CAVERNOSUM

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## Abstract

**Background and Objective:** *Prangos uechtritzii* Boiss&Hausskn is an endemic plant of Turkey, traditionally used as aphrodisiac in Anatolia. *Prangos* species are rich in coumarins which are reported to relax penile tissue. The plant roots were evaluated for their relaxation effect on swiss albino mouse corpus cavernosum (MCC). However, the mechanism of action remains ambiguous and needed to explain. So, the purpose of this study is to investigate the relaxation mechanisms of the chloroform extract of *P.uechtritzii* roots(Pu-CE) in MCC. **Methods:** The mechanism of action studies was carried out with Pu-CE ($10^{-7}$-$10^{-4}$ g/mL) which showed the highest relaxation in phenylephrine-precontracted MCC using strip myograph. Pu-CE-induced relaxations were repeated in the presence of synthesis inhibitors aminooxyaceticacid (AOAA,30min,$10^{-2}$M) and Nω-Nitro-L-argininemethylester (L-NAME,100 µM,30min) to understand the role of $H_2S$ and NO production, respectively. $Na_2S$ ($H_2S$ donor, $10^{-6}$-$3x10^{-3}$ M) and sodium nitroprusside (SNP,NO donor,$10^{-9}$-$10^{-4}$ M)-induced relaxations were obtained in Pu-CE (30 min,$10^{-4}$ g/mL) or vehicle preincubated MCC to investigate the contribution of downstream mechanisms of $H_2S$ and NO, respectively. KCl ($10^{-2,1}$-$10^{-0,9}$M), phenylephrine ($3x10^{-8}$-$3x10^{-5}$ M), $CaCl_2$ ($10^{-6}$-$10^{-4}$ M) induced contractions were obtained in the presence of Pu-CE (30 min,$10^{-4}$ g/mL) and vehicle to evaluate the effect of calcium entry. Results were analyzed by LabChart and GraphPad. **Results:** Pu-CE did not alter $Na_2S$ and SNP-induced relaxations ($P$>0.05,n=6-7). The relaxant effect caused by Pu-CE was not changed in the presence of AOAA and L-NAME ($P$>0.05,n=3). $CaCl_2$ ($E_{max}$=0.25±0.03mN), KCl ($E_{max}$=1.24±0.28mN) and phenylephrine ($E_{max}$=1.84±0.28mN) induced contractions were inhibited by Pu-CE ($E_{max}$=0.11±0.01mN, $E_{max}$=0.31±0.08mN, $E_{max}$=0.68±0.05mN, respectively)($P$<0.001, n=4-5). **Conclusions:** Pu-CE-induced relaxations are independent of NO and $H_2S$. The inhibition of contraction responses to $CaCl_2$, KCl and phenylephrine in the presence of Pu-CE showed that Pu-CE inhibits calcium entry or receptor-dependent contraction mechanisms in penile tissue. *P. uechtritzii* could be a promising natural source in the development of new drugs for erectile dysfunction.

**Key Words**: *Prangos uechtritzii*, aphrodisiac, corpus cavernosum, calcium channels.

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**ORAL PRESENTATION**

# *PRANGOS HEYNIAE* RELAXES MURINE PENILE TISSUE VIA INCREASING NO AND $H_2S$ SYNTHESIS

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## Abstract

**Background and Objective:** Erectile function is a complex neurovascular process that relevant to relaxation of smooth muscles of corpus cavernosum in penil tissue. *Prangos heyniae* H.Duman&M.F.Watson is an endemic plant and traditionally used as aphrodisiac in Turkey. The plant roots were investigated for their relaxation effect on swiss albino mouse corpus cavernosum (MCC). However, the mechanism of action remains unclear. So, the aim of this study is to reveal the relaxation mechanisms of the chloroform extract of *P. heyniae* roots (Ph-CE) in MCC. **Methods:** The mechanism of action studies was carried out with Ph-CE($10^{-7}$-$10^{-4}$ g/mL) which showed the highest relaxation in phenylephrine-precontracted MCC using strip myograph. To investigate the roles of $H_2S$ and NO synthesis, Ph-CE relaxations were repeated in the presence of inhibitors, aminooxyacetic acid (AOAA, $10^{-2}$ M, 30 min.) and L-nitro arginine methyl ester (L-NAME, 100 µM, 30 min.), respectively (n=4-5). Results were analyzed by LabChart and GraphPad. **Results:** Ph-CE-induced concentration-dependent relaxation in phenylephrine pre-contracted MCC ($P$<0.001, Two-Way ANOVA). Relaxation responses to Ph-CE ($E_{max}$=73.06±2.13, $pD_2$=4.794±0.047) was more than Ph-HE ($E_{max}$=64,41±1,341, $pD_2$=4.378±0.034) and Ph-ME ($E_{max}$=36,06±0,758, $pD_2$=3.058±0.045) ($P$<0.001). Since Ph-CE is the highest relaxant extract, the mechanisms of relaxant effect of this extract were investigated. Relaxation responses to Ph-CE were inhibited by L-NAME and AOAA (P<0.001). The maximum relaxation and $pD_2$ value of Ph-CE were significantly decreased in the presence of L-NAME ($E_{max}$=61.34±2.088, $pD_2$=4.359±0.065) and AOAA ($E_{max}$=50.32±1.25, $pD_2$=4.024±0.053) (P<0.001). These results show that NO and $H_2S$ production play important roles in the relaxing effect of Ph-CE. **Conclusions:** Ph-CE relaxed MCC by increasing the synthesis of $H_2S$ and NO. Further experiments including downstream mechanisms of $H_2S$ and NO, also calcium entry evaluations are going on. Ph-CE could be used for the treatment of endothelial dysfunction-related erectile dysfunction where NO and $H_2S$ productions are impaired.

**Key Words**: *Prangos heyniae*, aphrodisiac, corpus cavernosum, hydrogen sulfide, nitric oxide.

**Acknowledgements**
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**ORAL PRESENTATION**

# SYNTHESIS, CHARACTERIZATION, AND BIOCOMPATIBILITY OF GREEN-SYNTHESIZED SILVER NANOPARTICLES FROM *LAVANDULA STOECHAS*

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**Abstract**

Silver nanoparticles (AgNPs) play an important role in biomedical applications due to their enhanced optical, electrical and biological properties. Current methods for producing silver nanoparticles often require the use of potentially hazardous chemicals or excessive heat and produce environmentally harmful byproducts that limit large-scale nanoparticle production. In addition, the chemicals used in these methods may cause adverse effects on the biocompatibility of AgNPs and prevent their use in biomedical applications. For this reason, in recent years, the green synthesis method, called biosynthesis of nanoparticles, has attracted great interest for the production of environmentally friendly nanoparticles. Among the different approaches to AgNP biosynthesis, plant extracts attract attention due to the presence of many biomolecules such as polyphenols, flavonoids, vitamins, reducing sugars and terpeniodes. The biosynthesis approach of AgNPs with herbal extracts is a safe and environmentally friendly method, as well as providing nanoparticles with improved properties resulting from the synergetic effects of phytochemical compounds and silver in the extract.
In this study, silver nanoparticles were successfully synthesized and characterized from extracts of *Lavandula Stoechas* plant, which is known for its antimicrobial properties. Dynamic Light Scattering (DLS) analysis showed that nanoparticle sizes vary greatly depending on silver nitrate concentration and extract amounts. Biocompatibility studies of silver nanoparticles on L929 cells show that AgNPs synthesized with three different $AgNO_3$ concentrations, 1, 1.5 and 2 mM, exhibit varying cytotoxic and antibacterial effects.

**Key Words**: Silver nanoparticles, Lavandula Stoechas, Green-synthesized, Biocompatibility

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**ORAL PRESENTATION**

# IMPROVING NUTRITIONAL QUALITY AND ANTIRADICAL ACTIVITY OF BUCKWHEAT BY GERMINATION

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**Abstract**

Germination is an effective biotechnological process for improving nutritional quality of grains and seeds. The aim of our study was to determine the influence of germination on antioxidant activity and chemical composition in buckwheat (*Fagopyrum esculentum*) grains of Ukrainian varieties.

Buckwheat grains of several varieties (collection 2019-2020 yrs) were dehulled and germinated by the algorithm: disinfected by 1 % hypochlorite, washed to neutral pH by distilled water, soaked in distilled water for 4 hours, germinated in Petri dishes on a filter paper at 20 °C in dark with periodically sprinkled by distilled water. During germination time (7 d.), every day was taken part of grains, dried at 35 °C, melt into a powder. The antiradical activity of powders was investigated based on their ability to scavenge stable free radical DPPH; the content of total phenolics – using Folin-Ciocalteau reagent; ascorbic acid yield – by colorimetric assay with Tillman's Reagent.

Dynamic of the total phenolics content change showed the highest values on the 3$^{rd}$ and the 4$^{th}$ days of germination with increassing by 1.4-3.1 folds depending on the buckwheat variety. For the variety Viktoriia on the 3$^{rd}$ day it was 7.3 mg GAE/g comparing to with 2.5 mg GAE/g in non-germinated grains.

Ascorbic acid was not detected in non-germinated samples of buckwheat. In 3$^{rd}$-day samples the content of ascorbic acid ranged from 23.2 to 61.3 mg / 100 g depending on the variety.

The antiradical activity of germinated material samples was higher by 1.3-2.8 folds than in non-germinated. For example, the antiradical activity of Viktoriia buckwheat variety samples with a germination period of 4 days was 212 mM AAE / 100 g DW comparing with 98.6 mM AAE / 100 g DW of non-germinated materials.

Germinated buckwheat can be used for development new functional food and for improvement nutritional quality of traditional food products based on buckwheat.

**Key Words**: buckwheat, germination, Ukrainian varieties, antiradical activity, phenolic compounds, ascorbic acid

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**ORAL PRESENTATION**

# THE COMPUTATIONAL AND BIOLOGICAL INVESTIGATION OF INDOLE AND QUINOLINE BASED THIOSEMICARBAZONES TOWARDS α-GLUCOSIDASE ENZYME INHIBITION

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**Abstract**

Thiosemicarbazones are important classes of Schiff base ligands due to the presence of conjugated N-N-S system providing an important therapeutic potential and have been the subject of many structural and medicinal studies via the interactions of biomolecules. A wide variety of heterocyclic systems have been used for the structural modifications of new thiosemicarbazone based compounds. Due to the presence of the indole and quinoline structures in many natural products, studies have been directed towards investigations of the biological properties of natural indolic and quinolic compounds, and a range of medical uses has been identified.

In the present work, the synthetic procedures and chemical characterization of the targeted compounds derived from indole-3-carbaldehyde and 2-chloroquinoline-3-carbaldehyde systems with a range of thiosemicarbazides. The final compounds have been subjected to α-glucosidase enzyme inhibition assay to investigate the antidiabetic efficiency. A complementary study was carried out with the molecular docking study of targeted compounds on the catalytic side of the designated enzyme. The biological aspect of the study revealed that the indole-based compounds possessed more promising potency compared to the quinoline derivatives.

**Key Words**: Indole, Quinoline, Thiosemicarbazone, α-glucosidase, Diabetes

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**ORAL PRESENTATION**

# THE ROLE OF miR-146a-5p EXPRESSION ON TUMOR GROWTH IN EHRLICH ASCITES CARCINOMA-BEARING MICE TREATED WITH OLEUROPEIN

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**Abstract**

MicroRNAs are transcription regulators that can alter expression in many types of cancer. Some studies have shown that various phytochemicals used in cancer treatment affect microRNA levels, which causes changes in epigenetic and transcriptional regulation and molecular mechanism of the cell. This study aims to investigate the relationship between the effects of Oleuropein (OL) treatment on mice with Ehrlich ascites carcinoma (EAC) on tumor growth and the altering of the expression level of miR-146a-5p. miRNAs were isolated from untreated or OL-treated EAC cells using the appropriate commercial kit, and cDNA was synthesized by reverse transcription kit. The expression coefficient was calculated as the fold-change compared to the control group. There was a 4.88-fold increase in miR-146a-5p expression ($p<0.05$) due to the curative effect of 5-Fluorouracil (5-FU, day 2, single-dose, 20mg/kg) was observed. The miR-146a-5p expression of the OL-treated group was determined as 0.92 compared to the untreated-EAC-control group. It was understood that OL masked the effect of 5-FU by increasing 1.96-fold the expression of miR-146a-5p in the 5-FU+OL group [5-FU(single-dose, 20mg/kg) + OL(150mg/kg/day, 6days)]. Likewise, it was found that OL increased the number of EAC cells compared to the untreated group. While cell proliferation decreased as expected in the 5-FU-treated group, the OL suppressed the treatment effect of 5-FU in the 5-FU+OL group, bringing the cell number to a similar level as the EAC cell number in the untreated group. As a result of this study, it has been determined that OL, which has been suggested as a promising compound for the treatment of breast cancer in many other in vitro studies, may adversely affect the course of the disease when administered alone or in combination with a chemotherapy agent. In conclusion, it is considered that there should be more caution in using phytochemicals to prevent or treat breast cancer.

**Keywords**: Breast cancer, Ehrlich ascites carcinoma, oleuropein, miR-146a-5p.

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**ORAL PRESENTATION**

# DETECTION OF PREBIOTIC EFFECT OF PITAYA *N*-GLYCANS BY USING AN *IN-VITRO* DIGESTION SYSTEM

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**Abstract**

Pitaya is a fruit of the genus *Hylocereus* with rich content, and functional bioactive components. Recently, the production and consumption of pitaya have increased worldwide. It has important roles in human health such as cancer chemoprevention, anti-inflammatory, anti-diabetic effects, and decreasing risk of cardiovascular diseases. Pitaya is special for human gut health because of its glycan content. Glycans can be found as a free or conjugated form in nature. They can reach the colon in undigested form since humans do not have glycosidase enzymes to utilize glycans. They can be utilized by some symbiotics as a carbon source in gut microbiota and, cause selective growth among some probiotics. *Bifidobacterium infantis* has the capability of degradation and utilization of these glycans. It can release conjugated *N*-glycans by its unique enzyme Endo-*β*-*N*-acetylglucosaminidase (EndoBI-1). Studies about conjugated glycans are limited, thus released *N*-glycans from pitaya may have important prebiotic effect on gut health.

Therefore, pitaya samples that are obtained from Thailand and Mersin were analyzed for protein content. Protein quantity of samples measured with QUBIT 3.0 fluorometer and visualized. By using EndoBI-1 enzyme, pitaya *N*-glycans released and purified. Characterization of glycans will be performed by MALDI-TOF-MS and HPLC-HILIC-FLD analysis. In vitro digestion model will be used for the analysis of purified glycans' prebiotic activity. These glycans utilization as a prebiotic will be tested on several probiotics.

Consequently, we will have information about the pitaya *N*-glycan profile, and prebiotic activity studies of bioactive *N*-glycans will form the basis outputs of this study. In this context, how various glycoprotein-containing products are affected throughout the digestive tract and how they shape the intestinal microflora will be revealed. These findings on pitaya fruit may have a role in advancing research into the complex nature of fruit-drug interactions and their possible impact on the clinical effects of drugs.
**Key Words**: Pitaya, *N*-glycan, prebiotic, *in-vitro* digestion, Endo-*β*-*N*-acetylglucosaminidase

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**ORAL PRESENTATION**

# INFLAMMASOME ACTIVATION AND CANCER

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**Abstract**

Inflammasomes are intracellular multiprotein complexes and they are activated by pathogens or endogenous danger signals. Inflammasome activation results in the release of pro-inflammatory cytokines and inflammation. Although, inflammation and also inflammasome activation are cell protective mechanisms, chronic inflammation is harmful to the cell and may be the underlying mechanism of many autoimmune and diverse inflammatory disorders. Nowadays, it has demonstrated that inflammasomes play a significant role in tumor development and progression. However, recent studies also show that different inflammasomes may have not same roles in tumor development depending on tissue and also tumor types. The central roles of inflammasomes makes them significant therapeutic molecular targets in anticancer drug development. However, the physiological roles of inflammasomes and their components in different cancers should be exactly analyzed and possible therapeutic targets for the prevention and treatment of cancer are discussed.

**Key Words**: inflammasome activation, inflammation, cancer, therapy

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**ORAL PRESENTATION**

# DRUG-INDUCED ENDOPLASMIC RETICULUM STRESS

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## Abstract

Endoplasmic reticulum (ER) is a crucial organelle for cell homeostasis, protein synthesis, calcium storage, and lipid metabolism. ER is considered to be one of the main toxicity mechanisms of drugs and other chemicals due to its vital functions. It has significant role in the development of different adverse reactions in cell. Mitochondrial toxicity, lipid accumulation, inflammation, cytotoxicity, cell death may be induced when ER stress is occurred and this leads to numerous diseases such as cancer, diabetes, cardiovascular and neurological diseases. It is demonstrated that ER stress may be induced by commonly used drugs such as paracetamol, amiodarone, diclofenac, arsenic trioxide and other anticancer drugs, bleomycin, and antiretroviral compounds. In recent years, comprehensive assays and techniques are performed in screening ER stress potential of clinically used drugs and newly developed drug candidates. Researchers must select the most appropriate assay for accurate and reliable results. Therefore, it needs to summarize the all-current data for drug-induced ER stress, its different adverse effects and also valid methods for monitoring ER stress.

**Key Words**: Endoplasmic reticulum stress, adverse effects, cell toxicity, *in vitro* assays,

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**ORAL PRESENTATION**

# A GRAPE (*VITIS VINIFERA* L.) POMACE WATER EXTRACT MODULATES INFLAMMATORY AND IMMUNE RESPONSE IN SW-480 CELLS AND ISOLATED MOUSE COLON

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**Abstract**

Grape (*Vitis vinifera* L.) pomace is a residue deriving from the winemaking process which contains bioactive compounds displaying noteworthy health-promoting properties. The aim of the present study was to investigate the phenolic composition and protective effects of a water extract of grape pomace (WEGP) in colorectal cancer cell line SW480 and in isolated mouse colon exposed to E. coli lipopolysaccharide (LPS). The extract decreased SW-480 cell viability, as well as vascular endothelial factor A (VEGFA), hypoxia-induced factor 1α (HIF1α), and transient receptor potential M8 (TRPM8) LPS-induced gene expression. Moreover, the extract inhibited mRNA levels of nuclear factor kB (NFkB), cyclooxygenase (COX)-2, tumor necrosis factor (TNF)α, interleukin (IL)-6, IL-1β, IL-10, inducible nitric oxide synthase (iNOS), and interferon (IFN)γ, in isolated colon. Conversely, WEGP increased the gene expression of antioxidant catalase (CAT) and superoxide dismutase (SOD), in the same model. The modulatory effects exerted by WEGP could be related, at least in part, to the phenolic composition, with particular regards to the catechin level. Docking calculations also predicted the interactions of catechin towards TRPM8 receptor, deeply involved in colon cancer; thus further suggesting the grape pomace as a valuable source of bioactive extracts and phytochemicals with protective effects in the colon.

**Key Words:** *Vitis vinifera*; Grape Pomace; Inflammation; Colon cancer; TRPM8; Catechin

**Acknowledgements**
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**ORAL PRESENTATION**

# BETA ELEMENE CAUSES CYTOTOXICITY-MEDIATED CELL DEATH AGAINST FLT-3 ITD MUTATED ACUTE MYELOID LEUKEMIA

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**Abstract**

**Objective:** The FMS-like tyrosine kinase-3 internal tandem duplication (FLT3-ITD) is found in approximately 25% of all acute myeloid leukemia (AML) cases and is associated with poor prognosis. New and effective treatment options for FLT3-ITD AML are required. β-Elemene is an anticancer sesquiterpene which well known for strongly inducing cell death in cancer cells, and it is especially effective against drug-resistant and complex tumors. In this study, we aimed comprehensive investigation the anticancer potential of β-Elemene on FLT3-wild type (THP-1) and especially FLT3-ITD mutated (MV4-11) cells. **Methods**: Cellular viability analyses were performed with WST-1 assay in a time- and dose-dependent manner. Morphological assessment of β-Elemene treated MV4-11 cells were evaluated by microscopic analyses. The expression changes of three major apoptotic genes -Bax, Bcl-2, Caspase3- were analyzed with Quantitative real-time RT-PCR. For a more comprehensive elucidation of the cell death mechanism, high-throughput screening of targets that are responsible in cell death, survival and resistance was performed. The protein levels of 43 different targets were analyzed simultaneously with the Human Apoptosis Antibody Array. **Results**: Time and dose dependent cell viability analyses showed that β-Elemene has cytotoxic effects on both cell lines, but more selective to FLT3 ITD mutated cells. Morphological assessment indicated that especially doses of IC50 and above β-Elemene treatment led to a stressful phenotype in MV4-11 cells. mRNA data from apoptotic markers revealed resistance. Moreover, the antibody array showed that 18 of the 43 apoptotic protein targets changed in a statistically significant manner. β-Elemene caused cell death with the induction of p53 and cell cycle arrest with the induction of p21 and p27. Increase in the levels of HSP proteins were observed. We also detected an increase in the levels of HTRA protein, which is involved in apoptosis or drug-associated cytotoxicity. It did not cause activation of membrane receptors and mitochondrial membrane degradation. **Conclusion/Discussion:** We can conclude that β-Elemene causes cytotoxicity-mediated cell death in ITD mutated AML cells, together with the effects of stress factors and inhibiting cell division. Our findings suggest that β-Elemene could be a promising drug candidate against AML. For more effective therapies, β-Elemene can be used with novel pharmaceutical formulations.

**Key Words**: Beta Elemene, FLT3, Acute Myeloid Leukemia, Cytotoxicity, Anti-cancer activity

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**ORAL PRESENTATION**

# COMPARATIVE QUALITY CONTROL STUDIES OF RADIOLABELED SOLID LIPID NANOPARTICLES

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**Abstract**

The aim of this study is to develop a suitable quality control method for solid lipid nanoparticles labeled with technetium-99m ($^{99m}$Tc). For this purpose, solid lipid nanoparticles were first prepared using the hot homogenization technique. Briefly, the lipid phase (Gelucire 48/16 pellets) was melted at 75°C until a uniform and clear oil phase was obtained. Soy lecithin was used as surfactant. The aqueous phase (distilled water) containing surfactant was heated at 75°C then added to the oil phase. The aqueous and oily phases were mixed under high-speed stirring (7500 rpm) for 5 min using an Ultra-Turrax blender. After that, obtained pre-emulsion was sonicated at 500 W and 20 kHz in changing 20 s cycles for 10 min by using Vibracell tip sonicator. According to obtained results, solid lipid nanoparticles with zeta potential of -3.61 ± 0.15 mV, particle size of 117.1 ± 4.034 nm, and polydispersity index of 0.376 ± 0.053 were successfully developed. Then, nanoparticles were radiolabeled with $^{99m}$Tc using the stannous chloride method. To the nanoparticle formulation (1 mL), 25 µL of reducing agent (stannous chloride) was added under an atmosphere of bubbling nitrogen. Reduction of $^{99m}$Tc was performed at neutral pH (1 mg stannous chloride dissolved in 1 mL distilled water). Radiolabeling was performed with freshly eluted $^{99m}$Tc (37 MBq) in 0.9% sodium chloride solution (0.1 mL). The mixture was shaken for 30 s and incubated for 15 min at room temperature. After, to determine the radiolabeling efficiency, two methods were used: radioactive thin layer chromatography (RTLC) and centrifugation. For RTLC, acetone (100%) and pyridine:acetic acid:water (3:5:1.5) were used as mobile phases, and ITLC-SG plates were used as stationary phase. Aliquots (10 µL) of radio-mixtures were applied to the origin of ITLC-SG plates and dried at room temperature. The plates were then developed in appropriate solvent systems. Acetone was used for the determination of free $^{99m}TcO_4^-$, whereas pyridine:acetic acid:water (3:5:1.5) was used for determination of radiocolloid. After developing, the plates were dried, and radioactivity distribution was determined by TLC scanner. The centrifugation was the second method to determine the radiolabeling efficiency. The final radio-mixture was separated by centrifugation at 3000 rpm for 0.25 h at 25°C. The radioactivity content of free $^{99m}TcO_4^-$ was evaluated by counting supernatants by gamma counter. Then, the radioactivity content of nanoparticles was evaluated by subtracting the radioactivity of free $^{99m}TcO_4^-$ from 100. According to obtained results, the labeling efficiency of solid lipid nanoparticles for RTLC and centrifugation was found ≥98% and ≥95%, respectively. In conclusion, although RTLC is extensively used for the labeling efficiency of radioactive nanoparticles, the centrifugation method can also be used to determine the labeling efficiency of radiolabeled solid lipid nanoparticles.

**Key Words**: Solid lipid nanoparticles, technetium-99m, radiolabeling, quality control.

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**ORAL PRESENTATION**

# ASSESSMENT OF COMMERCIAL *MENTHA PIPERITA* L. (PEPPERMINT) ESSENTIAL OILS SOLD ON THE TURKISH MARKET IN TERMS OF EUROPEAN PHARMACOPOEIA 10.0 CRITERIA

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**Abstract**
**Objectives:** The essential oil of *Mentha piperita* L. is widely used in folk medicine. Antiemetic, antibacterial, antiviral, antifungal, antioxidant and cardiopulmonary regulatory activities of peppermint oil have been demonstrated [1]. Pharmacopoeias are official books that contain qualitative and quantitative analysis methods of active substances and excipients used in the manufacture of medicinal products, which contains international rules and methods that must be followed legally and scientifically. In this study, it is aimed to assess various commercial *M. piperita* essential oils on the market regarding the quality standards. Different pure peppermint essential oil samples purchased from pharmacies and other sources (herbal stores, internet) were evaluated in terms of the criteria in the "Peppermint Oil" monograph in the European Pharmacopoeia 10.0 (EP). **Materials and Methods:** Characteristic feature tests (appearance, odor, and solubility), spot control, relative density, refractive index, optical rotation and acidity index tests were applied to 14 different peppermint oils sold on the market as stated in the EP monograph [2]. In addition, Thin Layer Chromatography (TLC) was carried out for the qualitative determination of chemical profile while Gas Chromatography-Mass Spectrometry (GC-MS) analyzes carried out for quantitative determination of phytochemical contents. **Results:** Results demonstrated that none of the brands fully met the standards stated in the European Pharmacopoeia 10.0. However, it was observed that the rate of meeting the criteria for the products obtained from pharmacies was significantly higher when compared to other sources (80% and 71.96%, respectively). **Conclusion:** The quality insufficiency of *M. piperita* oils on the market to meet criteria of Pharmacopoeia indicates the requirement of higher standards for regulation and auditing mechanisms in Turkey. Until then, results showed that pharmacies are still the best option for public to obtain pure essential oils.

**Key Words**: *Mentha piperita* L., Essential oil, GC-MS, menthol, European Pharmacopoeia, Aromatherapy

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**ORAL PRESENTATION**

# THE EFFECTS OF VITAMIN C AND N-ACETYL CYSTEINE TREATMENT ON THE PREVENTION OF SATAVAPTAN CYTOTOXICTY IN THE CELL CULTURE

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**Abstract**

Nephrogenic Diabetes insipidus (NDI) is a rare genetical disease that is characterized by severe imbalance of body water homeostasis. Loss-of-function mutations of arginine vasopressin receptor 2 gene (AVPR2) which is a G protein coupled receptor (GPCR) can cause NDI. Mutations can affect conformational maturation process of receptor and mutant receptor cannot locate on cell membrane where it is functional since it cannot pass the Endoplasmic reticulum (ER) quality control mechanism of the cell. Lately, pharmacological chaperones are used to rescue of trapped mutant receptors from the ER to make them functional again. Satavaptan is one of these PCs and it can rescue mutant AVPR2s via its selective AVPR2 antagonist properties. Even if small concentrations of satavaptan can be enough to rescue mutant AVPR2s from the ER, it can somehow show cytotoxicity. Thus, prevention of its cytotoxicity using with antioxidants can be helpful to reverse bad effects of the satavaptan. The aim of this study is to analyze antioxidant effects of Vitamin C and N-acetyl cysteine (NAC) on cytotoxicity of satavaptan. To measure antioxidant potency of Vitamin C and NAC, MTT analysis was performed on COS-1 cells which are commonly used for PC studies. MTT assay was performed with the treatment of different concentrations of satavaptan, satavaptan + Vitamin C, and satavaptan + NAC on COS-1 cells. According to the results, treatment with different concentrations of satavaptan caused a decrease on cell viability. Results of compared percentages showed that this cytotoxicity could be prevented by using with Vitamin C and NAC as antioxidants. In conclusion, prevention of small levels of cytotoxic effects of satavaptan which is a promising pharmacological chaperone for the treatment of NDI is important for the understanding and developing of new therapeutics for NDI.

**Key Words**: Nephrogenic Diabetes insipidus, AVPR2, GPCR, Satavaptan, Cytotoxicity, Antioxidants

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**ORAL PRESENTATION**

# EVALUATION OF THE ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF PHENOLIC COMPOUNDS OF A MEDICINAL PLANT *GLOBULARIA ALYPUM* L.

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**Abstract**

This work is in keeping with the general pattern of bringing one's contribution to the development of the vegetable reign as a source of natural bioactive substances to discover new compounds of therapeutic interest. . In this study, we were interested in the extraction and analysis of phenolic extracts from the species *Globularia alypum* L., as well as in the study of their biological effects concerning antioxidant activity and their inhibitory effects on the two enzymes of the hydrolase class (α-amylase and α-glucosidase) responsible for sugar digestion. The total phenol content ranges from 1.36 to 14.84 mg gallic acid equivalent/g dry matter. While the flavonoid content expressed as quercetin equivalent is between 0.31 and 4.54 mg/g. The results of the antioxidant activity determined by DPPH test showed a good antioxidant capacity compared to antioxidants taken as reference.

All extracts showed inhibitory effects on both enzymes, with inhibition percentages ranging from 8.38% to 61.65% for α-amylase and from 12.52% to 71.22% for α-glucosidase with the best inhibition recorded for the butanolic extract with an $IC_{50}$ value = 0.22 mg / ml).

**Keywords:** *Globularia alypum* L., phenolic compounds, inhibition effect, antioxidant activity.

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**ORAL PRESENTATION**

# INVESTIGATION OF DIFFERENT SYNTHESIS PARAMETERS OF HYDROXYAPATITE FOR TISSUE ENGINEERING APPLICATIONS

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## Abstract

Hydroxyapatite undoubtedly has vital roles in tissue engineering applications. The fabrication methods and different treatments lead distinct properties in hydroxyapatite crystals, including particle, size, shape, and surface features. In this study, we applied sol-gel synthesis route for hydroxyapatite production which offers relatively cost available and high yield of product. The influence of initial pH parameter and various temperature treatments on properties of hydroxyapatite were investigated. The leading hydroxyapatite powders have been compared in terms of their morphological and chemical structures by XRD and SEM analyses. The incipient pH in which the precursor solutions introduced to one another had critical role in this synthesis reaction. This has determined major properties, such as the chemical composition, phase purity, product yield, and morphology. The reactions of precursor solutions with higher incipient pH contributed to high yield (86%) of pure HA possessing high thermal stability. On the other hand, in lower incipient pH (8) counterpart, β-TCP phase was detected upon treatment at 950 °C. We had used the acquired pure HA in dried form in chitosan based injectable hydrogel compositions with pro-angiogenic features designed for bone tissue regeneration and drug delivery applications.

**Key Words**: hydroxyapatite, sol-gel synthesis, incipient pH, microstructure, bone tissue engineering

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**ORAL PRESENTATION**

# STABILITY-INDICATING STRESS DEGRADATION STUDIES OF NATEGLINIDE BY USING UV SPECTROPHOTOMETRIC METHOD

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## Abstract

Nateglinide (NTG), chemically known as N-(trans-4- isopropylcyclohexylcarbonyl)-D-phenylalanine, is a D-phenylalanine derivative lacking either a sulfonylurea or benzimido moiety. It is a novel oral meal time glucose regulator that has recently been approved for the treatment of type II diabetes. NTG is an oral insulinotropic agent capable of restoring the physiological insulin secretion pattern lost in type II diabetes . It increases the insulin release from pancreatic β-cells through inhibition of potassium ATP-channels. Stability-indicating stress degradation studies have shown the stability-indicating nature of the method. Stability-indicating stress degradation studies are used for determining impurities and degradation pathways in pharmaceutical preparations and performed in accordance with the established ICH guidelines. In this study, NTG was exposed to acidic, basic and oxidative degradation. The effect of different extraction solvents on the absorbance of NAT was investigated using methanol, water, and acetonitrile. The drug was comparatively more resistant to acid hydrolysis than to basic and oxidative degradation. Severe decomposition of the drug on basic and oxidative degradation was determined.

**Key Words**:Nateglinide, UV Spectrophotometric method, acidic, basic and oxidative degradation.

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**ORAL PRESENTATION**

# TELMISARTAN LOADED PROTEIN-BASED NANOPARTICLES AND THEIR SIZE DEPENDENT CELLULAR UPTAKE

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## Abstract

Recently, continuous usage of angiotensin II receptor type 1 blockers such as telmisartan has been reported to inhibit the growth of cancer cells [1].
The focus of the current research is to design telmisartan-loaded protein-based nanoparticulate system and to investigate the effect of the particle size on cellular uptake and anticancer activity of the nanoparticles. Telmisartan-loaded bovine serum albumin nanoparticles were constructed by the desolvation method. The *in vitro* characteristics of telmisartan-loaded nanoparticles were evaluated in terms of encapsulation efficiency, particle size and size distribution, surface charge, and *in vitro* drug release. In addition, the influence of different particle sizes on the cellular uptake behavior of nanoparticles was investigated by fluorescent imaging and flow cytometry.
The negatively surface-charged nanoparticles with particle sizes of 127.8, 236.2, and 404.0 nm were obtained using bovine serum albumin at 4, 6, and 8% concentrations, respectively. The encapsulation efficiency of telmisartan was in the range of 73 and 87%. On the other hand, it was observed that the cellular uptake of the nanoparticles was time-dependent and decreased by the increased size of the nanoparticles. However, the anticancer activity of free telmisartan appeared to be higher than that of all sizes of nanoparticles, due to the prolonged *in vitro* telmisartan release from nanoparticles. Therefore, extensive research is being planned to improve the anticancer activity of telmisartan-loaded nanoparticles.

**Key Words**: Telmisartan, Protein-based nanoparticle, Cellular uptake.

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**ORAL PRESENTATION**

# SCREENING OF *IN VITRO* BIOLOGICAL ACTIVITIES OF *CALTHA PALUSTRIS* L. METHANOL EXTRACT

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**Abstract**
The genus *Caltha* is a member of the Ranunculaceae family and is represented by only one species *Caltha palustris* L. (Syn. *Caltha polypetala* Hochst.) in Türkiye [1]. Aerial parts of the plant have been used for food and treatment of lung disease, hemorrhoids, and rheumatism [2]. The genus is a rich source of triterpene derivatives, phenols, and cyanogenic compounds. Studies about biological activities showed that *Caltha* species have antioxidant, anthelmintic, antimicrobial, and antiinflammatory activities [3]. In this study, it was aimed to test total phenolic content, DPPH radical scavenging, anti-tyrosinase, anti-glucosidase, anti-cholinesterase, and DNA interaction effects of methanolic extract of aerial parts of *C. palustris*.

The plant material was collected from Trabzon during the flowering season in May 2015. Dried aerial parts were extracted with methanol at 40°C, then filtered and evaporated to dryness under vacuum. The total phenolic content, DPPH radical scavenging, anti-tyrosinase, anti-glucosidase, and anticholinesterase inhibitory properties were examined using spectrophotometric assay. In addition, kinetic parameters of extract were investigated using Lineweaver-Burk and Dixon plots on tyrosinase enzyme. Finally, DNA nuclease activity and DNA-damage protective actions of the extract on Fenton's reagent were examined using electrophoretic methods [4].

The total phenolic content of methanol extract was found to be 25.30 ± 2.45 mg GAE/g dry weight. At 200 µg/mL, methanol extract scavenged to DPPH radical with %71.38 ± 0.27. The extract inhibited tyrosinase enzyme concentration-dependent manner. Lineweaver-Burk and Dixon plots showed that the extract was a competitive inhibitor against tyrosinase with Ki value of 42.50 ± 0.30 µg/mL. On the other hand, the extract did not have inhibitory effects against cholinesterases and glucosidase at studied concentrations. Electrophoretic studies showed that the extract blocked plasmid DNA damage on Fenton's reagent. The results showed that the methanol extract of *C. palustris* might have a potential for the treatment of several diseases.
**Key Words**: *Caltha palustris*, anti-tyrosinase, Lineweaver-Burk, electrophoresis, DNA.

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**ORAL PRESENTATION**

# SALT TOLERANCE MECHANISMS and POTENTIAL USES of HALOPHYTES

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**Abstract**
Halophytes are a sustainable alternative to traditional crops in saline and arid regions, as they are well adapted to saline-arid soils and saline marshes. Since marginal soils are already increasing due to global warming, more practical uses of halophytes are being sought. These species represent a valuable resource with a potential role in landscape engineering, desalination and erosion prevention, or in commercial uses as ornamental plants. Such species create attractive models for fundamental research on the mechanisms of induced stress tolerance. Some types of halophytes have remarkable morpho-anatomical features, such as salt glands due to ion accumulation, simultaneous use of several osmolytes for osmotic regulation, and activation of effective antioxidant systems.

As salt-resistant plants have strong antioxidant defense systems based on enzymatic activities and non-enzymatic antioxidant compounds, halophytes have strong antioxidant defense systems, resulting in reduced oxidative stress associated with salinity; resulting in reduced oxidative stress associated with salinity. They also constitute a group of plants that are of particular interest as sources of nutraceuticals and functional foods. Some secondary metabolites contained in halophytes, including phenolic compounds, can delay the hazardous effects of oxidative stress. Halophytes are also potential candidates for phytoremediation programs. Unlike glycophytes, which cannot withstand prolonged exposure to salty environments, halophytes have ability to recover from salt stress and germinate after exposure to extremely salty conditions, a strategy that has great selective advantage.

As a result, they are expected to respond better to the decontamination of contaminated soils than glycophytes and are ideal candidates for the phyto-extraction or phytostabilization of heavy metal-contaminated soils, especially those affected by salinity. The wide variety of salt stress responses described in halophytes make these plants attractive models for fundamental studies on salt tolerance mechanisms, in addition to their potential uses as food, medicinal and ornamental plants for a sustainable, saline agriculture.

**Key Words:** halophytes, saline, antioxidant, phytoextraction, nutraceauticals

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**ORAL PRESENTATION**

# SYNTHESIS AND ANTIMICROBIAL EVALUATION OF NEW PYRAZOLINE DERIVATIVES

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## Abstract

Antimicrobial resistance has become a growing global public health problem [1]. It is estimated that there are more than 250 million cases of bacterial infections per year, resulting in approximately $1.6 billion in economic losses each year. There are difficulties in infection management due to the emergence of antimicrobial resistance and the transmission of resistance among the strains. As a result, the rates of hospitalization and mortality enhance significantly [2]. In the last decades, complications related to resistance and therapeutic difficulties have been expressed by some bacterial strains such as *Staphylococcus aureus*, *Enterococcus faecium*, *Streptococcus pneumonia*, *Mycobacterium tuberculosis* [3]. Therefore, the importance of discovery and development of novel antimicrobial molecules increases day by day. Pyrazoline structure has different pharmacological properties such as antifungal, antibacterial, antiviral, antitubercular, antileshmanial, antiprotozoal [4]. Due to its different biological properties, pyrazoline core is highly preferred in new drug candidate development studies. The aim of this study to synthesize new pyrazoline derivatives and evaluate their antibacterial and antifungal activity. Therefore, in this study, new compounds bearing pyrazoline ring were synthesized. Their chemical structures were confirmed by different methods such as IR, NMR and elemental analysis. The antimicrobial activity of all synthesized compounds was investigated against standard bacterial strains such as *Staphylococcus aureus* ATCC 25922, *Escherichia coli* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, and against standard yeast strains such as *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida parapsilosis* ATCC 90018, *Candida tropicalis* KUEN 1021. The minimum inhibitory concentration (MIC) test was performed in the concentration range of 800-0,391 µg/ml of compounds [5]. According to the activity results, it was determined that all synthesized compounds had higher antimicrobial activity against *E. faecalis* and *C. glabrata* than other bacterial and yeast strains.

**Key Words**: Pyrazoline, antibacterial, antifungal, MIC.

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**ORAL PRESENTATION**

# OXIDATIVE EFFECTS of HELICOBACTER PYLORI in ADENOCARCINOMA CELLS and PROTECTIVE EFFECTS of SODIUM SELENITE

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## Abstract

**Objectives:** *Helicobacter pylori* (*H. pylori*) was discovered in 1982 by two Australian researchers, Barry Marshall and Robin Warren. This Gram-negative human stomach pathogen causes infection that induces gastritis, gastric ulcer and gastric cancers. Different studies show that oxidative stress could be one of the mechanisms underlying the toxicity of *H. pylori*. Sodium selenite (SS) is an inorganic selenium compound which is suggested have antioxidant effects. This research aimed to evaluate the oxidative stress-causing effects of *H. pylori* on human adenocarcinoma cells. In addition, the protective effects of sodium selenite against oxidative stress caused by *H. pylori* were analyzed.
**Methods:** Study groups were formed as control (C), *H. pylori* (HP), sodium selenite (SS) and *H. pylori*+sodium selenite (SS+HP). To determine the oxidative effects of *H. pylori*, the levels of thiobarbituric acid (TBARS) as a marker of lipid peroxidation, protein carbonyl levels as an indicator of protein oxidation and total glutathione levels as a marker of cellular thiol levels were examined. The antioxidant effects of SS against the possible oxidative stress caused by *H. pylori* were also observed.
**Results:** Results were expressed as mean ± standard deviation (SD). p values $<0.05$ were considered as statistically significant. Carbonyl and TBARS levels were higher in HP group vs. C group and levels of these two parameters were decreased in SS+HP compared to the HP group ($p<0.05$). Total glutathione levels were lower in the HP group when compared to C group and total glutathione levels of SS+HP were higher vs. HP group ($p<0.05$).
**Conclusions:** The results of our study show that *H. pylori* may lead to oxidative stress. It can be concluded that oxidative stress may be one of the underlying mechanisms of gastritis, ulcer and gastric cancers that can be caused by this particular bacterium. Moreover, SS may have a protective role against the oxidant properties of *H. pylori* infection.

**Key Words**: *Helicobacter pylori*, oxidative stress, sodium selenite

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**ORAL PRESENTATION**

# EXPLORING THE IN VITRO PROBIOTIC POTENTIAL AND BIOPROCESS DEVELOPMENT COMPATIBILITY OF A NOVEL *PICHIA KUDRIAVZEVII* FOL-27

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## Abstract

The goal of this study to explore *Pichia kudriavzevii* FOL-27's: i) survival against artificial gastric acid (AGJ) and artificial bile juice (ABJ), ii) growth kinetics in batch trials (BT) and fed-batch trials (FBT). Survival of FOL-27 as measured by relative cell density (RCD) against AGJ and ABJ was performed at four different pH-levels (control, 3, 2, 1.5) and ox-bile concentrations (control, 0.2%, 1%, 2%), respectively. Growth kinetics was calculated by periodic measurement of $OD_{600}$ in BT or in FBT where pH, dissolved-oxygen and temperature were controlled at 5.5, 25%, and 30°C, respectively. Also, impact of dissolved oxygen level at 12.5% or 25% were tested against the growth and performance of FOL-27 in FBT using exponential feeding regimen. The doubling-time, maximum specific growth rate, and final cell densities achieved for BT were 101.8min, 8.202$h^{-1}$ and 28.7, respectively. FBT at 25% $O_2$ or 12.5% $O_2$ level resulted in doubling-time, maximum specific growth rate, and final cell densities of 90.18min, 3.95$h^{-1}$, 22.51 and 88.8min, 2.83$h^{-1}$, 26.6, respectively. RCDs calculated were similar for pH=3 and control vs both were remarkably higher ($p<0.05$) than pH=1.5 and pH=2 with the last two pH-levels were significantly different ($p<0.05$) from each other. RCDs were similar across control, 0.2%, 1%, and 2% ox-bile levels ($p>0.05$). *P. kudriavzevii* FOL-27 is a potential probiotic candidate showing resistance against AGJ and ABJ conditions. A remarkable increase in biomass when grown with FBT implies that *P. kudriavzevii* FOL-27 is compatible to bioprocess development therefore a yeast-based probiotic culture could perhaps be developed using this strain.

**Key Words**: *P. kudriavzevii* FOL-27, probiotics, fed-batch, bioprocess, dissolved-oxygen

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**ORAL PRESENTATION**

# ASSESSING PHYSICIAN-PATIENT COMMUNICATION SKILLS FOR EFFECTIVE MEDICAL HISTORY TAKING PROCEDURE

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## Abstract

Clear physical patient communication is important for achieving patient satisfaction, as is effective communication for successful outcomes in all areas. However, communication is greatly influenced by doctors' prejudices. Therefore, this process is considered art rather than science. This study determines how clinicians classify the results of medical history studies in relation to the patient's maternal and paternal medical history, the patient's own medical history, and their current profession. A total of 1270 clinicians were hired from the fields of otology, general surgery, internal medicine, cardiology, respiratory medicine, and psychiatry. The university website served as a useful resource for gathering clinician professional background and contact details. This study provided professional and medical history-based information. He also demonstrated the importance of balancing effective clinical expertise and communication. The study concluded that good communication skills are important for physicians to promote patient satisfaction and effective treatment.

**Key Words**: Medical history taking, Categorization, Free-text, Survey, Coherence, Clinician's biasness

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**ORAL PRESENTATION**

# EFFECTS OF FUMONISIN B1 ON INTERCELLULAR COMMUNICATION (GAP JUNCTIONS) IN HEK-293 CELLS

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## Abstract

Fumonisin B1 (FB1) is a mycotoxin produced by *Fusarium* species in maize and maize-based products. Although it is one of the most common mycotoxins that frequently contaminates corn products, it causes serious health problems on humans because it is the most important type in terms of toxicity among fumonisins. The International Agency for Research on Cancer (IARC) has classified FB1 as Group-2B ("possibly carcinogenic to humans"). FB1 causes toxic effects by causing accumulation of sphinganine, which plays an important role in the pathways associated with cancer development and apoptosis mechanisms, and disruption of sphingolipid biosynthesis. Also, little is known about the early molecular changes associated with FB1 carcinogenicity. Based on its non-genotoxic effect, it is thought that epigenetic mechanisms may play a role in the carcinogenic effect of FB1. In our study, it is planned to elucidate the key molecular mechanisms involved in the toxicity of FB1. For this purpose, the effects of FB1 on intercellular comminications in *in vitro* human embryonic kidney cells were investigated. Cytotoxicity tests (MTT and NRU) were performed and exposure concentrations were determined as 10, 50 and 100 µM. Cell proliferation (BrDU) and 5-methyl cytosine (5-mC%) levels were measured using the Elisa kit. Gene expression analyzes of genes related to intercellular communication-gap junction functions such as *Cx43*, *Cx45*, *Cadherin2* were performed. It was determined that FB1 caused an increase in 5-mC% and significantly decreased gene expressions related to gap junction functions. As a result, it is thought that FB1 may show toxic effects by affecting epigenetic modifications and intercellular communication, and exposure to mycotoxins such as FB1, which is dangerous for human health, becomes very important for both public health and risk assessment studies. The elucidation of the mechanisms of chemical carcinogenesis also contributes to the development of biomarkers suitable for early detection of cancer.

**Key Words**: Gap junctions, Fumonisin B1, cell culture, toxicity.

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**ORAL PRESENTATION**

# INVESTIGATION OF PHENOLIC COMPOUNDS AND PHARMACEUTICAL EFFECTS OF EDIBLE MUSHROOM *RAMARIA FLAVA*

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## Abstract

It is an edible mushroom species in the Gomphaceae family and the *Ramaria* genus. It is generally distributed in Europe. In Turkey, it is found in forest areas around Kastamonu, which is found in the Black Sea region. Mushrooms are very delicious and can be consumed fried, prepared in the form of salad, pickled and many more. This study investigated the ethanolic extracts obtained from *Ramaria flava* (RF) mushroom for phenolic content and antioxidant, anticancer and antimicrobial activities. The antioxidant potential of the extract was determined regarding DPPH radical scavenging activities. The phenolic content of the ethanolic extract was measured by using the ultra-performance liquid chromatography (UPLC). RF etanolic extract was also tested for aldose reductase, catalase and superoxide dismutase activities. The anticancer effect of the mushroom extract was tested on the colon (HT-29) and the breast (MCF-7) cancer cell lines by using the MTT assay. Besides, the antimicrobial activity of the mushroom extract was evaluated against Gram-positive and Gram-negative bacteria. According to the UPLC results, ethanolic extract of the RF contains vanillic, ferulic, p-coumaric, cinnamic, chlorogenic, caffeic acid, myricetin, apigenin and luteolin compounds. The ethanol extract of RF scavenged about 60% of the DPPH radicals. Also, *R. flava* ethanol extract activated the SOD enzyme by %3 to %9 at all concentrations. However, the ethanol extract of the same mushroom inhibited the CAT enzyme by 32% to 23% at all concentrations. Additionally, the RF extract inhibited aldose reductase (AR) by 75% – 20% at (10-2.5 mg/mL) concentrations. Besides, the RF extract showed moderately anticancer activity on HT-29 and MCF-7 cell lines. Moreover, the extract displayed antimicrobial activity against *Staphylococcus aureus* ATCC 25923, *S. epidermidis* ATCC 35984, *Enterococcus faecalis* 26, *E. faecalis* 25 and *Pseudomonas aeruginosa* ATCC 27853. Thus, it appears RF can be a potent inhibitor of the aldose reductase enzyme. In addition, it can be used as a food supplement because it contains high phenolic compounds.

**Key Words**: *Ramaria flava*, UPLC, aldose reductase, antioxidant, anticancer, antimicrobial

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**ORAL PRESENTATION**

# PREPARATION AND CHARACTERIZATION OF GLYCYRRHIZIC ACID LOADED PLGA NANOPARTICLES FOR ANTI AGING COSMETIC APPLICATIONS

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## Abstract

In recent years, the use and application of nanotechnology has been increasing in the cosmetic industry due to its great functionality. In cosmetic formulations, the encapsulation of active ingredients for efficient delivery through skin barriers is widely used. The fact that the antioxidant power of *Glycyrrhiza glabra L.* extracts and components is at a level that can correct aging changes such as loss of elasticity and wrinkles may be one of the reasons for their preference in anti-aging cosmetic products. Glycyrrhizic acid (GA) is a triterpenoid saponin that can be used as a medicinal plant and one of the components of licorice roots (*Glycyrrhiza glabra L.).* Poly-lactic-co-glycolic acid (PLGA) nanoparticles are also known to be used as nano-cosmetic carriers to improve the performance of cosmetic formulations. In this study, Glycyrrhizic Acid loaded PLGA nanoparticles were prepared as an anti-aging active ingredient candidate. In this purpose, GA was encapsulated by PLGA using double emission method. Ultraviolet spectrometer (UV), Zeta Sizer and Scanning Electron Microscopy (SEM) were used to characterize the nanoparticles. According to the results, the GA-PLGA NPs had a 212.6 ± 2.892 nm average particle size, 0.070 ± 0.042 PdI and -8.44 ±0.525 mV zeta potential. The encapsulation efficiency and loading capacity were calculated as 81.0% and 26.6% respectively and the in vitro drug release study showed a GA release of 96.4% within 48 hours in pH=5.5 media. Finally, GA-PLGA NPs were examined for genotoxicity in *S. typhimurium* TA98 and TA100 strains and no genotoxic effect was observed. In conclusion, GA-PLGA NPs may be used for anti-aging skin care topical formulations as an alternative active ingredient.

**Key Words**: Glycyrrhizic Acid, PLGA, anti-aging, skin care

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**ORAL PRESENTATION**

# NEOPTERIN LEVELS OF INDUSTRIAL WORKERS

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## Abstract

Neopterin is a well-established biochemical marker that provides information about the activation of the cellular immune system. Increased neopterin levels in biologic fluids are connected with various pathological conditions such as viral infections, autoimmune diseases, inflammatory diseases, neurological and cardiovascular diseases. It is well known that cell-mediated immunity has a close relationship with environmental factors. As the neopterin level serves as a biomarker for cell-mediated immune activity, the effects of occupational diseases related to environmental conditions on neopterin levels have become a popular research area. Previous studies reveal that there is a remarkable increase in neopterin levels of individuals who work in toxic environments. Thus, neopterin may be an effective biomarker in measuring toxic exposures of industrial workers. This study aimed to determine neopterin levels of industrial workers (n=33) working in auto painting, bodywork and furniture production. The control group (n=17) was selected from healthy adults. Urinary neoptrin levels were measured by high-performance liquid chromatography with fluorescence detection. Neopterin levels were found to be higher in workers than in the healthy controls (P>0.05). The highest and lowest values of urinary neopterin for industrial workers were obtained 908.96 and 119.86 µmol/mol creatinine, respectively. Workers in the auto painting, body and furniture business may have been exposed to various toxic chemicals in their working places. As a result, an increase in the concentration of neopterin in the urine may be an early critical marker in diagnosis of occupational exposure-related immune system disorders. Moreover, continuous monitoring of neopterin levels in workers may provide valuable information about worker's health status, resulting in the prevention of various disease progression.

**Key Words**: Neopterin, biomarker, urine, HPLC

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**ORAL PRESENTATION**

# CHEMICAL CONSTITUENTS AND BIOACTIVITIES OF *FERULA LYCIA* BOISS AERIAL PARTS DURING ITS PHENOLOGICAL CYCLE

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## Abstract

Phytochemicals which are commonly found at different levels in many medicinal plants, are natural strong antioxidants used in traditional medicine. In this research the variation in the quantity and quality of the essential oil of *Ferula lycia* during its life cycle stages is reported. The oils were obtained by hydrodistillation of air-dried samples. The yield of essential oil (w/w %) in different stages was in the order: floral budding (1.3%)> vegetative (0.9%)> flowering (0.7%)> immature fruit (0.7%)> ripen fruit (0.4%). The essential oils were analyzed by GC and GC-MS. In total, 33, 40, 42, 36, and 42 constituents were identified and quantified in the subsequent stages, respectively. Total phenolic and flavonoid contents were determined by spectrophotometric methods and antioxidant capacities were evaluated by DPPH, RP and MCA assay. In addition, the phenolic acid and flavonoid compositions were evaluated by RP-HPLC. This study presented a comprehensive report for the first time on evaluation of the phytochemical composition and the biological properties of *F. lycia* at different phenological stages. Full flowering stage was found as the richest period in terms of analyzed phenolic acid and flavonoid compositions of *F. lycia* for the first time. The species examined in this research showed a high antioxidant activity in comparison to other studies with Ferula species. Besides, a high correlation between antioxidant activity and phytochemical content of *F. lycia* was found. These results suggest that *F. lycia* can be used as a safe source in the cosmetic, food and pharmaceutical industries.

**Keywords:** Apiaceae, Developmental periods, Essential oil, Secondary metabolite

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**ORAL PRESENTATION**

# METHOD DEVELOPMENT FOR THE DETERMINATION OF NIFEDIPINE IN HUMAN GINGIVAL CREVICULAR FLUID AND PLASMA BY HPLC

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## Abstract

One of the widely known side effects of nifedipine is gingival overgrowth. The aim of this study is method development for determination of nifedipine concentration in gingival crevicular fluid (GCF) and plasma by HPLC, and to determine whether there is a relationship between plasma and GCF nifedipine levels.

Separation of nifedipine from GCF was performed by Microsphere, $C_{18}$ (100 x 4.6 mm, particle size 3 µm) analytical column and methanol, sodium acetate (pH=4.0, 10 mM) (60:40, v/v) containing mobile phase at 0.8 ml/min. Detection of nifedipine and nitrendipine (internal standard, 0.5 µg/ml) was performed by UV/Vis detector at 235 nm. GCF samples were extracted by using a mixture of methanol and water (50:50, v/v). Plasma nifedipine content were extracted by using a mixture of hexane and dichloromethane. The calibration curve for nifedipine was linear over the concentration range of 0.01-0.5 µg/ml. The mean recovery (±SD) from GCF was 99.05±3.72 % for nifedipine at a concentration of 0.1 µg/ml (*n=6*). The mean recovery (±SD) from plasma was 102.03±5.62 % for nifedipine at a concentration of 0.1 µg/ml (*n=6*).

No association was found between GCF and plasma levels, and nifedipine is not a risk factor for gingival enlargement. Consequently, this study describes a simple, sensitive, and practical HPLC-UV/Vis method which permits determination of nifedipine in human gingival crevicular fluid and plasma samples.

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**ORAL PRESENTATION**

# CYTOTOXIC ACTIVITY OF *CLEMATIS CIRRHOSA* L.

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## Abstract

The present study aims to investigate the cytotoxic activity of *Clematis cirrhosa* L. (Ranunculaceae) against the renal cancer cell lines.
*Clematis cirrhosa* was collected in the vicinity of Aydın. Air-dried and coarsely powdered aerial parts of the plant were sequentially extracted at room temperature with dichloromethane, ethyl acetate, and methanol. The extracts were separately concentrated in a rotary evaporator under reduced pressure to dryness. These extracts were subjected to cytotoxic activity testing. The assay used was a two-day, two cell line XTT bioassay, an in vitro antitumor colorimetric assay [1]. The renal cancer cell lines used were UO31 and A498.
All three extracts of *Clematis cirrhosa* aerial parts exhibited growth inhibition of more than 50% at a 25 ug/mL concentration on the renal cancer A498 and UO31 cell lines. The methanol extract was the most cytotoxic extract against both renal cancer cell lines, with higher activity against UO31 cells.
Bioactivity-guided fractionation of the methanol extract of the aerial parts of *Clematis cirrhosa* is planned to isolate and identify their cytotoxic principles. This is the first report on the cytotoxic activity of *Clematis cirrhosa* against renal cancer cell lines.

**Key Words**: *Clematis cirrhosa*, Cytotoxic activity

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**ORAL PRESENTATION**

# CRITICAL STEPS OF SOLUTION PREPARATION PROCESS FOR PARENTERAL DRUGS: TIGECYCLINE CASE

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## Abstract

**Objective:** Parenteral dosage forms frequently are used for formulation of drugs which are unstable in the gastrointestinal tract or have low bioavailability. In this study, it was aimed to analyze the critical process parameters of solution preparation process for parenteral drugs. For this aim a moisture, heat and oxygen sensitive drug, tigecycline was selected as model drug. **Material and Methods:** For this purpose, first of all, the formulation that can be used for tigecycline was examined. Since the formulation containing lactose has patent protection until 2026, a process has been developed with maltose monohydrate, another excipient that provide the stability of the active substance. The formulation was determined as 50 mg/vial tigecycline, 100 mg/vial maltose monohydrate and HCl/NaOH to adjust pH 4.5-5.5. Critical process parameters were determined as oxygen level of the solution, the order of adding the excipients and the active substance, the solution temperature. **Results and Discussion:** Since tigecycline is known to be sensitive to oxygen, the upper limit of the solution oxygen level was determined as 0.5 ppm. Because of tigecycline sensitivity against to heat, the solution temperature was kept constant within the range of 8°C±2°C. The addition of maltose before tigecycline was also determined as critical parameter. At the end of production with controlling critical process parameters as described above, tigecycline assay is not less than 100% in any product, and epimer of tigecycline was found less than ≤0.5%. All other data showed that the product can be manufactured in accordance with Tigecycline for Injection monograph in the US Pharmacopoeia. **Conclusion:** As a result of this study, critical process parameters of solution preparation process for tigecycline, a moisture, heat and oxygen sensitive drug, were determined and controlled successfully. These parameters are also a guide for the production of other drugs.

**Keywords:** Parenteral drugs, tigecycline, critical process parameters, solution preparation

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**ORAL PRESENTATION**

# PRODUCTION OF MELOXICAM NANOCRYSTALS BY NANOPRECIPITATION METHOD

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**Objective:** Meloxicam is a selective non-steroidal anti-inflammatory (NSAIDs) drug with an enolic acid structure. Although previously tablet and solution for intramuscular administration were commercially available, its formula (Anjeso), produced with nanocrystalline technology, received FDA approval in 2020. Thanks to its small particle size, it can be injected directly into the vein. This study aims to produce meloxicam nanocrystals by nanoprecipitation method. **Material and Methods:** For this purpose, firstly, the organic phase for preparation method was evaluated. For this purpose, acetone, ethanol, DMSO and DMF, which are frequently used solvents in nanoprecipitation method, were tested. In addition to the organic solvent, other excipients, used to stabilize the nanocrystals in the aqueous phase, were evaluated. Different amounts of meloxicam were dissolved in the organic phase and the effect of different parameters on the particle size of the obtained nanocrystals was investigated. **Results and Discussion:** The critical parameters affecting the particle size in the nanoprecipitation method generally was listed as the solvent, the drug/polymer concentration in the organic phase and the aqueous phase content. As a result of the trials in this study, DMSO was determined as the solvent that can dissolve the desired amount of meloxicam. Crystals with particle sizes varying between 266 nm and 1681 nm were obtained in the studies. A significant effect of the amount of meloxicam in the organic phase on the particle size was not determined. Nanocrystals were prepared by using PVA, PVP K12 or PVP K17 in the aqueous phase and the lowest nanocrystal size was achieved using PVA. **Conclusion:** As a result of this study, it was seen that the nanoprecipitation method could be an effective method for obtaining meloxicam nanocrystals which have suitable particle size for intravenous use.

**Keywords:** Meloxicam, nanocrystal, NSAIDs, nanoprecipitation, DMSO, PVA, PVP

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**ORAL PRESENTATION**

# INVESTIGATION ON THE COVID-19 PANDEMIC: HEALTH, INFECTION, AND VACCINATION

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## Abstract

COVID-19, a highly contagious and progressive infectious disease that is still posing a major global health challenge for our world, after the emergence of the seventh zoonotic pathogenic novel member of the human coronaviruses SARS-CoV-2. To assess the gravity of the situation and keep up to date with what is happening in this pandemic, we launched an "open to everyone" survey on social media, to estimate the use of preventive measures, or distinguish the rate of infection, and vaccine hesitation for members according to their gender, age, and place of residence; it contained questions on the novel human coronavirus, the applied preventive measures and vaccination, plus the impact of the pandemic on their lives. We found out that many believed in the disease and knew how to prevent it, but mostly didn't understand the transmission process, also some of the partakers did confirm the infection by the different available tests, others didn't, and unfortunately, half of the participants were not vaccinated and the other half refused to get a vaccine. Many sides of this pandemic are still unknown, and important data is needed, to understand the key to ending it.

**Key Words**: SARS-CoV-2, COVID-19, Epidemiology, Prevention, Vaccination.

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**ORAL PRESENTATION**

# EXTRACTION OPTIMIZATION OF BIOACTIVE COMPOUNDS OF THE SOLID RESIDUES FROM HYDRODISTILLATION OF LAVENDER BY BOX–BEHNKEN DESIGN

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## Abstract

A large amount of solid residues containing a considerable number of bioactive compounds is generated during the production of essential oils. Thus, the valorisation of these residues constitutes a promising alternative in terms of finished products rich in phenolic antioxidants. The aim of the present study is to find out the optimum extraction conditions for extraction of bioactive phenolic compounds and antioxidant activity from the solid waste of the essential oil extraction of *Lavandula angustifolia*. The effect (main and interactive) of extraction conditions on total phenolic and flavonoid content were studied using Box–Behnken design (three factors at three levels). The influence of extraction time (60-360 min), solid–liquid ratio (1:10–1:30 g/ml) and concentration of ethanol (40- 80%) on the extraction yield were investigated. Total phenolic compounds (TCP) and flavonoids (TCF) contents were estimated by Folin - Ciocalteu and aluminum chloride methods, respectively. Antioxidant activity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The results obtained showed that the extraction yield of bioactive compounds from lavender residues as well as the antioxidant activity were affected by the extraction parameters, a time of 4 hours, an ethanol/residue ratio of 20 and an ethanol concentration of 60% revealed a maximum content of 87.91 mg EAG/g in TCP and a maximum content of 7.71mg EQ/g in TCF. Under these conditions, the highest IC50 value (0.54 mg/mL) is recorded. In addition, the results showed that the contribution of the quadratic model was significant for all the responses. Second-order mathematical regression models were developed and were found to fit well with observed data. This study leads to confirm the possibility to valorize bioactive molecules present in the residues of the extraction of essential oils of lavender which are a potential source of bioactive compounds.

**Key Words**: Aromatic and Medicinal Plants, Bioactive Compounds, Waste valorization, Antioxidant activity, Lavender, Box–Behnken design.

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**ORAL PRESENTATION**

# SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATIONS OF NOVEL 2(3H)-BENZOXAZOLONE MANNICH BASES

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## Abstract

As a result of increasing antimicrobial resistance, the treatment of microbial diseases has become a challenging process. Studies on the conscious use of existing antibiotics and the discovery of new antimicrobial agents against this development of resistance have gained importance. Heterocyclic compounds are important pharmacophores in medicinal chemistry to form chemical structures with pharmacological activities. In this study, synthesis and antimicrobial activity of eight novel Mannich bases of 2(3H)-benzoxazolone derivatives with substituted piperazine moieties were investigated. The chemical structures of synthesized compounds were confirmed by FT-IR, elemental analysis, mass spectrometry, $^{1}$H NMR and $^{13}$C NMR spectra. The MIC (minimum inhibitory concentration) values of compounds were evaluated by broth microdilution method. According to the results, compound 3 and compound 4 which have 4-cyclopropyl piperazine substituent at position 3 of 2(3H)-benzoxazolone core structure were reported to have the highest activity in the series against *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis* with lower MIC values. Besides, all of the synthesized compounds were reported to have mild antifungal activity against *Candida albicans* compared to the reference drug, Ketoconazole. In general, all of the title compounds were reported to show moderate antimicrobial activities with the influence of different piperazine substituents.

**Key Words**: antimicrobial activity, 2(3H)-benzoxazolone, piperazine, Mannich reaction, Mannich bases

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ORAL PRESENTATION

# NOVEL SULFONAMIDES INCORPORATING β-LACTAM MOIETY AS CARBONIC ANHYDRASE INHIBITOR

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**Abstract**
Sulfonamides are a broad class of biologically active compounds with substantial pharmacological effects such as anticancer, antibacterial, antifungal, antiprotozoal, anti-inflammatory, and anticonvulsant that has been widely used in different therapeutic areas for nearly 100 years. β-Lactams, on the other hand, are essential heterocyclic compounds in medicinal chemistry, with anti-HIV, antimalarial, antibacterial, antioxidant, anti-inflammation, and anticancer properties. Our current efforts in this study aimed to design novel human carbonic anhydrase inhibitors (*h*CAIs) to lower the administration dose while staying within the safety limits of commercially available medications. In this direction, a novel series of sulfonamides incorporating β-lactam moiety (**5a-l**) were synthesized, characterized, and investigated the biological activities of these compounds on *h*CA I and II isoenzymes. Compared to the standard inhibitor acetazolamide, the synthesized derivatives (**5a-l**) were potent inhibitors against *h*CAs ($IC_{50}$s are in the range of 26.58-167.30 nM and 51.13-139.10 nM for *h*CA I and II, respectively). *In silico* studies were also carried out to evaluate the inhibition mechanisms of those inhibitors against *h*CAs. The new compounds described here could be promising lead molecules, and our findings could serve as a solid foundation for subsequent research into more potent *h*CAIs.

**Keywords**: Carbonic anhydrase, Sulfonamide, *in silico* study

**Acknowledgements**
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**ORAL PRESENTATION**

# EVALUATION OF THE *IN VIVO* WOUND HEALING ACTIVITY OF *ACHILLEA SINTENISII* HUB. MOR.

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**Abstract**

Wound is the deterioration of tissue or mucosal integrity as a result of damage to the normal anatomical structure and function of the body. Despite current clinical approaches, new, effective, accessible and economical alternatives in wound treatment are important. Plants that have been used since ancient times are among these treatment approaches. According to the results of ethno-pharmacological research, the genus *Achillea* L. has been used as wound healer in Turkish folk medicine. In our study, the wound-healing potential of *A. sintenisii* (AS), which has not been evaluated before and is endemic in the flora of Turkey, was evaluated in mice. After inducing full-thickness linear incision wound in the dorsal regions of experimental animals, thirty mice were divided into five groups: (1) negative control; (2) positive control (Madecassol®); (3) 0.5 g/day AS ointment; (4) 1 g/day AS ointment; (5) ointment base. Cream formulations were applied topically to the experimental animals twice a day for 12 days. At the end of the study, wound-formed back tissues of all euthanized animals were surgically removed for histopathological analysis. As a result of histopathological analysis, angiogenesis ($P < 0.01$), granulation tissue formation ($P < 0.01$), and re-epithelialization ($P < 0.05$) in the group receiving cream containing 0.5 g/day and 1 g/day extract, and the positive control group scores increased significantly compared to the control group, and edema decreased compared to the control group ($P < 0.05$). According to the LC-MS/MS analysis results of AS ethanol extract, it was determined that the plant is rich in phenolic compounds such as quinic acid, cynaroside, cosmosiin, chlorogenic acid etc. It is thought that the wound healing activity of the plant is due to the phenolic compounds it contains. Therefore, there is a need for bioactivity-directed studies to determine the compounds responsible for the wound repair effect.

**Key Words**: *Achillea sintenisii*, wound healing, *in vivo*, histopathology, LC-MS/MS

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**ORAL PRESENTATION**

# THE COMPARATION OF *IN VITRO* ENZYME INHIBITORY ACTIVITIES OF *PEUCEDANUM CHRYSEUM* FRUIT EXTRACTS

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## Abstract

*Peucedanum chryseum* is belongs to Apiaceae family and known as Hınzırotu in Turkish. Not only *P. chryseum* but also other *Peucedanum* species traditionally used for healing various diseases including sore throat, coughs, colds, headaches, asthma, cramps, epilepsy, gastrointestinal disorders, rheumatism, gout, and cardiovascular problems [1, 2]. In this study, we aimed to compare the phenolic and flavonoid content of the different *P. chryseum* fruit extracts. The antioxidant and enzyme inhibition activities of these extracts were also evaluated. The n-hexan, ethylacetate, and ethanolic extracts were prepared from *P. chryseum* fruits. The total phenolic contents of extracts were expressed as gallic acid equivalents, while flavonoid content was expressed as rutin equivalents. Antioxidant activities were determined by DPPH and ABTS radical scavenging methods. Cholinesterase, tyrosinase, inhibitory activities were investigated with different *in vitro* methods [3]. Sample results were statictically evaluated with One-way ANOVA followed by Tukey's multiple range. The results will be discussed.

**Key Words**: *Peucedanum chryseum*, enzyme inhibitory activity, antioxidant, total phenol, total flavonoid

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**ORAL PRESENTATION**

# ANTIOXIDANT, ANTICANCER ACTIVITY, AND MOLECULAR DOCKING INVESTIGATION OF TERPENOIDS RICH EXTRACT FROM *SAGE OFFICINALIS*

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## Abstract

*Sage officinalis* leaf extract was exposed to phytochemical screening to determine antioxidant and anticancer properties on (MCF-7) human breast cancer cells. To support antioxidant and anticancer effects, the phytoconstituents previously discovered were subjected to molecular docking investigations against 3ERT, 2J6M, 4OAR, 4DRH, and 3RCD protein as target receptors. **Material and Methodology:** *Sage officinalis* leaf was evaluated for total terpenoid content, DPPH, and ABTS assays. Invitro anticancer potential of the terpenoids rich extract was studied on human breast cancer cells (MDA-MB-231). Molecular docking studies were also performed to evaluate the binding interactions of phytoconstituents on 3ERT, 2J6M, 4OAR, 4DRH, and 3RCD protein using AutoDock Vina. **Results:** The results showed that hexane: ethyl acetate extract had the highest total terpenoid content 825.17 µg/ml. *Sage officinalis* leaf extract exhibited prominent antioxidant activity with a significant correlation between total terpenoid content and DPPH ($IC_{50}$) scavenging ($R = 0.976$, $P < 0.05$), and ABTS ($IC_{50}$) ($R = 0.962$, $P > 0.05$). In the MTT assay, Sage Officinalis leaf extract exhibited the highest antiproliferative activity ($IC_{50}$: $48.89 \pm 7.05$ µg/mL in the MDA-MB-231 cell line. The calculated cell viability was decreased with an increase in extract concentration. In silico toxicity studies revealed that fourteen active compounds in the plant extract have acceptable drug-like properties. α-Humelene was found to be best docked to three targets Human Estrogen Receptor, Progesterone Receptor, and Mammalian target of rapamycin (mTOR). In contrast, α-Ledene was the best-docked compound for EGFR Kinase, Progesterone Receptor, and HER 2. **Conclusion:** Our findings demonstrate that *Sage officinalis* leaf extract has significant antiproliferative properties in MDA-MB-231 cells, mediated by cell cycle disruption and pro-apoptotic effects. Furthermore, due to the presence of terpenoids, this study demonstrates the antioxidant potential of Sage Officinalis leaf extract. We got structural insights into putative binding mechanisms of drug-like bioactive molecules of *Sage officinalis* against primary molecular targets that play a critical role in cancer pathogenesis using an integrated strategy of virtual screening, molecular docking, and dynamics simulation investigations.

**Key Words**: *Sage officinalis;* DPPH assay; terpenoids; MDA-MB-231; Molecular docking 4OAR, protein

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**ORAL PRESENTATION**

# EXPRESSION OF PROTEASE ACTIVATED RECEPTORS IN STREPTOZOTOCIN-INDUCED DIABETIC RAT BLADDER

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**Objective:** Protease activated receptors (PARs), which are a class of G protein-coupled receptors (GPCRs), seem to be associated with bladder dysfunction [1, 2]. The aim of the present study was to evaluate the gene expression of all protease activated receptors (PAR1 to 4) in normal and streptozotocin (STZ)-induced diabetic rat bladder. **Material and methods:** A total of 14 male Sprague-Dawley rats were divided into two equal groups as control and STZ-induced diabetic rats. A single dose of STZ (35 mg/kg) was administered by intravenous injection for diabetes induction. After 4 weeks of STZ injection, expression of PAR1 to 4 mRNA in the rat bladder was estimated using reverse transcription-polymerase chain reaction (RT-PCR). The protein expression of transforming growth factor (TGF)-β1 was determined using western blotting. **Results:** Our results showed elevated fasting blood glucose levels and bladder weight in the diabetic groups compared with the control groups ($p<0.001$ and $p<0.05$, respectively). The mRNA expressions of bladder PAR1 and PAR4 in the diabetic rats were significantly higher than in the control group ($p<0.05$). There were no statistically significant differences in PAR2 and PAR3 gene expressions between control and diabetic rats (p values 0.212; 0.417, respectively). Diabetic rat bladders exhibited significantly higher expression of TGF-β1 protein compared with controls ($p<0.05$). **Conclusion:** Both PAR1 and PAR4 gene expression and TGF-β1 protein levels were significantly increased in the bladder of STZ-induced diabetic rats. These results indicated that increased expression of PAR1, PAR4 and TGF-β1 may contribute to the underlying mechanisms of diabetic bladder dysfunction.

**Key Words**: Protease activated receptors, diabetes, bladder, transforming growth factor β1

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**ORAL PRESENTATION**

# THE EFFECT OF ROSUVASTATIN ON LUNG TISSUE IN THE SEPSIS MODEL INDUCED BY CECAL LIGATION AND PUNCTURE

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## Abstract

Sepsis continues to be an important health problem due to its mortality rate of 30-70% despite modern treatments. One of the organs most affected by sepsis, the pathogenesis of which includes the systemic inflammatory response to infection, is the lung. Statins have pleiotropic effects with their antioxidant, anti-inflammatory and immunomodulatory aspects. Rosuvastatin exhibits higher pleiotrophic effects as well as greater enzyme suppression properties. Our aim was to evaluate the dose-dependent effect of rosuvastatin against lung injury in an experimental model of sepsis induced by cecal ligation and puncture (CLP). Sprague Dawley rats were randomly divided into six groups: Sham, CLP, CLP+rosuvastatin (10 mg/kg), CLP+rosuvastatin (20 mg/kg), control+rosuvastatin (10 mg/kg), control+rosuvastatin (20 mg/kg). Rosuvastatin was given orally 4 hours before the CLP protocol, and at the same time in the control group. The rats were sacrificed 16 hours after the CLP protocol by monitoring their mortality. MDA (Malondialdehyde) and GSH (Reduced glutathione) assays were performed to evaluate oxidative stress and antioxidant status in lung tissue. The lung specimens were evaluated for the presence of alveolar inflammation, interstitial inflammation, vascular congestion and alveolar septal thickness. Expression levels of caspase-3, nuclear factor kappa B (NF-kβ/p65) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) are evaluated using immunohistochemistry analysis. MDA, which was increased in the CLP group compared to the sham group, showed a statistically significant decrease in the CLP+rosuvastatin groups ($P<0.05$). The decreased GSH levels in the CLP group were significantly higher in the CLP+rosuvastatin groups ($P<0.05$). Lung tissue damage decreased significantly, especially in the CLP+rosuvastatin (10 mg/kg) group, compared to the CLP group. The increased levels of caspase-3, NF-kβ/p65 and 8-OHdG in the CLP group decreased to the level of the sham group in the rosuvastatin administered CLP groups. Rosuvastatin may represent a promising means of preventing sepsis-induced lung injury via antioxidant and anti-inflammation effects.

**Key Words**: Rosuvastatin, sepsis, lung, cecal ligation and puncture

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**ORAL PRESENTATION**

# *IN VITRO* ANTIRADICAL ACTIVITY OF *RUMEX PATIENTIA* L.

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## Abstract

*Rumex patientia* L. belongs to Polygonaceae family. The leaves of this plant are used as green vegetable and commonly called ‘‘labada’’ in Turkey. The antiradical activities of *Rumex patientia* L. extracts were examined in this study by different *in vitro* assay including DPPH free radical, $H_2O_2$ (non free radical) and superoxide anion radical scavenging effects. The results clearly indicated that *Rumex patientia* L. extracts had an effective radical scavenging activity and consumption of this plant is benefical for human health due to their activities and it can be used to prevent the damage caused by free radical.

**Key Words**: *Rumex patientia* L.; antiradical; DPPH; antioxidant; $H_2O_2$, superoxide anion radical.

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**ORAL PRESENTATION**

# EFFECT OF *Melaleuca alternifolia* ON CYTOTOXICITY AND NPY GENE EXPRESSION

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**Abstract**

*Melaleuca alternifolia* plant's essential oil, tea tree oil (TTO), is used as an active ingredient in the topical formulation in the pharmaceutical and cosmetic industries because of its anti-microbial properties. Neuropeptide Y (NPY) has an important role in the molecular mechanisms of obesity, anxiety disorders, depression, addiction, and epilepsy. NPY has recently received much attention as an endogenous anti-epileptic and anti-depressant agent. The SH-SY5Y (ATCC® CRL-2266™) cell line, used in this study contains many neuron cells responsible for NPY synthesis. Analysis of the toxicity of TTO is limited in the literature. Considering how widely it is used in the field of cosmetics and health today, it is considered important to determine the toxic effects of this substance.

The cytotoxic/proliferative effects of TTO solutions prepared at different concentrations by volume on the SH-SY5Y cell line were investigated. SH-SY5Y cells were treated with tea tree oil for 24 and 48 hours. Then, RNA isolation and cDNA synthesis were performed in cells treated with TTO at the determined concentrations. Expression analysis of the NPY gene was analyzed using the Real-Time PCR method.

When the Real-Time PCR results were evaluated, it was observed that the 24-hour diluting at a ratio of 1:32 and 1:64 TTO application had a greater effect on NPY gene expression compared to the other doses. While TTO increased the gene expression 1.14 times at a ratio of 1:32 applied for 24 hours compared to the control group; gene expression increased 2.24 times at a TTO ratio of 1:64.

In this study, the effect of TTO on neuroblastoma cells was investigated by cytotoxicity studies and NPY gene expression analysis. Thus, it is aimed to contribute to the literature by revealing the cytotoxicity of TTO and its effect on NPY gene expression, about which there is insufficient information.

**Key Words**: Tea tree oil, gene expression, NPY, neuroblastoma, SH-SY5Y

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**ORAL PRESENTATION**

# TOWARDS MORE POTENT ANTICANCER DRUGS: PHARMACOPHORE MODEL ACCOMPANIED BY CONFORMATIONAL DYNAMICS REVEALS NEW P53 ACTIVATORS

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## Abstract

Targeting the interaction between tumor suppressor p53 and murine double minute 2 (MDM2) protein has been an attractive therapeutic strategy of recent cancer research. There are a few number of MDM2-targeted anticancer drug molecules undergoing clinical trials right now, however none of them have been approved so far. In this study, a new approach in which global dynamics of MDM2 obtained by elastic network models are used as a guide in the generation and validation of the ligand-based pharmacophore model prior to virtual screening was employed in order to search for novel MDM2 inhibitors. Virtual screening, rigid and induced-fit molecular docking strategies were then conducted to account for the very flexible and intrinsically disordered nature of MDM2 protein, so as to capture several hit molecules exhibiting high affinity. Application of a rigorous molecular mechanics-generalized born surface area (MM-GBSA) method provided a more accurate prediction of the binding free energy values. Two leading hit molecules which have shown better docking scores, binding free energy values and drug-like molecular properties as compared to seven clinical trial MDM2 inhibitor molecules were identified by screening the drug libraries with this methodology. It was worth noting that besides their high docking scores, the two leading hits obtained have extra intermolecular interactions with MDM2 which indicates a stable complex formation as compared to the clinical trial MDM2 inhibitors. Having molecular properties in suitable ranges contributes positively for the hit compounds to be drug-like. Therefore, combined computational strategy employed in generating a pharmacophore model based on the active available ligands undergoing clinical trials and validating the model by the conformational dynamics background to screen libraries can be a promising tool in the initial stage of computational drug design or drug-repurposing which would save time and money in the discovery of potential new hit molecules.

**Key Words**: protein dynamics, elastic network model, molecular docking, binding free energy, computational drug design, MDM2**.**

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**ORAL PRESENTATION**

# ANTIMICROBIAL EFFECTS OF NON-STEROID ANTI-INFLAMMATORY DRUGS

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**Abstract**

Microorganisms, known as the oldest living things in the world, can easily adapt to changing conditions. The resistance that develops due to the unconscious use of antibiotics in bacteria has increased seriously and has become a global problem in recent years. Although this situation leads to the need for new antibiotic discovery, studies are being carried out on the idea of using drug groups whose main effect is not antimicrobial for this purpose, since the process is long, difficult and costly. Examples of these groups are local anesthetics, antipsychotics, antidepressants, statins, antihistamines, opioids and non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs are a very wide group of drugs with antipyretic, analgesic and anti-inflammatory effects. In in vivo and in vitro studies, effect of some NSAIDs such as aspirin, diclofenac sodium, ibuprofen, flurbiprofen, paracetamol, meloxicam, etodolac, naproxen, celocoxib, tolfenamic acid, flunixin meglumine alone or in combination with drugs (seftriakson, amoksisilin, eritromisin, tetrasiklin, gentamisin, flukanozol, mikonazol, ekanozol) to which the agent is resistant has been evaluated on viruses (*influenza, dang, japon ensefalit*), fungi (*T.asahii, C.albicans, C.parapsilosis*), bacteria (*H.pylori, S.aureus, E.coli, S.pneumoniae, M.tuberculosis, P.aeruginosa*) and biofilms formed by some of these agents. NSAIDs have a direct effect by affecting the growth and replication, adhesion, metabolism and motility of the agent, or have an indirect effect by increasing the sensitivity of the agents to the drug by forming synergism with the drugs to which the agents are resistant. As a result, the antimicrobial effect of non-antimicrobial drugs, such as NSAIDs, seen at different degrees, should not be considered as a side effect. Although the use of NSAIDs for antimicrobial purposes has shed light on clinical applications as a promising therapeutic approach, more studies are needed because their mechanism of action has not yet been fully elucidated.

**Key words:** NSAID, Antimicrobial effect, Resistance

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**ORAL PRESENTATION**

# INVESTIGATION OF ANTIVIRAL ACTIVITIES OF SOME FISH MUCUS

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## Abstract

Fish skin mucus contains innate immune factors and acts as the first line of physical or chemical defense against pathogens. This study aimed to determine the antiviral activity of sea bream, rainbow trout and sea bass fish skin mucus against Herpes Simplex Virus (HSV)-1. The non-cytotoxic dose of the fish mucuses were determined in Vero cell culture and their antiviral activity against HSV-1 virus was evaluated. Then, the cells in the control, mucus, HSV-1 and HSV-1+mucus were centrifuged at high speed and the MDA level and CAT and SOD activity were determined. In addition, SOD and CAT activities, cathelicidin, hepcidin, galectin 2, C10ORF99 and immunoglobulin M levels were also measured in fish mucus. Antiviral activity values of mucus of sea bream, rainbow trout and sea bass against HSV-1 were determined as $2^{-4}$, $2^{-5}$ and $2^{-2}$, respectively. It can be stated that the antiviral activity power of the mucus of the other two fish is higher when compared to the mucus of sea bass fish. In addition to the fact that the mucus of sea bream and rainbow trout reduce the MDA level increased by the virus more than the mucus of sea bass, the AMP peptide levels in this mucus are generally higher, which supports this view. As a result, it has emerged that the skin mucus of sea bream and rainbow trout can be combined with antimicrobial agents both in aquatic and other organisms, but this needs to be supported by further research.

**Key words:** Fish mucus, antiviral, antimicrobial peptide, immunoglobulin M

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## ORAL PRESENTATION

# SYNTHESIS OF SCHIFF BASES AND NEW SECONDARY AMINE DERIVATIVES OF *P-V*ANILLIN AND EVALUATION OF THEIR NEUROPROTECTIVE AND ANTIDEPRESSANT POTENTIALS

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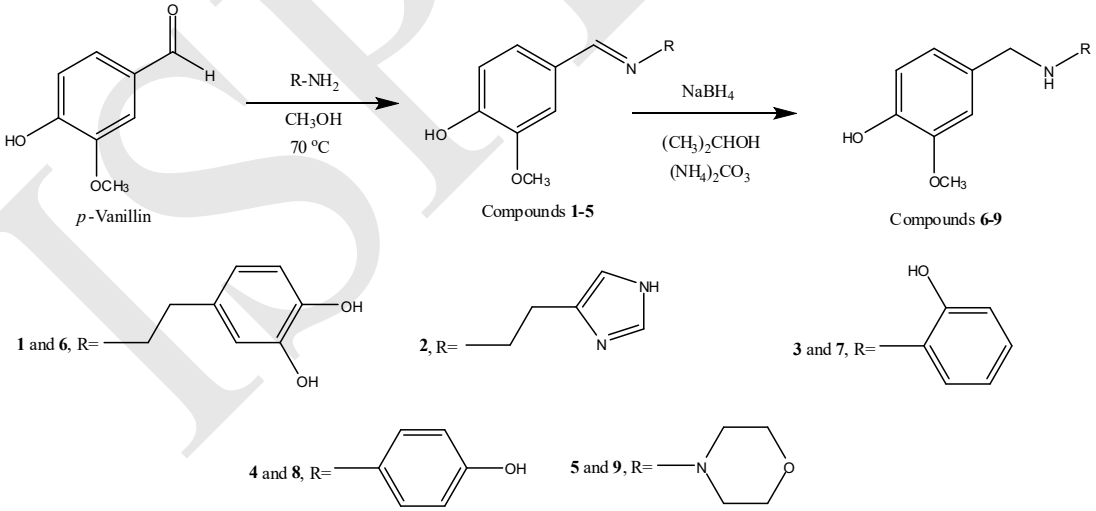
**Abstract**

In the current study, five Schiff base derivatives (**1-5**) of *p*-vanillin reacting with dopamine, histamine, 2-aminophenol, 4-aminophenol and 4-aminomorpholine were synthesized. Then, these Schiff bases were reduced with $NaBH_4$ to produce secondary amines (**6-9**). The chemical structures of the synthesized molecules were characterized by UV-Visible, FTIR, $^1$H-NMR, $^{13}$C-NMR, 1D- and 2D-NMR spectroscopic methods. The neuroprotective effects of the synthesized molecules (**1-9)** and *p*-vanillin evaluating the enzymes inhibitory effects on cholinesterase's (AChE and BChE) were determined for the first time. The neuroprotective potentials of the tested molecules were also compared with the commercial anticholinesterases, neostigmine and galantamine. The results on the inhibitory effects of the molecules on the AChE showed that the neuroprotective effects of all molecules, except for compound **1** are much weaker than the commercial anticholinesterases, neostigmine and galantamine. Compound **1** showed a potent inhibitory effect on the AChE activity with $IC_{50}$=1.53 mg/mL. Whereas all tested molecules exhibited the stronger inhibitory effects against BChE enzyme than AChE enzyme, **1** and **3** were found to be the most effective inhibitors against both AChE and BChE enzymes among the tested molecules. Based on the present results, compound **1** stands outs as a target molecule for *in vivo* as well as clinical studies due to its potent neuroprotective potential.




Furthermore, the antidepressant properties of *p*-vanillin, the Schiff bases and the secondary amines were tested for the first time against the MAO-A enzyme and their antidepressant properties were compared with the MAO inhibitor, clorgiline. The current results showed that among the tested molecules, *p*-vanillin ($IC_{50}$=0.72 mg/mL), **3** ($IC_{50}$=0.71 mg/mL), **7** ($IC_{50}$=1.22 mg/mL) and **8** ($IC_{50}$=2.36 mg/mL) are the potential antidepressant agents, although their antidepressant effects were lower than that of clorgiline ($IC_{50}$=0.34 mg/mL). However, the *in vivo* antidepressant properties, safeties and toxicities of the molecules should be investigated with further studies.

**Key Words:** *p*-Vanillin, Schiff bases, secondary amines, dopamine, anticholinesterases, antidepressant.

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**ORAL PRESENTATION**

# *IN VIVO* STUDY OF COMBINED EFFECT OF FENUGREEK EXTRACT (*Trigonella foenum-graecum* L.) WITH PROBIOTIC (*Bifidobacterium breve)* AGAINST *HELICOBACTER PYLORI*

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## Abstract

*Helicobacter pylori* is a Gram-negative bacterium that has been linked to chronic active gastritis, stomach ulcers, and gastric cancer. Although, conventional treatment achieved a great advancement in controlling *H. pylori* infection without any efficient. Nowadays, it's intended to find some other alternative sources that may be used alone or in combination with antibiotics to eradicate the infection.

In this study, we highlighted the *in vivo* antibacterial effect of fenugreek extract (*Trigonella foenum-graecum* L.) and probiotic (*Bifidobacterium breve*) on *H. pylori* colonization using Wistar rats as an animal model. On the other hand, we confirmed the enhanced effect of combination between *B. breve* and fenugreek extract on *H. pylori,* which have been reported to exert antibacterial and gastric mucosal protective effects.

Fenugreek extract was found to inhibit the growth of *H. pylori* in a concentration dependent manner, Also, when *H. pylori*-infected rats were administered *B. breve*, the infection rate of *H. pylori* was significantly reduced, while the combination of *B. breve* and fenugreek extract effectively inhibited *H. pylori*.

In addition, the *B. breve* and fenugreek extract complex mixture significantly reduced the stomach inflammation in *H. pylori* infected rats. These results suggest that this complex mixture may be an alternative to treating diseases caused by *H. pylori* infection.

**Key words:** *Helicobacter pylori*, Fenugreek exract, *Bifidobacterium breve*, *In vivo*, Combined effect, Inflammation.

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**ORAL PRESENTATION**

# DEVELOPMENT AND EVALUATION OF PEDIATRIC ORODISPERSIBLE TABLETS OF PRAMIPEXOLE

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## Abstract

**Introduction:** Restless leg syndrome (RLS) or Ekbom's syndrome is a sensory motor disorder characterized by a compelling urge to move the limbs. There has been a limitted number of studies on RLS in children, and much less on Pramipexole (PRA), and the therapy of RLS in children is mostly unexplored. Furthermore, PRA is presented only as tablet form at varied strengths, which are not specified for pediatric use, therefore there is a requirement for the development of more child appropriate dosage forms. Child appropriate dosage forms are indispensable in modern medicine and are a prerequisite for successful pediatric drug therapy. Since orodispersible tablets prove to be the ideal pediatric dosage form offering the possibility for personalized dosing. The aim of this study was to take the advantage of convenient direct compression method for preparation of Orodispersible Tablets (ODTs) containing 0.125 mg PRA per tablet. **Materials and methods:** For direct compression, six ready-to-use commercial tablet excipients (F-Melt®, Pearlitol® Flash, Pharmaburst® 500, Prosolv® Easytab SP, Ludiflash®, Parteck®) were employed, and their compatibility was assessed. Additionally simulated wetting test, disintegration time and *in vitro* dissolution test was examined too. **Results:** All the examined excipients were successful in compressing ODTs, and all the formulations had appropriate crushing strength, low friability, and a notably short disintegration time. ODTs with a disintegration time of less than 30 seconds were judged appropriate for future research. **Conclusion:** In vitro dissolving investigations revealed that ODTs produced from Pharmaburst® 500 released the medication completely after 15 minutes. The drug's short-term stability studies revealed no significant changes in the formulations tested. Finally, the most promising formulation was found to be PRA-containing ODTs produced with Pharmaburst® and Prosolv®.

**Key Words**: Pramipexole Dihydrochloride Monohydrate, Pediatric dosage forms, Orodispersible tablets, Direct compression

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**ORAL PRESENTATION**

# USE OF MEDICINAL PLANTS BY PREGNANT AND POSTPARTUM WOMEN: PREVALENCE, ASSOCIATED FACTORS AND TRADITIONAL PRACTICES (IN THE PROVINCE OF GUELMIM-SOUTH MOROCCO)

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**Abstract**

Women have long used herbal medicines during pregnancy and childbirth for a variety of purposes. This study aims to estimate the prevalence of the use of medicinal plants by pregnant women, to describe the traditional practices of self-medication and to determine the associated factors. This is a multicenter cross-sectional study with a descriptive and analytical purpose. The study was conducted at the level of all first-level health care establishments, hospital maternity and birthing centers in the province of Guelmim. Data was collected using an interview questionnaire.

**Results.** A total of 560 participants were included. The median age of the women interviewed was 30 years old(IQR). Prevalence of the use of medicinal plants was 72% distributed as follows: 67.45 % during pregnancy, 26.82% during childbirth, 5.73% postpartum cases. The plants frequently used by the women interviewed were: Artemisia herba-alba (Asso.), *Thymus., Lepidium sativum* L., *Trigonellafoenum-graecum* L., *Aloysia citriodora* Palau et *Olea europaea* L.var. *sativa* Loud.
Pain, genital infections, facilitating childbirth, flu syndrome, anemia, gestational diabetes and high blood pressure were the most common reasons for use. The consumption of medicinal plants is significantly associated with the level of education (Chi square =15.651; p =0.004) on the one hand and with pregnancy monitoring (Chi square =5.283; p =0.028) on the other hand. The prevalence of the use of medicinal plants during pregnancy and childbirth is high in the province of Guelmim. Hence the interest of deepening the investigations in the sense of exploring the risks and complications related to the use of plants during pregnancy and childbirth.

**Keywords :** Medicinal plants ; Pregnancy ; Labor and delivery; Prevalence; Associated factors; Traditional practices; Morocco

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**ORAL PRESENTATION**

# FREQUENTLY USED CYTOTOXICITY ASSAYS

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## Abstract

Cytotoxicity refers to hazardous effects via physical, chemical, and biological agents in cells. After exposure to these agents, cells might lose morphological structure or physiological functions. These alterations leading to cytotoxicity can be determined by cytotoxicity assays. These assays are performed in drug discovery, cosmetic, environmental and ecological studies in order to investigate cell viability or death. Many cytotoxicity assays have been developed to observe morphological and functional changes up to date. Cytotoxicity assays might be categorized in different aspects. Dye exclusion (Trypan blue etc.), colorimetric (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide), fluorometric (2'-7'dichlorofluorescin diacetate) methods and luminometric (Adenosine triphosphate) methods are the most popular technics determining cell viability and toxicity. Cytotoxicity assays ensure rapid screening in a short period of time and valuable information for further animal studies. These methods have advantages and disadvantages when comparing each other. For this purpose, researchers must select the most appropriate assay in accordance with the purpose.

**Key Words**: Cytotoxicity, Cytotoxic Assays, Cell Viability

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**ORAL PRESENTATION**

# HISTORY OF COLISTIN USAGE AND ITS TOXICITY

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## Abstract

Colistin is a kind of polymyxin antibiotic initially marketed in the 1950s. Colistin mainly targets gram-negative pathogens, also gram-positive organisms and some fungi. Moreover, colistin is used to treat infections of multidrug-resistant (MDR) strains of *E. coli*, *P. aeruginosa*, *K. pneumoniae* etc. However, its usage is restricted because of high rates of nephrotoxicity and neurotoxicity. Colistin is excreted mainly by the kidneys and a high dose of the drug might impair renal function, however, the mechanism of its nephrotoxicity is unknown. Colistin might also cause neurotoxicity resulting from hypoxia, concomitant medication or impaired renal function. Due to its mentioned toxicities Colistin was primarily replaced by aminoglycosides for the treatment of infections in the 1970s. Hence today, Colistin is clinically preferred as a 'last-line' therapy for the treatment of infections caused by MDR Gram-negative pathogens. Many antibiotics such as Penicillins, Fluoroquinolones, and Aminoglycosides available for clinical use have failed to treat resistant strains. Also, there is no novel and an effective antibiotic against Gram-negative bacteria. Hence old drugs such as Colistin are reconsidered for the treatment of multidrug-resistant (MDR) Gram-negative bacteria infections despite having significant toxicities. Novel colistin derivatives with less toxicity are needed to be synthesized to fight against MDR Gram-negative bacterial infections in order to protect human health.

**Key Words**: Colistin, Antibiotic, Colistin Toxicity

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**ORAL PRESENTATION**

# PREPARATION AND CHARACTERIZATION OF COMBINED DRUG CONTAINING TOPICAL NANOEMULGELS FOR SKIN DISEASES: A PRELIMINARY STUDY

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## Abstract

Today, there is a severe increase in skin diseases. Among the reasons that cause this increase, environmental factors and malnutrition types/resources are common. Along with the increase in skin diseases, new ways of treatment and new dosage forms continue to be sought. In recent years, nanoemulsions, one of the new generation nano-sized drug systems, have attracted much attention. Nanoemulsions are oil-in-water (O/W) or water-in-oil (W/O) dispersions of two immiscible liquids stabilized using a suitable surfactant. Nanoemulsions have the potential to overcome many disadvantages of conventional drug formulations. Nanoemulgels are emulsion-based topical gel formulations in which nano-sized emulsion droplets can be prepared with the help of high-energy or low-energy methods and converted into nanoemulsion by adding a suitable gelling agent. The aim of this study is to prepare and characterize nanoemulgel formulations containing salicylic acid and povidone iodine in combination. Combined drug containing nanoemulgels have been successfully prepared. Some characterization studies have been carried out on these nanoemulgels. However, additional characterization studies will be done in the future. In this study, salicylic acid and povidone iodine were combined for the first time. Nanoemulgels containing this drug combination can be developed further and used in the treatment of skin diseases. Combining the therapeutic properties of both salicylic acid and povidone-iodine would provide many advantages for the treatment of many skin diseases.

**Key Words**: Nanoemulsion, nanoemulgel, salicylic acid, povidone iodine, characterization.

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**ORAL PRESENTATION**

# MEDICINAL PLANTS AND THE TREATMENT OF DIABETES IN MOROCCO: SURVEY WITH PATIENTS

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## Abstract

Diabetes is a serious chronic and metabolic disease that occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. In Morocco diabetes have significantly high frequency, with more than one and half million types 2 diabetics in 2010, and would reach 2.5 million by 2030. Despite the development of modern medicine, it is still difficult to achieve adequate glycemic control in many diabetic patients due to the gradual decline in β cell function. All existing therapies for the treatment of diabetes, however, have limited efficacy and / or significant side effects. The use of drugs and their side effects are of great concern, and most patients have perceived negative side effects of conventional medicine. Therefore, patients often resort to alternative treatments such as herbal remedies. This study was conducted in public healthcare establishments in Guelmim city in south of Morocco to report medicinal plants used in folk medicine to treat diabetes. Three hundred sixty-two informants were interviewed through semi structured interviews. The inventory includes scientific, popular and common names of the plants, used parts and method of preparation. The survey shows that 24.6% of the patients use these plants. Twenty-seven medicinal plants belonging to seventeen families were inventoried and three species were cited for the first time in the treatment of diabetes in Morocco. *Olea europea, Artemisia herba-alba* and *Trigonella foenum-graecum* are the most plant species used to treat diabetes, and the two most cited families are *Lamiaceae* (5 species) and *Apiaceae* (4 species). Leaves represented the most utilized part of plants and decoction was the most cited mode of preparation of drugs. The result indicated that some plants are extremely toxic at high doses and chronic treatment.

**Keywords**: Medicinal plants; Ethnopharmacological survey; Ethnobotany; Diabetes; Guelmim city

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**ORAL PRESENTATION**

# OLIVE TREE: A NOVEL SOURCE OF PLANT-BASED PHARMACEUTICALS FOR FUTURE

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**Abstract**

From the first human and may be to the end of the humanity some mystical plants; like olive, fig, grape could be together with human being. Having food, health and all purposes, these plants were mentioned at holy books, as well. Recent scientific reports have mentioned valuable phytochemicals, biological activities, nutritional values and healing powers of these plants' products. Olive tree is a unique food and medicinal plant and its all the plant parts have been used for many different purposes for centuries. Olive fruit, olive oil, olive leaf, olive stone and olive flowers are well known olive tree products. Although an ancient and well-known plant, olive tree and its valuable plant parts has been started to rediscover by recent scientific studies. Addition to scientifically reported fatty acids in the olive oil, a novel omega-7 fatty acid, Paullinic Acid (*cis*-13-Eicosenoic acid, $C_{20}H_{38}O_2$, 310.522 g·mol$^{-1}$) was found in Kilis Yaglik olive oil by Sekeroglu et al. (2020). As an Anatolian Folkloric Herbal Medicine olive kernel was examined in detail and a novel fatty acid, Nervonic Acid (*cis*-15-tetracosenoic acid, Selacholeic acid, $C_{24}H_{46}O_2$, 366.62 g/mol$^{-1}$) was found first time by Sekeroglu et al. (2021) in the same olive cultivar. Scientific studies on olive flowers are ongoing, and preliminary results indicate that olive flowers could have distinguished and valuable phytochemicals. Thus, former scientific reports and ongoing studies tell us that all the plant parts of the olive tree are waiting to be rediscovered for future for novel pharmaceuticals and valuable natural herbal raw materials.

**Keywords:** Olive tree, paullinic acid, nervonic acid, Anatolian Folk Medicine, pharmaceuticals.

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**ORAL PRESENTATION**

# ANTHOCYANIN-RICH BLACK CURRANT JUICE INHIBITS CELL PROLIFERATION IN HUMAN COLORECTAL ADENOCARCINOMA THROUGH INDUCTION OF APOPTOSIS

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## Abstract

Epidemiological studies over the past decades have revealed that regular intake of dietary antioxidants is helpful in prevention and control of cancer. Black currant (*Ribes nigrum* L., Grossulariaceae) fruits are edible berry fruits that known to contain high amounts of anthocyanins, which significantly contribute to anticancer, antiproliferative, anti-inflammatory and radical scavenging properties of the fruit. Black currant juice (BCJ) was prepared and semi-purified by solid-phase extraction (SPE). Sugars, acids, and other water-soluble compounds, and polyphenols (other than anthocyanins) were removed, then the purified extract was tested against human colorectal adenocarcinoma cells (HT-29 and DLD-1), and normal human colonic epithelial cell (NCM460). Cell viability and anticancer effect of BCJ against the cancer cells was analyzed using MTT assay. Apoptotic bodies in BCJ-treated human colorectal cancer cells were determined using immunologic based ELISA method. BCJ have been found to suppress cell proliferation towards the tested cancer cells

**Keywords:** Apoptosis, black currant, cytotoxicity, colorectal cancer, dietary antioxidants, cell death

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**ORAL PRESENTATION**

# VALUE CHAIN OF BILBERRIES IN KELMENDI REGION

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## Abstract

The bilberry value chain analysis in the northern part of Albania represents an overview and in-depth analysis of the value chain linkages, resulting in the categorization of a number of issues as well as findings, but also giving general recommendations for the bilberry development program.

The results of a bilberry study in the Kelmendi region are presented in this report. The study's purpose was to establish Kelemendi's bilberry area as a product with unique attributes and characteristics associated to the region, adding to the brand of quality recording while also maintaining and improving the area's biodiversity. The research examines the commercialization of forest products using the value chain method. The study is useful in evaluating the relevance of stakeholders or groups like collectors, processors, businesses, and exporters in driving the market in wild goods from the Kelmendi region. The goal is to first create a broad image of the diverse and wide group of enterprises who work with forest products. The goal is to learn how businesses in Kelmendi feel about various issues relating to the forest products industry.

**Key Words**: *Vacinium myrtillus*, Kelmendi, product definition

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**POSTER PRESENTATION**

# ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *SELAGINELLA DENTICULATA*

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**Abstract**

A number of species of the genus *Selaginella* have been traditionally used for medicinal purposes throughout the world. The present study aimed to extract the active compounds of collected *Selaginella denticulata* in Tiaret region (Algeria) using maceration and decoction method and to test their antioxidant activity and antibacterial effect against some bacterial strains (*E. coli*; *S. aureus*; *S. epidermidis* and *P. aeruginosa*).

The organic extracts were obtained by maceration and decoction using methanol, ethanol and distilled water as solvents, the yield extraction of methanolic, ethanolic and aqueous extract was 5.9%, 4%, and 3.2% respectively, while decoction gave 7.1%. However, the content of total polyphenols in methanolic, ethanolic and aqueous extracts was 2.85± 0.46 GAE/g extract, 0.85±0.027 GAE/g extract, 2.29±0.38 mg GAE/g extract respectively, and 2.15±0.38 mg GAE/g extract for decocted extract.

The phytochemical screening highlights the presence of flavonoids, saponins, sterols, tannins and proteins. Although the antioxidant activity carried out using the DPPH free radical reduction method showed that $IC_{50}$ was estimated at 04 µg /ml. The results of antibacterial activity of methanolic extract carried out by Agar Disk Diffusion method revealed the sensitivity of the studied strains to our extract especially for *Staphylococcus aureus* with DZI of 19.33 ± 2.51 mm and MIC of 25 mg/ml. This study showed that *Selaginella denticulata* has promising antioxidant and antibacterial activities.

**Key words:** *Selaginella denticulata*, phytochemical screening, antibacterial effect, antioxidant activity

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**POSTER PRESENTATION**

# DESIGNING PRIMER AND PROBES FOR MULTIPLEX REAL-TIME PCR FOR SARS-COV-2, INFLUENZA AND RESPIRATORY SYNCYTIAL VIRUS

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## Abstract

**Background:** Quantitative Real-Time Polymerase Chain Reaction (qPCR) is a type of Nucleic Acid Amplification Test (NAAT) which has become the gold standard in the diagnostic process as it allows the genetic material of the target pathogen to be amplified multiple times in a single reaction. SARS-Co-V-2, influenza viruses and Respiratory Syncytial Virus (RSV), which causes severe respiratory diseases, show clinically similar symptoms. A sensitive and effective diagnosis process is vital in terms of applying the right treatment process, controlling the course of the disease and providing immunity. **Aim:** We aimed to develop an *in silico*-based multiplex diagnostic kit with high accuracy and sensitivity, considering that the correct determination of the pathogen is very important in the selection of the treatment to be applied against viral-induced upper respiratory tract diseases with similar symptoms. **Method:** In this study, the most current gene and genome data of the viruses were downloaded from GISAID, Influenza Research Database and GenBank databases, and were analyzed using bioinformatics and big data processing methodologies. **Result:** With the analysis, primer and probe sequences that can be used in the diagnosis of SARS-CoV-2, influenza A and B and RSV A and B were determined, their multiplex capabilities were tested *in silico* and so designed an open panel that could be applied in any combination depending on the needs. **Conclusion:** A qPCR-based kit has been made ready for optimization and validation studies to enable diagnosis of these three viruses in a single reaction.

**Key Words:** SARS-CoV-2, Influenza, RSV, qPCR, Panel

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**POSTER PRESENTATION**

# THE ANTI-PROLIFERATIVE EFFECTS OF INDOLIN-2-ON DERIVATIVES IN *IN VITRO*

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## Abstract

*Objective:* Long-time exposure to asbestos and erionite arises malignant transformation of mesothelial cells in thoracic or abdominal cavities leading to Malignant Mesothelioma (MM). MM is difficult to treat since its late diagnosis, absence of specific serum biomarkers, anatomical location, and the limitations of current drugs. Therefore, it is crucial to develop new approaches for the treatment of the disease. It is well-known that approximately 60% of the drugs used for cancer treatments are heterocyclic compounds. Indole derivatives are such complexes with various pharmacological properties including antibacterial, anti-fungal, anti-malarial, anti-viral and anti-cancer activities. In this study, the anti-carcinogenic action of originally designed and synthesized Indolin-2-on derivatives has been investigated in MM cells and, in their normal counterparts. *Methods:* To reveal the effect of Indolin-2-on derivatives on cell viability and to understand the migration capacity of cells the MTT and the wound healing assays were performed in 72h, respectively. *Results:* All four compounds reduced the cancer cell viability and prevented the wound healing in a dose-dependent manner. In addition, observed that cancer cells were more sensitive to these derivatives than non-cancerous cells. *Conclusion:* Indolin-2-on derivatives indicated anti-cancer potential in MM cells. Thus, it will be worth investigating the effects of these derivatives more for future work.

**Key Words**: Malignant Mesothelioma, Indolin-2-on, proliferation, migration

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**POSTER PRESENTATION**

# SYNTHESIS AND CHARACTERIZATION OF INULIN-POLY (ε-CAPROLACTONE) COPOLYMER FOR USE IN CONTROLLED DRUG DELIVERY

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**Abstract**

In cancer therapy, the targeted drug delivery is of important strategy in terms of releasing the drug into cancer cells without damaging healthy cells. Therefore, the amphiphilic, self-assembly nano-drug delivery formulations play an essential role to overcome biological barriers by reducing toxicity and achieving controlled delivery. Because of having amphiphilic nature, they tend to form nanoparticles immediately in physiological fluid. While the hydrophilic head oriented to the outside of the formed particle, the hydrophobic part provides homogeneous dispersion of hydrophobic anticancer drugs in the inner core.

Polysaccharides are the main source of cellular energy and the cancer cells need more energy than normal cells. Therefore, they are more preferred energy source by cancer cells. Additionally, they have high cellular adhesion and easily interact with cell carbohydrate-binding proteins on the cell surface, providing the advantage of the enhanced permeability and retention (EPR) effect. [1,2,3] Therefore, the nanoparticles possessing hydrophilic polysaccharide surface and hydrophobic inner core have been considered as promising materials for the targeting chemotherapeutic drug delivery and cancer cell imaging systems.

Inulin is a class of fructan composed of β(2→1) linked fructose units with glycosidic bonds and typically has terminal glycose groups. It is economically produced by extraction from chicory plant. This polysaccharide has been considered as dietary fiber in food industry and shows the environmentally resistance to digestion and absorption through upper gastrointestinal system (GIS), but absorbed and enzymatically decomposed to short-chain fatty acid in colon (large intestine), to metabolize in body.
Polycaprolactone (PCL) is a biodegradable synthetic polyester. Due to the slow biodegradation capability, low molecular weight PCL has been widely used to construct the hydrophobic core part of controlled drug delivery formulations.

In this study, considering the aforementioned remarkable properties of inulin and PCL, we synthesized the PCL grafted inulin copolymer (PCL-g-In) and prepared a nano formulation in the presence of hydrophobic anticancer model drug, curcumin. The detailed physicochemical characterization of the PCL-g-In copolymer and the drug carrying nano-particles prepared was performed.
**Key Words**: Inulin, Polycaprolactone, Copolymer, Polysaccharides, Drug delivery

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**POSTER PRESENTATION**

# SYNTHESIS AND CHARACTERIZATION OF HYALURONIC ACID (HA) BASED NANOPARTICLES FOR USE IN DRUG DELIVERY SYSTEM

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**Abstract**

Medical treatments are most of the time unique to the patient and the stage of illness, however, still, each treatment has a side-effect. Therefore, there are researches to develop methods many of which benefit from the drug delivery systems that aim to minimize and even eliminate the side effects. Those methods include but are not limited to encapsulation of the drug within carriers made of biodegradable substances, nanoparticles, micro/nanospheres, polymers, etc.

In the metabolic processes, polysaccharides play an important role as the main energy source of the cells, and cancer cells need more energy than healthy cells. Therefore, polysaccharides are more preferred by tumor cells and they have high cellular adhesion providing more effective receptor-mediated endocytosis at cancer cell membranes resulted overcoming the mucosal barrier. Hyaluronic acid (HA) is one of the most important polysaccharides, which has a linear anionic molecular structure consisting of repeating disaccharide units of β-1,4-D-glucuronic acid and β-1,3-N-acetyl-D-glucosamine[1], which is a naturally occurring polymer throughout the body of all vertebrates. Its molecular weight changes from 4000 to 8x106 Da [2] and is mainly found in skin, the extracellular matrix of cartilage tissues, the vitreous humor of the eyes, and the umbilical cord [3-5]. The superior properties of HA like high water absorption capacity, lubricant ability, non-toxicity, biocompatibility, and biodegradability make it an ideal candidate for various biomedical and cosmetic applications. It carries free carboxylic acid and hydroxyl groups that can be easily modified with various agents for different purposes. These properties made HA a promising tumor-targeting material in the preparation of active drug delivery systems.

Considering the unique metabolic properties of the HA, we prepared HA-based amphiphilic chemotherapeutic drug carriers. For this purpose, we chemically modified HA to give an amphiphilic nature by attaching the hydrophobic tails to the main chain. Therefore, HA had the ability to self-assemble into micelles. We used curcumin as a model drug and a release profile from the HA-based micelles was observed.

**Key Words**: Hyaluronic acid, Nanopolymer, polymeric matrix, drug delivery systems.

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**POSTER PRESENTATION**

# DEVELOPMENT OF A MICROFLUIDIC PLATFORM TO MAINTAIN VIABILITY OF MICRO-DISSECTED TUMOR SLICES IN CULTURE

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## Abstract

One of the issues limiting the development of personalized medicine is the absence of realistic models that reflect the nature and complexity of tumor tissues. We described a new tissue culture approach that combines a microfluidic chip with the microdissected breast cancer tumor. 'Tumor-on-a-chip' devices are suitable for precision medicine since the viability of tissue samples is maintained during the culture period by continuously feeding fresh media and eliminating metabolic wastes from the tissue. However, the mass transport of oxygen, which arguably is the most critical nutrient, is rarely assessed. According to our results, transportation of oxygen provides satisfactory in vivo oxygenation within the system. A high level of dissolved oxygen, around 98-100% for every 24 hours, was measurable in the outlet medium. The microfluidic chip system developed within the scope of this study allows living and testing tumor tissues under laboratory conditions. In this study, tumors were generated in CD-1 mice using MDA-MB-231 and SKBR-3 cell lines. Microdissected tumor tissues were cultured both in the newly developed microfluidic chip system and in conventional 24-well culture plates. Two systems were compared for two different types of tumors. The confocal microscopy analyses, LDH release and glucose consumption values showed that the tissues in the microfluidic system remained more viable with respect to the conventional well plate culturing method, up to 96 hours. The new culturing technique described here may be superior to conventional culturing techniques for developing new treatment strategies, such as testing chemotherapeutics on tumor samples from individual patients.

**Key Words**: Microfluidic system, tumor tissue culture, breast cancer

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**POSTER PRESENTATION**

# DETERMINATION OF ANTIMICROBIAL AND ANTIBIOFILM ACTIVITIES OF NATURAL WHEY-BASED PROBIOTICS

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## Abstract

**Objective / Purpose:** Probiotic microorganisms are used as an alternative to antibiotics by various mechanisms that control pathogen microorganism growth and biofilm formation. Recently, there is a strong interest in obtaining probiotics from fermented dairy products by using practical methods. Whey is the liquid that remains during the production of cheese from milk and contains important nutrients and probiotic microorganisms. It is a complex mixture of many valuable ingredients: protein, lactose, fat, calcium and phosphorus, organic acids and vitamins. Due to its special properties, whey can be an excellent medium for probiotic bacteria. Recent studies have reported the beneficial effects of whey on human microbiota development. **Methods:** In this study, cell-free supernatants (CFS) of probiotic bacteria (*Enterococcus faecium, Lactobacillus fermentum, Enterococcus lactis*) isolated from natural whey were screened for antimicrobial and antibiofilm properties. The antimicrobial and antibiofilm activities of CFSs were determined by the minimum inhibitory concentrations (MICs) and minimum biofilm eradication concentrations (MBEC) assays. **Results:** The results showed a significant antimicrobial effect with 2000 µg/mL CFS concentration on the growth of *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* NRRL B47 and *Listeria monocytogenes* ATCC 19111. The CFS of *Lactobacillus fermentum* exhibited eradication activity on the biofilm of *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 14990, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 90028 with 3750 and 7500 µg/mL concentration. **Conclusion:** The test results demonstrate that whey-based probiotic CFSs were highly efficient against pathogenic microorganisms growth and biofilm structures. These results are highlighting the potential utility of probiotic CFS for the prevention and treatment of infections.

**Key Words**: Antimicrobial agents, dairy products, whey-based probiotics, antimicrobial, antibiofilm.

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**POSTER PRESENTATION**

# SYNTHESIS AND CHARACTERIZATION OF NORFLOXACIN CONJUGATED SINGLE-CHAIN POLYMER NANOPARTICLES

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**Abstract**
With the increasing public health awareness about the effect of bacteria and microorganisms, polymers with antimicrobial activity have aroused great interest. Modern antimicrobial polymers have found their way into a wide variety of practical applications, including food packaging, sanitary and medical devices. Compared with low molecular weight biocides, polymeric agents have advantages such as enhanced antimicrobial activity, efficiency, reduced toxicity and long-term stability [1].
The broader spectrum of fluoroquinolones and reduced bacterial resistance to beta-lactam-deactivating enzymes have made them the most studied class of antibiotics in terms of polymer chemistry. Norfloxacin (NOR), a quinolone carboxylic acid derivative, is an orally absorbed fluoroquinolone antibacterial agent with a fluorine ring at position 6 and a piperazine ring at position 7. However, NOR is a synthetic antibacterial drug used for the treatment of diseases caused by *E. coli, Salmonella* and *V. cholera* [2].
Manipulation of the molecular structure of the polymer backbone enables the formation of small single-chain polymeric nanoparticles (SCNP) with interesting physical properties such as low viscosity, low hydrodynamic volume and controlled affinity. The field of SCNP has seen significant growth in recent years for the synthesis of functional precursor macromolecules, the most prominently reversible deactivation radical polymerization in combination with versatile modular ligation processes [3].
In this study, we aimed to synthesize a hydrophilic SCNP bearing NOR groups. Reversible addition-fragmentation chain transfer (RAFT) polymerization was used for the synthesis of precursor polymer. SCNP was formed via click reaction in a dilute medium ($c$= 1 mg mL$^{-1}$). Precursor polymer and SCNP were characterized using gel permeation chromatography (GPC), fourier transform infrared (FT-IR) spectroscopy, proton nuclear magnetic resonance ($^1$H NMR) spectroscopy and dynamic light scattering (DLS).
**Key Words**: Single-chain polymer nanoparticles, norfloxacin, antimicrobial polymers, polymer-drug conjugates

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**POSTER PRESENTATION**

# EVOLUTION OF CLINICAL PHARMACY ACTIVITIES IN ADULT INTENSIVE CARE UNIT

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## Abstract

**Objective:** Patients hospitalized in Intensive Care Unit (ICU) are patient groups that may have many comorbid diseases (CD) and use more than one drug at the same time. Polypharmacy can cause potential drug-drug interactions (DDI). DDI can be evaluated at many stages, from life-threatening levels to treatment effectiveness. In this study, it was evaluated to determine the level of potential DDI seen in patients hospitalized in the ICU and to determine the degree of significance from the point of view of the clinical pharmacist. **Methods:** The study was carried out observationally in ICU of a hospital between 01 March and 01 May 2022. Patients with at least one CD were included in the study. The drugs taken by the patients during the hospitalization were classified using the Micromedex and Drugs.com databases, and the level of significance was determined. **Results:** In the study, 52 patients were evaluated during their hospitalization. 57.50% of the patients are male. The mean age of the patients was 67, and the mean age of male patients was higher than the mean age of female patients. A total of 427 interactions were detected in both databases. 4 of the interactions in the Micromedex database are contraindicated; 85 of them are Major, 117 of them are Modarate, 28 of them are Minor, 13 of them are no interaction. In the Drugs.com database, 73 Major, 91 Modarate and 14 did not interact. When major interactions were examined in the Micromedex and Durgs.com database, 17% were found to be clinically significant. The drugs with the most common interactions are anti-arrhythmic drugs, anti-thrombotic drugs, and anti-infective drugs respectively. **Conclusion:** The clinical pharmacist's contribution is very important in determining and managing DDI. The presence of a clinical pharmacist in a multi-disciplinary team will greatly contribute to the development of pharmaceutical care practices.

**Keywords:** Adult Intensive Care Unit, Clinical Pharmacy, drug interactions.

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**POSTER PRESENTATION**

# PRELIMINARY PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF VARIOUS EXTRACTS OF *PHYSALIS ANGULATA* ROOTS

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## Abstract

**Objectives:** *Physalis angulata* L belongs to the family Solanaceae and is distributed throughout the tropical and subtropical regions. In the present study, the ethyl acetate and butanol extract of the roots of *Physalis angulata* L were evaluated for its antioxidant activities and the total phenolic and flavonoids contents and cytotoxic activity against human breast cells (MCF-7), human cervical cancer cell lines (HeLa). **Methods:** Extraction of the roots of *Physalis angulata* wre carried out by soxhlet method using ethyl acetate and butanol successively. Total phenolic content of all the extracts of the roots of *Physalis angulata* were determined by Folin-Ciocalteau method using Gallic acid asthe standard.The total flavonoid content of all the extracts of the roots of *Physalis angulata* were determined spectrophotometrically by aluminium chloride method using Quercetin as the standard. The in vitro anti-oxidant screening of all the extracts of the roots of *Physalis angulata* were carried out by different spectrophotometric methods such as DPPH and ABTS assays. The cytotoxic evaluation of *Physalis angulata* were done using HeLa (Cervical carcinoma) and MCF-7 (Human Breast Adeno carcinoma) cell lines. **Results:** Both the extracts of the roots of *Physalis angulata* showed moderate antioxidant activity when compared with the standard ascorbic acid. The butanol aextract showe a cell viability of 76.69 and 73.85 for HeLa and MCF-7 respectively. The ethyl acetate extract showed 87.18 and 85.12 for HeLa and MCF-7 respectively. **Conclusion:** The various extracts of the leaf and fruits of P. angulata showed moderarte antioxidant activity. The cytotoxic activity was evaluated by the MTT assay method against various cell lines such as HeLa (cervical) and MCF-7 (breast). Both the extract showed moderate cytotoxic activity aganist the cell lines this may be due to the low total phenolic and flavonoid content of the roots of *Physalis angulate.*

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**FULL PAPER – ORAL PRESENTATION**

# IN VITRO ANTIRADICAL ACTIVITY OF RUMEX PATIENTIA L.

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## Abstract

*Rumex patientia* L. belongs to Polygonaceae family. The leaves of this plant are used as green vegetable and commonly called ‘‘labada’’ in Turkey. The antiradical activities of *Rumex patientia* L. extracts were examined in this study by different *in vitro* assay including DPPH free radical, $H_2O_2$ (non free radical) and superoxide anion radical scavenging effects. The results clearly indicated that *Rumex patientia* L. extracts had an effective radical scavenging activity and consumption of this plant is benefical for human health due to their activities and it can be used to prevent the damage caused by free radical.

**Key Words**: *Rumex patientia* L.; antiradical; DPPH; antioxidant; $H_2O_2$, superoxide anion radical.

**Abbreviations**
WEDL; Water extract of dried leaves
MEDL; Methanol extract of dried leaves
WEFL; Water extract of fresh leaves
MEFL; Methanol extract of fresh leaves

## 1. Introduction

*Rumex patientia* L., a member of Polygonaceae family (Kumar & Singh, 2020). The leaves of this plant are consumed as green vegetable and commonly called ‘‘labada’’ in Turkey. According to the data in literature, it contains a lot of bioactive compounds which have various pharmaceutical effects such as diuretic, anti-inflammatory, antipyretic and antioxidant activities (Vasas, Orbán-Gyapai, & Hohmann, 2015). Because of these effects, this plant is used in traditional medicine (Uzun & Demirezer, 2019). The leaves of *Rumex patientia* L. are shown in Figure 1.

**Figure 1.** *Rumex patientia* L.

Free radicals are chemically reactive species because they contain one or more uncoupled electron in their outermost orbital. These radicals can be caused by oxygen or nitrogen (Bursal, Koksal, Bilsel, Gulcin, & Goren, 2013). Oxygen content such as superoxide anion radical and hydroxyl radical are called reactive oxygen species. Similarly, nitrogen contents such as nitric oxide radical is called reactive nitrogen species. There are other species like $H_2O_2$ and singlet oxygen that are not considered radically. They known as non free radicals (Kılıc, Yesiloglu, Bayrak, Gülen, & Bakkal, 2013). In the organism, cells are damaged as a result of the increase of these substances. This is an important reason for the emergence of many different diseases including hypertension, cancer, diabetes, depression and immune system decline (Chang, Cheng, Chiang, & Chen, 2018) (Cecerska-Heryc, et al., 2021). Therefore, we aimed to determine the antiradical activities of *Rumex patientia* L. extracts.

## 2. Material and Methods

### 2.1. Chemicals and sample preparation

In this study, DPPH (1,1-diphenyl-2-picrly-hydrazyl), butylated hydroxyanisole (BHA), nitroblue tetrazolium (NBT), α-tocopherol, hydrogen peroxide ($H_2O_2$), phenazine methosulphate (PMS), ascorbic acid and butylated hydroxytoluene (BHT) were used analytical grade.

The samples of *Rumex patientia* L. leaves were collected in a village of Musellim (Kırklareli, Turkey). The dust on the leaves was cleaned with distilled water. Afterwards, cleaned leaves were dried at 25 ºC. Fresh leaves were stored at -18 ºC to be used in analysis. Fresh and dried *Rumex patientia* L. leaves were extracted with boiling water and methanol. These extracts were stored in the freezer at -18 ºC and were dissolved in solvent or distilled water before analysis. Concentration range of extracts and standards were selected as 50-250 µg/mL.

### 2.2. DPPH radical (1,1-diphenyl-2-picryl-hydrazyl) scavenging activity

DPPH radical scavenging effects of *Rumex patientia* L. extracts were measured following the procedure of Azhari et al. (Azhari, Xu, Jiang, & Xia, 2014) with a minor modification. Ethanolic solution of DPPH (3.5 mL, 0.1 mM) was added to *Rumex patientia* L. extracts (1 mL). These mixtures were vortexed and then incubated in darkness at 25 ºC for 30 minutes. Absorbance values of these mixtures were recorded at 517 nm against ethanol. DPPH free radical scavenging effects of samples were computed by this equation:

$$\text{Scavenging effect of samples (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

$A_{control}$ and $A_{sample}$ were the absorbances of the control solution and sample solution

### 2.3. Superoxide anion radical scavenging ability

Superoxide anion radical scavenging abilities of *Rumex patientia* L. extracts were measured using previous report of Chun et al (Chun, Kim, & Lee, 2003). All the solutions were prepared in phosphate buffer (0.1 M, pH 7.4). *Rumex patientia* L. extracts (1 mL) were mixed with NADH solution (1 mL of 468 µM solution) and NBT solution (1 mL of 156 µM solution). These mixtures were vortexed for 1 minute and then PMS solution (10 mL of 60 µM solution) was transferred to the reaction mixture. The mixed solutions were incubated at 25 ºC for 5 minutes and then their absorbances were measured at 560 nm at spectrophotometer.

### 2.4. $H_2O_2$ scavenging capacity

$H_2O_2$ scavenging capacities of *Rumex patientia* L. extracts were measured using previous report of Amir et al (Amir, Khan, Mujeeb, Ahmad, & Siddique, 2011). $H_2O_2$ solution (0.04 M) was prepared in phosphate buffer solution (0.1 M, pH 7.4). 1 mL of *Rumex patientia* L. extracts were mixed with 600 µL of $H_2O_2$ solution. 10 minutes later, absorbances of samples were recorded at 230 nm.

## 3. Results and Discussion

### 3.1. DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activity

DPPH radical scavenging effects of BHA, α-tocopherol, BHT, ascorbic acid, WEFL, WEDL, MEFL and MEDL at 250 µg/mL were found to be 42.40 ± 0.39, 39.70 ± 0.43, 34.70 ± 0.27, 32.60 ± 0.27, 28.90 ± 0.67, 26.20 ± 0.93, 23.70 ± 0.42 and 26.70 ± 0.12, respectively (Figure 2). DPPH radical scavenging effects of samples followed the order: BHA > α-tocopherol > BHT > ascorbic acid > WEFL > MEDL > WEDL > MEFL. Nevertheless, when compared to other four standards, the DPPH scavenging effects of the *Rumex patientia* L. extracts were found to be lower.

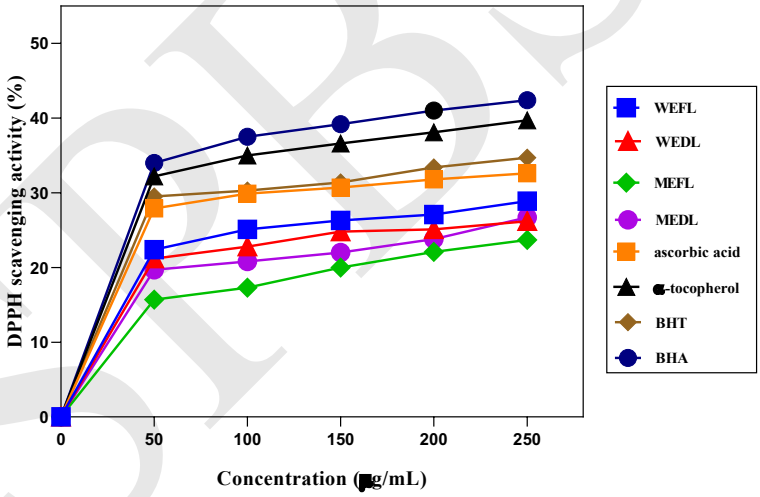


**Figure 2.** DPPH radical scavenging activities of *Rumex patientia* extracts. BHA, BHT, *α*-tocopherol and ascorbic acid were used as reference antioxidants.

### 3.2. Superoxide radical scavenging ability

As can be seen from Figure 3, superoxide anion radical scavenging activities of WEFL, WEDL, MEFL, MEDL, BHT, ascorbic acid and BHA were 32.00 ± 0.34, 29.20 ± 0.28, 28.60 ± 0.33, 27.00 ± 0.20, 22.10 ± 0.04, 33.60 ± 0.22 and 26.20 ± 0.04 at 250 µg/mL, respectively with ascorbic acid ≈ WEFL > WEDL > MEFL > MEDL > BHA > BHT. These results revealed that *Rumex patientia* L. extracts had superoxide anion radical scavenging effects.

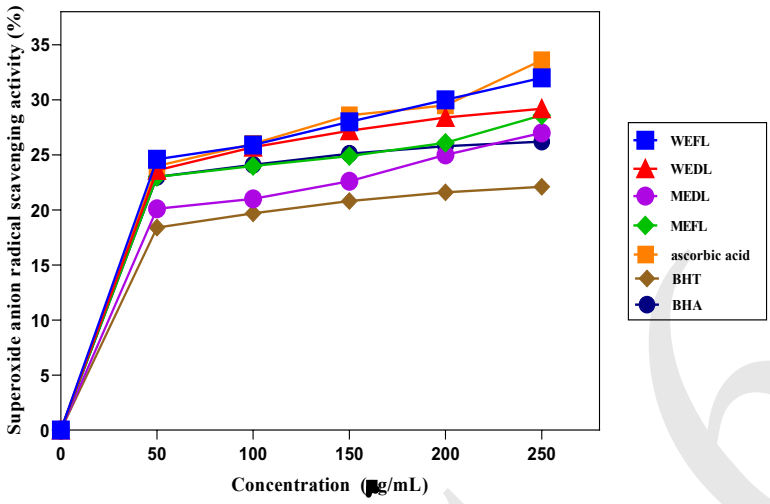


**Figure 3.** Superoxide radical scavenging activities of *Rumex patientia* extracts. Ascorbic acid, BHA and BHT were used as reference antioxidants.

### 3.3. $H_2O_2$ scavenging capacity

Because $H_2O_2$ can lead to hidroxyl radical formation, it can has toxic effects on cells (Amir, Khan, Mujeeb, Ahmad, & Siddique, 2011). Figure 4 presents the order of the $H_2O_2$ scavenging activities of samples: BHT (83.96 ± 0.03) > BHA (79.11 ± 0.17) > ascorbic acid (71.55 ± 0.01) > α-tocopherol (67.70 ± 0.56) > WEFL (60.00 ± 0.43) > WEDL (55.80 ± 0.04) > MEDL (50.80 ± 0.04) > MEFL (47.60 ± 0.84) at 50 µg/mL. The data confirm that the $H_2O_2$ scavenging activities of *Rumex patientia* L. extracts were lower than standards.

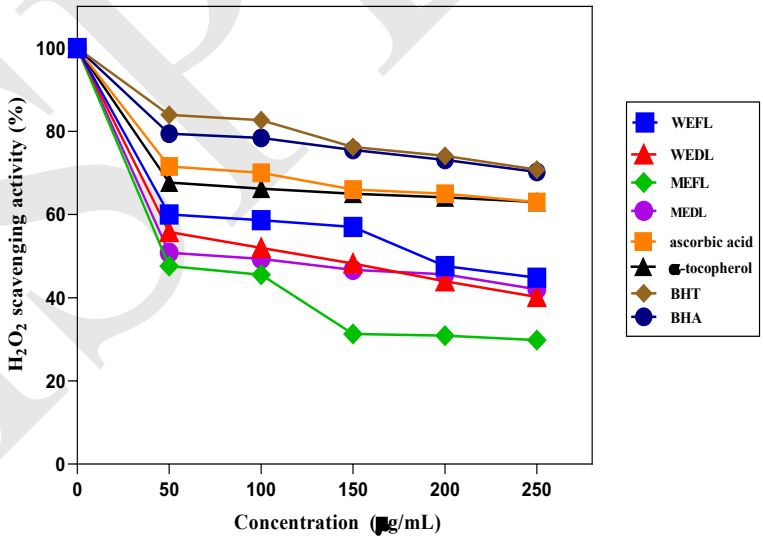


**Figure 4.** $H_2O_2$ scavenging capabilities of *Rumex patientia* extracts. BHA, ascorbic acid, BHT and $\alpha$-tocopherol were used as reference antioxidants.

## 4. Conclusion

The results of present study confirm that the *Rumex patientia* L. extracts have shown antiradical activities in different assays including $H_2O_2$, DPPH and superoxide anion radical scavenging activities when it is compared to synthetic antioxidants such as ascorbic acid, BHA, a-tocopherol and BHT. In conclusion, consumption of this plant is benefical for human health due to the activities mentioned above and it can be used to prevent the damage caused by free radical.

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**FULL PAPER – ORAL PRESENTATION**

# DESIGN, SYNTHESIS AND α-GLUCOSIDASE INHIBITORY ACTIVITY OF SOME QUINAZOLIN-4(3*H*)-ONE & 4-AMINO BENZENESULFONAMIDE HYBRID COMPOUNDS

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## Abstract

**Background and Objective:** Diabetes is a chronic metabolic disease that has a high prevalence rate and can cause fatal complications. Therefore, it's necessary to treat diabetes effectively. Diabetes treatment protocol aims to reduce high blood glucose levels in patients and α-glucosidase inhibitors play an important role in managing the disease. The efficacities of the drugs currently used as α-glucosidase inhibitors are limited and high-cost synthesis procedures are needed for producing them. So, there is an urgent need for new α-glucosidase inhibitor drugs which are more efficient and can be obtained with low-cost synthesis procedures. For this purpose, some novel quinazolin-4(3*H*)-one & 4-aminobenzenesulfonamide hybrid compounds were synthesized and evaluated for their α-glucosidase inhibitory activities in this study. **Methods:** The title compounds were synthesized by coupling of 2-chloroquinazolin-4(3*H*)-one and appropriate 4-amino-*N*-(substitutedphenyl) benzenesulfonamide intermediates, each obtained with three-steps reactions. Their structures were confirmed by spectral analysis and α-glucosidase inhibition assays were performed by spectrophotometrical method using a microplate reader. Results were expressed % inhibition of α-glucosidase inhibitory activity at 100 µM concentration of tested compounds and the reference drug acarbose **Results:** According to the biological activity results, all the synthesized compounds (1-4) showed α-glucosidase inhibition equal to or higher than the reference drug acarbose at 100 µM concentration. **Conclusions:** Preliminary activity screening results indicated that quinazolin-4(3*H*)-one & 4-aminobenzenesulfonamide hybrid molecules could be promising compounds for further studies in the development of new α-glucosidase inhibitors.

**Key Words**: Synthesis, Quinazolin-4(3*H*)-one, 4-Aminobenzenesulfonamide, α-Glucosidase Inhibitors

## 1. Introduction

Diabetes is one of the fastest-growing health emergencies of the 21st century. As of 2021, there are 537 million diabetics in the world (International Diabetes Federation, 2021). Diabetes is important not only because of its high prevalence but also because of its complications such as cardiovascular disease, nephropathy, retinopathy and neuropathy that reduce the individual quality of life and can be fatal (Luthra *et al*., 2018). In 2021, 6.7 million deaths were reported due to diabetes and diabetes-related

complications (International Diabetes Federation, 2021). The high-rate prevalence and fatal complications of the disease necessitate treating diabetes effectively. The main goal of the diabetes treatment procedure is to reduce high blood glucose levels thereby preventing its chronic complications (Ayan *et al*., 2021). One of the pharmacological alternatives for reducing blood glucose levels is α-glucosidase inhibitors.

α-Glucosidase enzyme, located on the brush-bordered surface of the small intestine has a critical role in the breakdown of carbohydrates into glucose. When α-glucosidase enzyme is inhibited, the breakdown of carbohydrates into glucose slows down and absorption of glucose is delayed (Hameed *et al*., 2019). In this way, hyperglycemia can be controlled. Currently, only three α-glucosidase inhibitor drugs named acarbose, voglibose and miglitol are used for the treatment of diabetes (Kazmi *et al*., 2018). However, the efficacities of these sugar-mimic compounds are low and high-cost multi-step synthesis procedures are required to obtain them (Gurram *et al*., 2015). Moreover, some of these drugs have been reported to show serious adverse effects such as hepatotoxicity and increased incidence of renal tumors (Hollander, 1992; Nakamura *et al*., 2012). Based on these reasons, there is an urgent need for safer, more efficient and can be synthesized more readily new α-glucosidase inhibitor drugs (Uysal *et al*., 2018; Wang *et al*., 2016). So far, many compounds with various skeletal structures including quinazolin-4(3*H*)-one and benzenesulfonamide derivatives have been reported for their α-glucosidase inhibitory activities (Dhameja & Gupta, 2019; Javaid *et al*., 2015; Seo *et al*., 2005; Wei *et al*., 2017).

Considering this, in this study, we designed and synthesized four novel quinazolin-4(3*H*)-one & 4-aminobenzenesulfonamide hybrid compounds and evaluated their α-glucosidase inhibitory activities. The synthesized compounds (**1-4**) are shown in Figure 1.

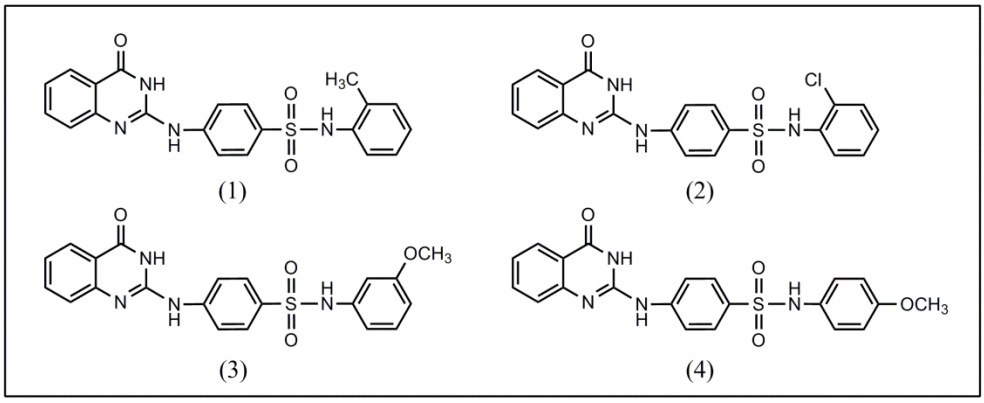


**Figure 1.** Chemical structures of the title compounds (**1-4**)

## 2. Material and Methods

All chemicals, solvents and reagents were high-grade commercial products. So they were used without further purification. α-Glucosidase enzyme (derived from *Saccharomyces cerevisiae*) was purchased from Sigma Aldrich. p-Nitrophenyl-α-D-glucopyranoside (pNPG) (from TCI) was used as the substrate and acarbose (from Acros Organics) was used as the reference drug.

### a. Chemistry

The title compounds (**1-4**) were synthesized with a reaction of 2-chloroquinazolin-4(3*H*)-one and appropriate 4-amino-*N*-(substitutedphenyl) benzenesulfonamide intermediates. Each of 2-chloroquinazolin-4(3*H*)-one and 4-amino-*N*-(substitutedphenyl) benzenesulfonamide intermediates were obtained by reactions with three steps. The structure of the final compounds (**1-4**) was confirmed by spectral analysis (IR, MS, $^{1}$H-NMR, $^{13}$C-NMR).

***Synthesis of 2-chloroquinazolin-4(3H)-one***

Anthranilic acid (1 equiv) and urea (10 equiv) were heated at 160 ˚C for 5 h to obtain quinazolin-2,4(1*H*,3*H*)-dione (Bozdag *et al*., 2017). Then, this compound (2 mmol) and phosphorus oxychloride (16 mmol) were refluxed in the presence of *N*,*N*-dimethylaniline (0.15 ml) for 5 h to yield 2,4-dichloroquinazoline (Samrin *et al*., 2012). Finally, 2,4-dichloroquinazoline was stirred with 1M NaOH aqueous solution at room temperature for 3 h and 2-chloroquinazolin-4(3*H*)-one was obtained (Ayan *et al*., 2021; DeRuiter *et al*., 1986).

***Synthesis of 4-amino-N-(substitutedphenyl)benzenesulfonamide intermediates***

Initially, acetanilide (14.8 mmol) and chlorosulfonic acid (78.27 mmol) were refluxed at 60 ˚C for 30 min to afford 4-acetamidobenzenesulfonyl chloride (Barbosa *et al*., 2014). Then, this compound (22 mmol) was coupled with appropriate anilines (20 mmol) at room temperature to yield 4-acetamido-*N*-(substitutedphenyl)benzenesulfonamide derivatives (Masevicius *et al*., 2012). Finally, 4-amino-*N*-(substitutedphenyl)benzenesulfonamide intermediates were obtained by deacetylation reaction of 4-acetamido-*N*-(substitutedphenyl)benzenesulfonamide derivatives by heating with 5M NaOH aq. solution (20 ml)-methanol (12 ml) mixture at 70 ˚C (Yu *et al*., 2012).

***Synthesis of the final compounds (1-4)***

2-Chloroquinazolin-4(3*H*)-one (1 mmol) and appropriate 4-amino-*N*-(substitutedphenyl) benzenesulfonamide intermediates (1 mmol) were refluxed at 105-110 ˚C until the TLC showed that one of the reactants was over. The precipitate was filtered and recrystallized from acetonitrile-water mixture (1:1) (Abouzid & Shouman, 2008).

**b. Biological Activity**

α-Glucosidase inhibitory activity of the title compounds was carried out spectrophotometrically by using a microplate reader. Firstly, the test compounds were dissolved in DMSO and diluted in half with the equal amount of water. Then, 30 µL of the test compounds and 70 µL of the enzyme (71.4 mU/ml) solution in phosphate buffer (pH: 6.8) were mixed in a 96-well plate and incubated at 37 ˚C for 5 min. After the incubation, 50 µL of the substrate solution in the buffer (2.5 mM) was added and the absorbance was measured spectrophotometrically at 405 nm for 10 min at 30 seconds intervals. All measurements were triplicated. Absorbance values were plotted versus time, and the slope of the lines was calculated ($r^2$>0.95). DMSO (10% of total volume) was used as a standard and acarbose was used as a reference. % Inhibition was calculated as follows:

% Inhibition = [(slope of the standard-slope of the tested compound) / slope of the standard] x 100

Results were expressed % inhibition of α-glucosidase inhibitory activity at 100 µM concentration of tested compounds and acarbose (Ayan *et al*., 2021; Ranilla *et al*., 2010).

## 3. Results and Discussion

In this study, we combined the quinazolin-4(3*H*)-one and 4-aminobenzenesulfonamide structures via molecular hybridization method and synthesized four hybrid compounds (**1-4**). The compounds were synthesized by the method reported in Material and Methods section. The spectral findings were in accordance with the declared structures. All of the title compounds are novel and their synthesis procedures and biological activities have been reported for the first time in this study.

According to the biological activity results, all the synthesized compounds, except compound **1** showed higher α-glucosidase inhibition rate than the reference drug acarbose at 100 µM concentration. Compound **1** inhibited the enzyme almost equally to acarbose at the same concentration. Among the tested compounds, compound **4** bearing 4-methoxyphenyl substituent on the sulfonamide nitrogen was found to show the highest inhibitory % activity. Biological activity results were summarized as % inhibition values in Table 1.

**Table 1.** % Inhibition values of the title compounds (**1-4**)

| Compound | % Inhibition (100 µM) ± SEM[a] |
|---|---|
| **1** | **15.7 ± 0.8** |
| **2** | **17.5 ± 0.8** |
| **3** | **19.4 ± 0.6** |
| **4** | **21.7 ± 1.5** |
| **Acarbose** | **15.5 ± 1.9** |

[a]The data means ± standard error of the main of triplicate independent experiments.

## 4. Conclusion

Herein, some quinazolin-4(3*H*)-one & 4-aminobenzenesulfonamide hybrid compounds were synthesized and evaluated for their α-glucosidase inhibitory activities. Preliminary activity screening results indicated that this class of hybrid molecules could be promising compounds for further studies in the development of α-glucosidase inhibitors.

## Conflict of Interest

The authors declare no conflict of interest, financial or otherwise

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**FULL PAPER – ORAL PRESENTATION**

# INVESTIGATION OF DIFFERENT SYNTHESIS PARAMETERS OF HYDROXYAPATITE FOR TISSUE ENGINEERING APPLICATIONS

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## Abstract

Hydroxyapatite undoubtedly has vital roles in tissue engineering applications. The fabrication methods and different treatments lead distinct properties in hydroxyapatite crystals, including particle, size, shape, and surface features. In this study, we applied sol-gel synthesis route for hydroxyapatite production which offers relatively cost available and high yield of product. The influence of initial pH parameter and various temperature treatments on properties of hydroxyapatite were investigated. The leading hydroxyapatite powders have been compared in terms of their morphological and chemical structures by XRD and SEM analyses. The incipient pH in which the precursor solutions introduced to one another had critical role in this synthesis reaction. This has determined major properties, such as the chemical composition, phase purity, product yield, and morphology. The reactions of precursor solutions with higher incipient pH contributed to high yield (86%) of pure HA possessing high thermal stability. On the other hand, in lower incipient pH (8) counterpart, β-TCP phase was detected upon treatment at 950 °C. We had used the acquired pure HA in dried form in chitosan based injectable hydrogel compositions with pro-angiogenic features designed for bone tissue regeneration and drug delivery applications.
**Key Words**: hydroxyapatite, sol-gel synthesis, incipient pH, microstructure, bone tissue engineering

## 1. Introduction

Hydroxyapatite (HA) with some ionic substitutions comprise the main inorganic phase of natural bones and teeth. The unique bioactive properties of HA provide anchorage with native tissues and stimulate their regeneration. Being contributing to osteoconductivity and osteoinductivity, HA triggers attachment and proliferation of osteoblasts and construction of new bones (Arun Kumar et al., 2015; Kattimani et al., 2016). Therefore, HA is a significant biomaterial at bone tissue engineering applications. HA is utilised in wide range of biomedical applications including bone fillers or substitutes and coatings. Also, HA can be used as scaffold or injectable composites to sitimulate osteogenesis (Arun Kumar et al., 2015) and angiogenesis (Kocak et al., 2020; Unger et al., 2007). In addition, HA is used in drug delivery systems

involving treatments of bone diseases, such as osteoporosis and tumours. In addition, other biological molecules e.g. proteins, vitamins, hormones and genes can be also delivered by HA based biomaterials (Munir et al., 2021).

There are various approaches to produce HA for biomedical applications. HA can be obtained from bones of biological species by deprotenation and calcination (Boutinguiza et al., 2012; Khoo et al., 2015), or it can be produced synthetically. The solid state and wet synthesis methods are present for HA production. The main drawback of solid-state synthesis is shortage of chemical interactions during the reaction. In addition, it is reported that HA produced by solid-state techniques do not support generation of apatite layer upon contact to biological fluids (Sadat-Shojai et al., 2013). In contrary, wet synthesis routes (e.g., chemical precipitation, hydrothermal and sol-gel) ensure molecular level mixing of reagents. Among these methods, sol-gel technique offers production of homogeneous, pure nano-sized products in low reaction temperature and pressure, and control of particle size and shape. Furthermore, as reported, HA synthesised with sol-gel method ensure better bioresorption resebling biological apatite (Fathi et al., 2008). In this study, hydroxyapatite powders were produced by sol-gel synthesis utilizing ethanol and water as solvent. To our knowledge, there is not much report demonstrating the critical effect of incipient pH in which precursor solutions has met. Therefore, we investigated the effect of two different incipient pH values while keeping the pH stable in the rest of the reaction. Different heat treatments applied on these HA products. The results showed that incipient pH was a crucial factor in this sol-gel synthesis directly determining the final significant properties of HA particles, such as thermal stability, phase purity, reaction yield, particle size, shape, and crystallinity.

## 2. Material and Methods

### a. Materials

The reagents used for HA synthesis include calcium nitrate tetra-hydrate (Acros Organics, Belgium); di-ammonium hydrogen phosphate (VWR-Prolabo Chemicals, Germany); ammonium hydroxide of 35% (Thermo Fisher Scientific, UK); and ethanol (≥99.8%, AnalaR NORMAPUR®, VWR-Prolabo Chemicals, France). In all experiments, de-ionised ultrapure (Type-I) water (Veolia Water Technologies, PURELAB® Chorus, 18.2 MΩ.cm, Wycombe, UK) was utilised.

### b. Synthesis of Hydroxyapatite by Sol-Gel Method

Synthesis of HA powdes by a sol-gel technique was applied by modification from the literature (Kuriakose et al., 2004). Reaction was carried out in three-necked baloon placed in a heat bath kept at 85 °C. For a stochiometric reaction, equal volumes of 0.5 M of calcium nitrate tetrahydrate and 0.3 M of di-ammonium hydrogen phosphate were dissolved in ethanol and ultrapure water, respectively. In one group, pH of both precursor solutions was 8 initially and kept it the same once they met. In the second group, pH of the solutions was increased to 10.5 by adding ammonia. Then, phosphate precursor solution was dropwise added into calcium precursor solution in reactor by maintaining a constant stirrring. After addition was completed, the pH was adjusted to 10 and this was repeated every hour during the reaction. After complete reaction in 4 h, the white hydroxyapatite solution was filtrated and purified by a serial washing.

HA powders were obtained upon drying at 80 °C. Some powders were obtained after freeze drying (-20 °C). HA specimens were also sintered at different temperatures (950 °C, 1100 °C and 1300 °C).

**c. X-ray diffraction (XRD) Analyses**

HA particles were analysed by X-ray diffractometer (Bruker D2 Phaser, Dublin, Ireland UK with a LNEXEYE detector). An angle range of 2θ=5-80° with 0.02° step size were applied to get diffraction patterns in DIFFRAC.SUITE™ Software. ICDD® and ICSD Fiz Karlsruhe GmbH databases were used to obtain standards and graphical data was drawn in GraphPad Prism (Version 7.0, San Diego, CA, USA) software.

**d. Scanning Electron Microscopy (SEM)**

Morphological analyses of HA powders after conductive coating were perfomed by using a FE-SEM instrument (FEI Inspect$^{TM}$ F50, Hillsboro, Oregon, USA).

## 3. Results and Discussion

The effects of different incipient pH values (8 vs. 10.5) caused major alterations at thermal stability which is leading the formation of additional phases to HA at different temperatures. The higher incipient pH provided a high yield of HA product which amounts to 86% whereas the lower counterpart has resulted in a 78 % of yield. The effects of freeze drying were also investigated and compared with oven drying process in terms of crystal features of HA particles.

**a. Chemical Analyses by XRD**

The chemical analyses of HA samples were conducted by XRD analyses. XRD patterns of HA particles obtained at two different incipient pH of 8 vs. 10 were matched with standard HA patterns (ICDD® data base, PDF card no: 01-073-84-19). The identical peaks to HA were detected at 2θ angles with corresponding planes as: 26°(002); 32-34° (211), (112), (300), (202); 40° (310); and 46-55° (222), (213), and (411) (Chaudhry et al., 2006; Choi et al., 2006).

Figure 3.1 shows XRD spectra of HA samples after sintering at 950 °C obtained at two different incipient pH of 8 vs. 10.5 which are symbolised as HA-1 and HA-2, respectively. Results showed that HA-1, lower pH product sintered at 950 °C showed less thermal stability due to detection of additional phase of β-tricalcium phosphate (TCP). The β-TCP phases were identified at 2θ angles of 13.60°, 16.94°, 20.19°, 27.75°, and the major peak of 30.99° (ICSD Fiz, code#97500).

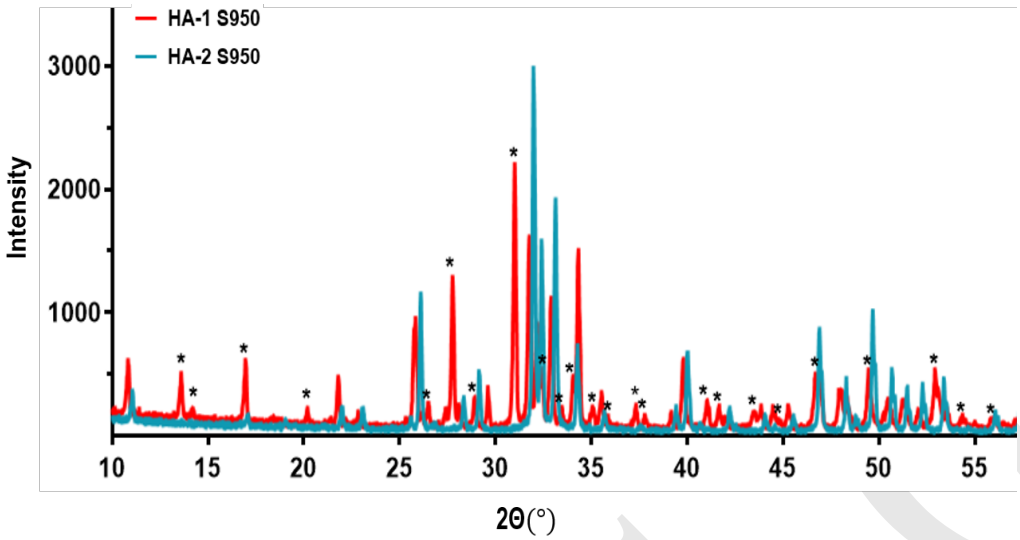


**Figure 3.1.** XRD patterns of HA samples after sintering at 950 °C for 6 hours, which were prepared with different incipient pH: HA-1: (8) and HA-2: (10.5) (Stars indicate the β-TCP phases)

In contrary, high incipient pH specimen (HA-2) sintered at different temperatures (950 °C and 1100 °C) maintained its purity without other phase detection in XRD. (Figure 3.2). Despite the phase purity, the peak intensities in patterns of HA particles have significantly decreased at samples sintered at 1100 °C and 1300 °C, which indicates decline in particle crystallinity. Raman analyses of these samples showed formation of an additional shoulder peak in the spectra of HA samples treated at 1300 °C (this data has not icluded in this report).

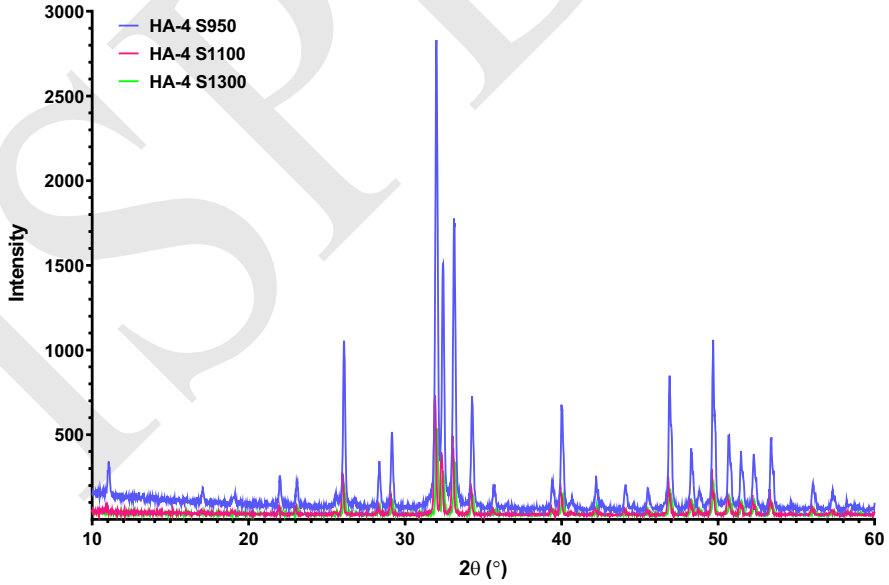


**Figure 3.2.** XRD patterns of a HA samples (HA-4) prepared with an incipient pH of 10.5 sintered at different temperatures: 950 °C, 1100 °C and 1300 °C (for 6 hours) which are denoted as HA-4 S950, HA-4 S1100, and HA-4 S1300.

b. **Microstructure Analyses by SEM**

The morphological structure of HA powders wAS characterised by SEM technique. Figure 3.3. demonstrates the comparative effect of oven-drying and freeze-drying processes on the microstructural features of HA particles. The particles acquired by freeze-drying process possess led to finer crystalline size with less agglomeration. Though freeze-drying seems to give more uniform particles, particle shape of both powders resembles to each other leading spherical particles generated from needle-like crystals. This could be due to the shrinking of particles by the effect of high temperatures during oven drying. As reported, freeze-drying reduces this contraction effect minimizing agglomeration (Wang et al., 2010). However, higher cost of freeze-drying might somehow limit its usage.

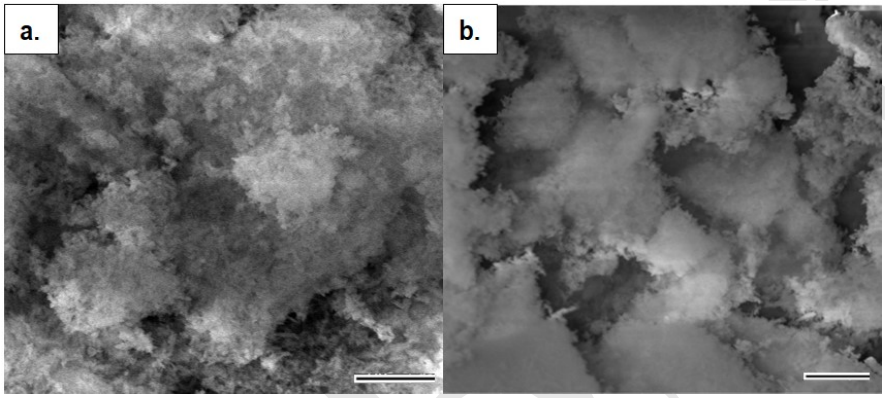


**Figure 3.3.** SEM morphological analyses of HA powders prepared with an incipient pH of 10.5 and obtained by **a.** freeze drying (-20 °C**)** and **b.** oven drying (80 °C**)**

Figure 3.4 compares the impact of pH factor on morphological features of HA particles upon treating at 950 °C. The lower incipient pH specimen which constitutes β-TCP phase showed rod-like porous crystal morphology. In contrast, the round, nano sized, porous and interconnected microstructure was observed in higher pH specimen.

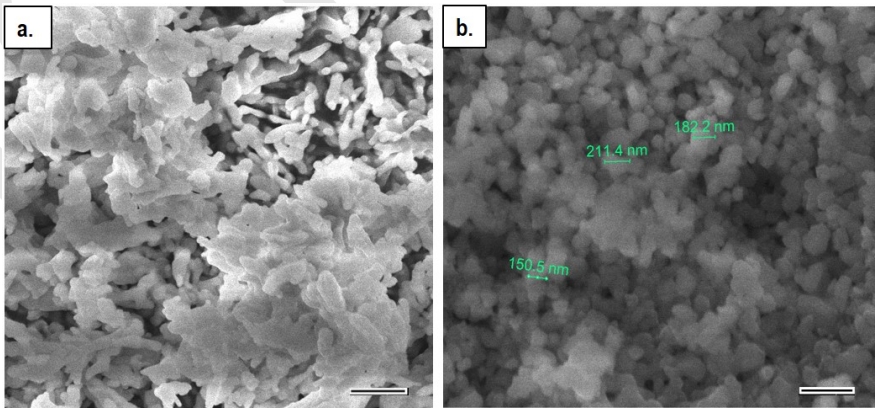


**Figure 3.4.** SEM morphological analyses of HA samples after sintering at 950 °C for 6 hours, which were prepared with an incipient pH of **a.** 8 and **b.** 10.5

## 4. Conclusion

This study revealed that incipient pH is a crucial factor determining essential characteristics of HA particles synthesised by sol-gel route involving a hydroalcoholic media (water/ethanol). This has direct impacts on the reaction yield, phase purity, crystallinity, crystal size and shape. The low initial pH (8) solutions have given rise to acquisition of bi-phasic HA/β-TCP formation observed at 950 °C-sintered samples with low HA yield (78%). Biphasic HA can be also used to adjust bioresorption rate *in-vivo* due to desired reduced stability of HA. However, more thermally stable (at 1100 °C) pure HA powders with high product yield (86%) were obtained owing to higher initial pH (10.5). Both types of HA product might be facial for use in different biomedical applications including scaffold or injectable bone replacement and regenerative compositions which can also be compatible as drug delivery systems for different targets.

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**FULL PAPER – ORAL PRESENTATION**

# A MATHEMATICAL QSAR MODEL TO PREDICT THE SAFE USE OF ANTIHISTAMINES DURING PREGNANCY

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## Abstract

Antihistamines are a pharmacological group frequently prescribed during pregnancy, as allergic reactions are common during pregnancy. In order to use a drug during pregnancy, it must be included in the US Food and Drug Administration (FDA) pregnancy category, in groups A and B. On the other hand, C, D, and X group drugs should not be used during pregnancy due to the risk of developmental toxicity. We constructed a mathematical model to predict the safe use of antihistamines during pregnancy. Since current antihistamines are only in groups B and C as FDA pregnancy categories, our model made predictions over these two groups. If the drug is in group B or C, it gives us information about whether the drug can be used or not. In our model, we included all antihistamines with a determined pregnancy category on the market. The polynomial interpolation developmental model is constructed based on the two descriptor values of the antihistamines data, AlogP and MW. With this new model, we achieved a very high estimation success of 85%. Our work is highly innovative among predictive toxicology studies, as we focused on a specific drug group, such as antihistamines. Our study supports non-animal-based studies and contributes to the literature for new drug development studies using such methods.
**Key Words**: mathematical toxicology; polynomial interpolation; QSAR; developmental toxicology; antihistamine; FDA pregnancy category

## 1. Introduction

Allergic reactions may occur in approximately 20-30% of women in pregnancy (1). Therefore, antihistamines are a pharmacological group commonly prescribed during pregnancy (2). The most important issue to be considered in the safe use of drugs during pregnancy is the risk of developmental toxicity, especially teratogenicity. A pregnancy category was created by the US Food and Drug Administration (FDA) to avoid the risk of developmental toxicity. In the FDA pregnancy category consisting of five groups (A, B, C, D, X) with different risk levels, the use of drugs in groups A and B during pregnancy is considered safe, while the use of drugs in groups C, D, and X is risky (3). While some of the currently used antihistamines are in group C and some are in group B, the developmental toxicity tests of some of them have not been completed (4). Moreover, novel molecules with an antihistamine effect, which have the potential to be converted into medicine in the future, continue to be synthesized (5). Because of ethical problems of in vivo systems, non-animal-based tests,

mathematical models, and predicting strategies should be primarily chosen for developmental toxicology tests of antihistamines whose pregnancy category has not yet been specified.

The aim of this study is to develop a mathematical model to predict the FDA pregnancy category (B or C) of antihistamines by using two types of molecular descriptors (LogP and Molecular weight). These groups were chosen because all currently used antihistamines are in groups B or C. Our study contributes to predictive toxicology studies. The main idea of the model is to derive a polynomial to identify the pregnancy categories (B or C) of antihistamines by using polynomial interpolation. The mathematical model we have created will provide information on whether molecules whose developmental toxicology studies have not been completed can be used in pregnancy.

## 2. Material and Methods

### a. Materials

FDA-approved 36 antihistamine drugs determined as reproductive toxicity risks B and C were collected using the FDA's official database (https://www.fda.gov/) (6). In order to obtain the chemical structure and physical properties of all these compounds, we downloaded the two-dimensional structure data file (SDF) in 2D form, from the chemistry database PubChem (https://pubchem.ncbi.nlm.nih.gov/) (7). We used the 2D SDF files to calculate the molecular descriptors of these compounds by using the open-source software PADEL (8). We have 28 molecules left, after the inorganic compounds, salts, aromaticity, and finally duplicated compounds were removed. In this work, we only used Ghose-Crippen LogKow (AlogP) and the molecular weight (MW) of each compound that we used to design our polynomial model.

### b. Methods

In this work, we construct a polynomial to identify the categories (B and C) of antihistamines drugs by using polynomial interpolation. The mathematical background of how we derived the polynomial interpolation is as follows.

If $(x_0, y_0)$, $(x_1, y_1)$,…, $(x_n, y_n)$ are n+1 distinct values and $f(x, y)$ is a function whose values are given at these values, then we can find a unique multivariate interpolation polynomial $P(x, y)$ such that

$$f(x_i, y_i) = P(x_i, y_i), \quad (1)$$

where, for each i=0,1,…,n.

The polynomial of two variables of the total degree of n is given by

$$P(x, y) = \sum_{i=0}^{n} \sum_{j=0}^{k} a_{j,i}\, x^j y^{i-j} \quad (2)$$

where, for each i=0,1,…,n and j=0,1,…,k (9).

In this work, we used 15 distinct $(x, y)$ values to find a multivariate interpolation polynomial $P(x, y)$. Suppose $(x_i, y_i)$ be the interpolation points then we can write the following system of equations for i=0,1,…,15 (10).

$$\begin{aligned} f(x_1, y_1) &= a_{0,1} + a_{1,1}x_1 + a_{1,2}y_1 + \cdots + a_{4,4}x_1{y_1}^3 + a_{4,5}{y_1}^4 \\ f(x_2, y_2) &= a_{0,1} + a_{1,1}x_2 + a_{1,2}y_2 + \cdots + a_{4,4}x_2{y_2}^3 + a_{4,5}{y_2}^4 \\ &\vdots \\ f(x_{15}, y_{15}) &= a_{0,1} + a_{1,1}x_{15} + a_{1,2}y_{15} + \cdots + a_{4,4}x_{15}{y_{15}}^3 + a_{4,5}{y_{15}}^4, \end{aligned} \tag{3}$$

where, $a_{0,1}, a_{1,1}, \ldots, a_{4,5}$ are the coefficient values that are to be determined to form the interpolation polynomial $P(x, y)$.

The system of equations (3) takes the form,

$$Aa = \begin{bmatrix} 1 & x_1 & y_1 & \cdots & x_1{y_1}^3 & {y_1}^4 \\ 1 & x_2 & y_2 & \cdots & x_2{y_2}^3 & {y_2}^4 \\ \vdots & \vdots & \vdots & \cdots & \vdots & \vdots \\ 1 & x_{15} & y_{15} & \cdots & x_{15}{y_{15}}^3 & {y_{15}}^4 \end{bmatrix} \begin{bmatrix} a_{0,1} \\ a_{1,1} \\ a_{1,2} \\ \vdots \\ a_{4,4} \\ a_{4,5} \end{bmatrix} = \begin{bmatrix} f(x_1, y_1) \\ f(x_2, y_2) \\ \vdots \\ f(x_{15}, y_{15}) \end{bmatrix} = f, \tag{4}$$

where, $A \in \mathbb{R}^{15\times15}$, $f \in \mathbb{R}^{15}$, and $a \in \mathbb{R}^{15}$ is the unknown vector to be found. The system has a unique solution if the coefficient matrix $A \in \mathbb{R}^{15\times15}$ is non-singular.

## 3. Results and Discussion

### a. Results

In present study we generated the interpolation polynomial $P(x, y)$ by using two molecular descriptor values of each antihistamine i.e. AlogP and MW. We normalized the two descriptor values to scale the data between 0 and 1. $x$ values represented the normalized AlogP and y values represented the normalized MW of each compound that we used to generate the $P(x, y)$. Seven category B and eight category C drugs were used for our model. We set the output function values $f(x_i, y_i)$, according to the category of antihistamines. If the drug is categorized as B the output value is set 1 and if the drug is categorized as C the output value is set -1. By inserting the $(x_i, y_i)$ interpolation points in (4) we obtained a system of equations with a coefficient matrix $A \in \mathbb{R}^{15\times15}$, an output vector $f \in \mathbb{R}^{15}$, and an unknown vector $a \in \mathbb{R}^{15}$. In order to have a unique solution $a \in \mathbb{R}^{15}$, the coefficient matrix $A \in \mathbb{R}^{15\times15}$ must be non-singular. We checked the singularity of $A \in \mathbb{R}^{15\times15}$ by calculating the linearly independent columns of $A \in \mathbb{R}^{15\times15}$. The number of linearly independent columns of $A \in \mathbb{R}^{15\times15}$ was found 15 which indicated that the matrix $A \in \mathbb{R}^{15\times15}$ is non-singular in other words the matrix is invertible. That guarantees that the system (4) has a unique solution. We used MATLAB to calculate the $a_{j,i}$ values.

The coefficients $a_{j,i}$ of the model $P(x, y)$ with two variables are given in Table 1.

Table 1. Coefficients of the model polynomial $P(x, y)$

| Coefficient | Calculated Value of Coefficient |
|---|---|
| $a_{0,1}$ | 81.94 |
| $a_{1,1}$ | -602.38 |
| $a_{1,2}$ | -587.53 |
| $a_{2,1}$ | 1673.56 |
| $a_{2,2}$ | 2916.96 |
| $a_{2,3}$ | 887.45 |
| $a_{3,1}$ | -2053.86 |
| $a_{3,2}$ | -4659.91 |
| $a_{3,3}$ | -3105.83 |
| $a_{3,4}$ | -412.83 |
| $a_{4,1}$ | 935.29 |
| $a_{4,2}$ | 2384.66 |
| $a_{4,3}$ | 2587.649 |
| $a_{4,4}$ | 832.77 |
| $a_{4,5}$ | 29.97 |

Once the $a_{j,i}$ values calculated, the interpolation polynomial $P(x, y)$ was found as follows:

$$P(x,y) = 1.0\text{e}+03 * (0.0819 - 0.6024x - 0.5875y + 1.6736x^2 + 2.9170xy + 0.8874y^2 - 2.0539x^3 - 4.6599x^2y - 3.1058xy^2 - 0.4128y^3 + 0.9353x^4 + 2.3847x^3y + 2.5876x^2y^2 + 0.8328xy^3 + 0.03y^4). \quad (5)$$

We categorized the antihistamines drugs that we collected as pregnancy risks as groups B or C, by inserting the descriptor values AlogP as $x$-value and MW as $y$-value of the antihistamines drugs in the interpolation polynomial $P(x, y)$. After substituting the $x$ values representing the AlogP and the $y$ values representing the MW in $P(x, y)$, if the output value is greater than zero, we considered the drug as category B and if the output value is less than zero, we considered the drug as category C. Using the AlogP and MW values of all the drugs that we collected for the test, we determined which category they were in with the help of the interpolation polynomial.

The category information of the drugs we collected as data and the category information found by the model are given in the following tables. Classification of selected drugs as B and C categories according to FDA developmental category and the Polynomial interpolation developmental model, respectively, are given in Table 2 and Table 3.

Table 2. Classification of selected drugs as B and C category according to FDA developmental category

| | Drug Category | Name of the Drugs |
|---|---|---|
| **FDA developmental category** | **B-Category** | Pheniramine maleate, Clemastine, Diphenhydramine, Methdilazine hydrochloride, Cetirizine, Pyrilamine, Tripelennamine hydrochloride, Triprolidine, Phenyltoloxamine citrate, Chlorpheniramine, Loratadine, Rupatadine, Buclizine hydrochloride |
| | **C-Category** | Fexofenadine, Terfenadine, Antazoline, Azelastine, Astemizole, Carbinoxamine, Brompheniramine, Promethazine, Azatadine, Trimeprazine, Desloratadine, Ketotifen, Dimethindene maleate, Cyproheptadine, Meclizine |

Table 3. Classification of selected drugs as B and C categories according to the Polynomial interpolation developmental model

| | Drug Category | Name of the Drugs |
|---|---|---|
| **Polynomial interpolation developmental model** | **B-Category** | Pheniramine maleate, Clemastine, Diphenhydramine, Methdilazine hydrochloride, Cetirizine, Pyrilamine, Tripelennamine hydrochloride, Triprolidine, Phenyltoloxamine citrate, Chlorpheniramine, Loratadine, Rupatadine, Buclizine hydrochloride |
| | **C-Category** | Fexofenadine, Terfenadine, Antazoline, Azelastine, Astemizole, Carbinoxamine, Brompheniramine, Promethazine, Azatadine, Trimeprazine, Desloratadine, Ketotifen, Dimethindene maleate, Cyproheptadine, Meclizine |

According to the above tables, the interpolation polynomial we derived has classified 24 of 28 molecules correctly, while 4 of them have been classified incorrectly. The two category B compounds Cyproheptadine, and Meclizine were found incorrectly in category C and the two category C compounds Rupatadine, and Buclizine hydrochloride were found incorrectly in category B. All the other compounds are correctly categorized. According to the results, the percentage of the success of the model was found to be 85%.

**b. Discussion**

Predictive toxicology studies have been quite popular in recent years. However, in the literature, we do not reach a study focusing on the pregnancy category as in our study. Studies in this area have generally focused on reproductive and developmental toxicity (11). In this respect, our study is quite innovative. Although our data set may seem to be small compared to other studies, we included all currently used antihistamines whose pregnancy category was determined. Since the antihistamines available in the market are only in groups B and C as FDA pregnancy categories, our study was modeled using these groups. Our polynomial interpolation developmental model has a high success rate.

## 4. Conclusion

In conclusion, this study presented robust and reliable prediction models with an 85% success rate. These encouraging results will inspire further studies on drug use during pregnancy. The current research is the first step in predicting the safe use of antihistamine drugs during pregnancy. In the near future, the development of new non-animal-based in silico models is quite crucial to ethical issues for the risk assessment of drugs.

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FULL PAPER – ORAL PRESENTATION

# VALUE CHAIN OF BILBERRIES IN KELMENDI REGION

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## Abstract

The bilberry value chain analysis in the northern part of Albania represents an overview and in-depth analysis of the value chain linkages, resulting in the categorization of a number of issues as well as findings, but also giving general recommendations for the bilberry development program. The results of a bilberry study in the Kelmendi region are presented in this report. The study's purpose was to establish Kelemendi's bilberry area as a product with unique attributes and characteristics associated to the region, adding to the brand of quality recording while also maintaining and improving the area's biodiversity. The research examines the commercialization of forest products using the value chain method. The study is useful in evaluating the relevance of stakeholders or groups like collectors, processors, businesses, and exporters in driving the market in wild goods from the Kelmendi region. The goal is to first create a broad image of the diverse and wide group of enterprises who work with forest products. The goal is to learn how businesses in Kelmendi feel about various issues relating to the forest products industry.
**Key Words**: *Vacinium myrtillus*, Kelmendi, product definition

## 1. Introduction

Bilberry, is a plant deciduous or evergreen perennial shrub grows in the northern part of the Kelmendi mountains area where the study is conducted. Heath landscapes, as well as open forests are preferred by the plants. (Paparisto et al. 1988; Paparisto et al 1002; Doko et al 2014).
Bilberry is a wild-collected plant, so the farmed bilberry is yet in its infancy in our short history. This opens up a lot of development opportunities. The global average yield is 3 886.4 kg per hectare. During 2001, the Canada and the United States represented about 79.90% of all farmed land on the planet. Throughout the last ten years, the world's cultivated area has expanded by 30.38 percent, with increases of 10.13 percent, 31.21 percent, and 126.42 percent in the United States, Canada, and Europe, respectively. Production has climbed by 68.48 percent globally, while it has increased by 55.44 percent in the United States, 58.13 percent in Canada, and 150.72 percent in Europe, respectively. The bilberry market is currently divided into two segments: fresh fruit and processed fruit. Fruits, whether fresh, processed, or used as a raw ingredient in cosmetics, have recently developed and have a promising future. The market demand for fresh bilberries and their processed goods has expanded year after year as a result of their exceptional health benefits. Between 1981 and 1990, per capita consumption of fresh fruit and processed foods in the United States climbed by 50%. (M.A.F.F.I.C. 2003; ORAC 2007)

According to scientists from the United States, Japan, and Europe, regular bilberry usage can improve vision, nutrition, and skin; improve heart function, delay brain aging, and prevent Alzheimer's disease; and treat arthritis, allergies, cardiovascular disease, cancer, and diabetes. No other fruit compares to bilberry fruits in this regardBilberry has been dubbed the "fruit of the twenty-first century" due to its numerous health benefits. (M.A.F.F.I.C. 2003)

Bilberries are currently picked in Albania's isolated mountainous regions and brought to collectors who operate temporary collecting centers or sell the product fresh. Bilberries are highly fragile and must be chilled, frozen, or preserved immediately to avoid waste and deterioration.
The current supply of bilberries is irregular, leaving a market for local fresh blueberries that is generally unstable and unmet. Furthermore, wild berry collection tactics may cause environmental damage, which local authorities must be aware of it. (Dervishi 2012; Beqaj 2012)

In Albania, and particularly in the Kelmendi region, the collection of blueberries, as well as medicinal crops, is permitted in public forests for private purposes. Selling these to a consumer is permitted for a collector, processor, or exporter. A number of local businesses gather, prepare, and export berries.
The use and cultivation of bilberries are not well investigated or explored in Albania. Until today, Albanians have consumed modest amounts of these fruits, largely from the wild. (Doko 2012)
Bilberry is wild harvested, like many other medicinal plants, in public forests administered by state organizations, especially for the area that is taken into account in this research. The authorization system, which is maintained by public agencies, governs bilberry collection. (Dervishi 2012)

The government, on the other hand, has yet to be able to manage this industry. Furthermore, wild berry wild collecting practices may cause environmental harm, which local governments must be aware of. As a result, working with collectors/traders is critical in order to enrich the collection and ensure its long-term viability. Implementing the Code of Practice, as well as identifying the bilberry's provenance as Bilberry of Kelmend, is one approach to accomplish this.
In Albania, the bilberry value chain is now unreliable, creating a market for local fresh bilberries that is generally unstable and unfilled. (Doko 2012)

## 2. Material and Methods

The bilberry value chain study in the Kelmendi region of Albania is an overview and in-depth assessment of value chain links, leading in the identification of a number of difficulties and findings, as well as recommendations regarding the bilberry development program. The relevant value chain stakeholders, which included producers, harvesters, processors, and commune representatives, were included in the research at all phases.

In comparison to other national regions, the northern section of Albania has had less development, particularly in the agricultural sector (related this with the agricultural land they posses). Considering that more than half of the population of the region lives in rural regions, agriculture potential, particularly in terms of wild fruit development, are important. The market-oriented approach will improve agriculture performance while also improving the socioeconomic circumstances of these regions. In the bilberry value chain analysis, some unique concerns and obstacles have been highlighted that should be considered for the sector's future growth.

The majority of those participating in production are small-scale farmers. As a result, output is dispersed and diverse, resulting in greater cost per unit than larger farms with specialized production.

Farmers' land, which is also tiny in size, is frequently unsuitable for commercial production due to barriers to mechanization and the organization of subsequent production methods. There aren't enough producer organizations, which could help boost production.

Post-harvest operations are not given enough attention, and most of these tasks are left to the processors to complete. This part lacks suitable equipment, and each farmer's investment in this approach is too expensive. To facilitate the development of the bilberry value chain, organizations of simple post harvest facilities and appropriate post harvesting techniques are required. It is important to note that proper post-harvesting methods should raise the market price of this crop. Investing in competent collection centers, storage and processing facilities, as well as exploring value-added options, is critical. The bilberry manufacturing is organized by a few small processors that are still in the early stages of development and are assuming leadership roles in the production region. This industry is in desperate need of financial assistance to meet its infrastructure needs and permit future business expansion. Training in processing technology, new product development, and food safety standardization are also critical for their future export market position.

The processors sell their products in the domestic market from a marketing standpoint. The need for assistance in this chain is critical since it affects their market competitiveness and gives a direct access point into overseas markets. The resources and skills required to engage directly into export markets are lacking. To promote the Albanian bilberry products in the foreign market, the participation on trade fairs is crucial.

In terms of indirect support for this sector, the extension service is unable to provide support for new manufacturing technologies that are emerging due to market trends. The communes lacked a method to track the growth of the bilberry sector and increase the interaction between value chain operators at the local level.

The bilberry Value Chain study was conducted using a range of sources. There isn't much hard data as this is a new value chain. Due to a lack of commercial bilberry production in the United States, there are no relevant domestic producer or consumer data sets. Face-to-face interviews with all possible bilberry producers, processors, producer associations, and traders were done by the research team. Few reports were used as information sources for the value chain analysis such as: the report of the project “The potentials of bilberry in the areas of Kelmendi in the context of quality signs and development of the respective value chain”.

The desk activity entailed gathering and comparing all available data from reliable sources such as official data, published studies, and reports related to the bilberry VC. The information was collected at the local level and relates to both overall agricultural development and the development of specific sub-sectors. The data which has been collected covered information related to the production quantity, varieties, geographic distribution (location), number of hectares were bilberry grows, altitude above the sea level, export and import data for some bilberry products.
The field work has provided data that would not be available otherwise and it was conducted via interviews with all the relevant actors of the value chain including: producers, processing facilities, bilberry collectors, and commune agricultural specialists. A field survey was carried out by the group using clear and concise questionnaires, proving information that would be understandable and which has covered all the product value adding activities such as: harvesting, production, processing, and marketing. The field survey was conducted for a period of 4 weeks with all the relevant actors of the VC in two districts involving: households, processing companies, as well as collectors of wild blueberries and specialist of the extension service. The group discussions have led to draft certain comments and conclusions, so that key issues on the analyzed data could be reached based on the

information gathered from the field research. Both the desk work and the field research information were as an important data tool to prepare the detailed action plan which will act as an important document for the further support of the bilberry crop in Albania. Research on the bilberry VC indicated that there was a need for intervention and recommendations to improve the performance of bilberry sector.

## 3. Results and Discussion

Blueberries, as wild fruits of the forest and pasture, appear to be necessary for their taste, smell, and ecological characteristics, according to expert group meetings with people of the above places. It is classified as an organic product because it was discovered and evolved naturally in our country's mountainous region. Pesticides and fertilizers are not used during the growing stages, making them bio products. Bilberry fruit is only used as a fresh fruit when it is mature, and as a dry fruit during the rest of the year.

### a. Product Definition

At present, supply is inconsistent, leaving supply of local fresh blueberries, a market largely unstable and unfilled. Moreover, wild bilberry collection practices pose a potential risk of environmental damage, which official authorities should start to recognize.

The bilberry fruit collection in Kelmendi has a long tradition. The geography of the region is mountainous and hilly, rich in variety of vegetation. The environment is unpolluted which has a positive impact on the quality of this plant species. Bilberry plant is harvested each year naturally in substantial quantities. The product types include fresh bilberry, dried bilberry, and processed (especially jam). From an employment point of view the value chain represents an opportunity for rural inhabitants starting with the collection process and a need for seasonal workers to handle the post-harvest processes. With support to the relevant processors and in the diversification of the marketed products collection activities can start in spring and and in summer.

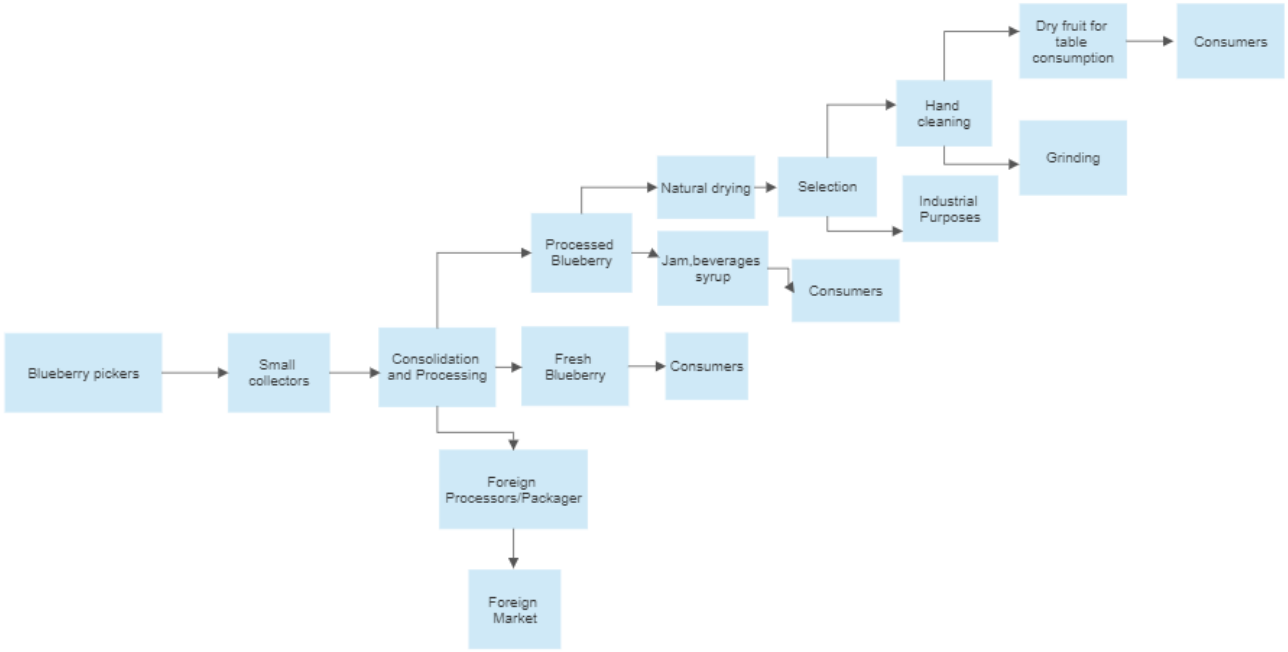


**Figure 1. Bilberry Marketing Structure in Albania**

Bilberry pickers: The harvesting procedure is a labour-intensive activity that is entirely done by hand, and pickers are known for their disorganization. Individuals or rural families perform the harvesting

procedure on a part-time basis. Many pickers supply wild bilberry to minor collecting stations or directly to the main operators around the area. It is a seasonal job that takes place throughout the bilberry season. There are roughly 300 rural families picking blueberries in Kelemendi. To summarize, the collection is carried out solely by residents of such area.

Collectors: Small-scale collectors oversee the collection process, organizing collection stations where many pickers deliver raw, wild blueberries. The main quantity of wild bilberry is not collected in the Vermoshi region due to poor infrastructure. When the collected blueberries are not sent to small scale collectors, they are sent directly to processing centres. Finally, the pickers in Vermosh do the collection directly, and the quantity harvested is sold to small-scale collectors who accumulate fresh blueberries directly from the pickers.

Processors: The majority of bilberry goods are sold on the market with no added value. The processor's function is to receive products from collectors and provide further services like packaging, drying, storing, or freezing. Currently, only a few processors get their wild-bilberry supply from their suppliers. Small processors are currently available and are often responsible for primary processing, which includes natural drying and packaging, while others add value to the commodity by making jam and other drinks. Albkalystian and Filipi Company, for example, own their own processing equipment. The processors drive the bilberry industry, injecting enormous sums of money into rural areas by purchasing the harvest, processing the raw material to maintain the quality of the blueberries, and finally, by exporting the product. This way, they generate high revenues for the business as well as for the Albanian economy.

Post harvesting/storage: According to the survey, there is insufficient storage capacity. Due to a shortage of equipment at the collecting sites, such as refrigeration and cleaning equipment, a considerable amount of potential profit is lost. The temperature of the blueberries should be decreased using a refrigerated chamber in order to retain their freshness. To avoid decomposition, the fruits must be transferred from the collection centers to the processing facilities after this process. Investment in refrigerating facilities will boost the value of bilberry products while also allowing them to be processed and shipped internationally. The market value of the bilberry will rise as cleaning facilities are built. The quality and diversity of the products collected will increase as investment in collection sites is boosted. The goal is to add more value for the product, resulting in better market prices.

The competitive landscape: Bilberries grown in the Albanian Alps are in high demand both domestically and internationally. The demand for this commodity is significantly larger than the supply. This demand has been steadily expanding in recent years, beginning with public awareness of the nutritional and medical benefits of this product for the internal market. Because of the bilberry's great reputation, it was difficult for early collectors in metropolitan areas to find specific quantities during the bilberry harvest season.

Outside of the harvest season, finding any number of fresh bilberries on the market is impossible. This is not due to a lack of demand, but rather to a lack of local infrastructure that would provide more time to trade the fresh product. However, as demand for bilberry from Tropoje and Kukes grows in the domestic market, there is a significant increase in demand for its exports. It is simple to spot because practically all fresh bilberries for export are sold out as soon as they arrive in urban regions. Traders from Kosovo, who export to Kosovo and then to European markets, are common in these locations. These businesses purchase all the available stock. In no situation bilberry batches remained, but the sole issue remains in the lack of product quality.
According to our interviews, the reputation of Albanian Alps Bilberry is pretty strong, and the product is quite sought after. Based on the highest values typical of bilberry produced in our Albanian Alps, this

product appears to be marketed exclusively for the purposes of high-quality pharmaceutical industries in industrialized countries. Bilberries are used to make the best pharmaceutical goods in Albania.

Strategy and execution: Collectors frequently lack knowledge of proper collection techniques. There is a need for collectors to be trained in order to improve their practical abilities, knowledge, and attitude toward effective collecting and agricultural practices. Collection methods must ensure the long-term viability of wild populations and their habitats. Collection management plans should include a framework for determining sustainable harvest levels as well as descriptions of proper collection procedures for the plant species and plant parts used (such as leaves, roots, fruits, and so on).
Processing facilities are scarce in these places. To safeguard the competitive advantage that nature and climate have produced, as well as to avoid any loss of value due to human error or contamination, the processor must apply and be compliant with international food safety regulations.

The development of the trademark "Albania Bilberry Alps" is a prerequisite from a marketing standpoint. There is a lack of marketing plan, as well as promotion and commercial connections.

Other Promotion: The promotion methods are quite necessary in order to make the bilberry products well known and appealing to buyers. Bilberry goods may be out of reach for the typical Albanian customer due to their high cost. The intended market in this circumstance must be international. The collectors must bring high-quality wild bilberry to the processors, who must then process and deliver the proper products to the international market in terms of quality, packaging, labeling, and any other information requested by customers.

**Table 1: Characteristics of the Marketing channel**

| No | Characteristics | Kelmendi region |
|---|---|---|
| 1 | Main marketing channels used by farmers | Collectors and processors in Albania |
| 2 | Number of buyers | High |
| 3 | Time employed in selling activities for farmers | Short |
| 4 | Transaction cost (farmers – collector) | Low |
| 5 | Perceived level of satisfaction of farmers regarding price/quality ration | Low |
| 6 | Main marketing channels used by processor/exporters | National buyers and other countries (Processors) |
| **7** | Level of transaction costs | Moderate |
| **8** | Potential to upgrade channels | Low due to lack of processors |
| **9** | Type of channel upgrade | Domestic market and diversification of foreign |

Because the majority of "educated consumers" get information via the internet, an online advertising campaign is essential. Media advertising, website development, and social media advertising must all be part of the marketing plan. The curative value of the Bilberry from the Albanian Alps, as well as its unique traits, should be highlighted as part of the digital marketing strategy.

The importance of Quality Signs on Market Channels: The creation of the geographic indication quality sign of bilberry produced from Kelmendi region is a very important step toward consolidating the marketing channels and increasing the value of products. The linkages between quality signs and higher product value in the market have been proven as positive by many studies and authors.

## 4. Conclusion

The relevance of Quality Signs on Market Channels is highlighted by the findings of this study. Given some of the most essential fruit quality attributes, the bilberries in the Kelmendi region have the potential to become an important and valuable wild shrub. It is critical to establish a geographic indication quality sign for bilberry grown in the Kelmendi region, named "Albania Bilberry from the Highland."

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FULL PAPER – ORAL PRESENTATION

# THE COMPUTATIONAL AND BIOLOGICAL INVESTIGATION OF INDOLE AND QUINOLINE BASED THIOSEMICARBAZONES TOWARDS α-GLUCOSIDASE ENZYME INHIBITION

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## Abstract

Thiosemicarbazones are important classes of Schiff base ligands due to the presence of conjugated N-N-S system providing an important therapeutic potential and have been the subject of many structural and medicinal studies via the interactions of biomolecules. A wide variety of heterocyclic systems have been used for the structural modifications of new thiosemicarbazone based compounds. Due to the presence of the indole and quinoline structures in many natural products, studies have been directed towards investigations of the biological properties of natural indolic and quinolic compounds, and a range of medical uses has been identified.
In the present work, the synthetic procedures and chemical characterization of the targeted compounds derived from indole-3-carbaldehyde and 2-chloroquinoline-3-carbaldehyde systems with a range of thiosemicarbazides. The final compounds have been subjected to α-glucosidase enzyme inhibition assay to investigate the antidiabetic efficiency. A complementary study was carried out with the molecular docking study of targeted compounds on the catalytic side of the designated enzyme. The biological aspect of the study revealed that the indole-based compounds possessed more promising potency compared to the quinoline derivatives.
**Key Words**: Indole, Quinoline, Thiosemicarbazone, α-glucosidase, Diabetes

## 1. Introduction

Most of the diabetic population has type 2 diabetes which is noninsulin-dependent and considered more difficult than type 1 to control effectively. There are available oral antidiabetics to prevent hyperglycaemia in these patients and protect them from complications affecting several organs including heart, kidneys, eyes, and blood vessels. Acarbose and miglitol, two of these drugs, inhibit the hydrolysis of the polysaccharides to oligo- and monosaccharides by inhibiting the enzymes α-amylase and α-glucosidase. These inhibitions result with a delay on the postprandial glucose absorption. However, these drugs are non-selective on these enzymes and have several side effects such as stomach-ache, meteorism, emesis and diarrhoea (Apostolidis & Lee, 2010; Trinh, Staerk, & Jäger, 2016).

Indole and quinoline heterocyclic systems are pharmacologically valuable scaffolds and appear in many natural compounds (Kamal, Rao, Laxman, Ramesh, & Reddy, 2002). Considerable efforts have been devoted to the synthesis of complex and pharmacologically active indole and quinoline alkaloids that are prevalent in a variety of biologically active natural and synthetic compounds. The literature reveals interesting biological properties, such as antibacterial, antifungal, anti-inflammatory, antimalarial, analgesic and anticancer activities (Gözler & Shamma, 1990; Küçükgüzel, Mazi, Sahin, Öztürk, & Stables, 2003; Loncle, Brunel, Vidal, Dherbomez, & Letourneux, 2004; Melnyk, Leroux, Sergheraert, & Grellier, 2006; Todeschini, de Miranda, da Silva, Parrini, & Barreiro, 1998). Due to the promising and wide range of biological aspects of indole and quinoline-based compounds, the design and synthesis of analogues have attracted the great attention of researchers.

On the other hand, thiosemicarbazone moiety (–(C=N)-NH-C(=S)-$NR_1R_2$) has also been identified as an important fragment and many structural and medicinal studies have been subjected due to the biological properties namely antiviral, antibacterial, antifungal antioxidant and anticancer activities (de Oliveira et al., 2008; Đilović et al., 2008; Hu, Zhou, Xia, & Wen, 2006; Pavan et al., 2010; Yu et al., 2009). The hybrid molecules derived from the indole and quinoline heterocycles with the thiosemicarbazides to generate targeted indole and quinoline based thiosemicarbazones were reported as as α-amylase / α-glucosidase inhibitors (Bakherad et al., 2022; Kawde et al., 2020; Taha et al., 2021; Taha et al., 2019).

In the current work, the preparation of eight indole and quinoline based heterocyclic systems with the thiosemicarbazone functionality have been reported and the α-glucosidase inhibition potency properties have been evaluated. The synthetic pathway was designed via Schiff base reaction of corresponding carbaldehydes with the appropriate thiosemicarbazides. Although the indole-based thiosemicarzbazones were reported previously, to the best of our knowledge, the quinoline-based counterparts have been reported for the first time. The current work is also novel for the inhibition evaluation towards the α-glucosidase enzyme. Our manuscript is also valuable due to the computational contribution to determine the anti-diabetic potency of targeted compounds.

## 2. Material and Methods

### a. Chemicals and Physical measurements

All commercially available reagents and the standards used for the biological assays were purchased from Sigma Aldrich and used without further purification. The general synthetic procedure was written for the synthesized compounds and the known compounds were reported with the appropriate references. The TLC chromatographic method was used to monitor the reactions. Merck was the supplier for the Silica gel 60 (particle size: 0.040-0.063 mm, 230-400 mesh ASTM) and the Thin Layer Chromatography plates. d6-DMSO was the solvent for the 1H and 13C NMR spectra were recorded on a Bruker DPX 400 MHz spectrometer at 300 K. Chemical shifts were reported as ppm and the solvent peak d6 -DMSO was given as 1H d 2.50 ppm, 13C d 39.52 ppm. (J) was given as coupling constants in Hertz (Hz) unit. m= multiplet, t= triplet, d= doublet, s= singlet, dd= doublet of doublets illustrated the standard conventions indicating multiplicity. The Thermo Scientific Nicolet IS10 FT-IR spectrometer was used forthe Infrared spectroscopy data between 600 and 4000 cm-1. The Mel-Temp melting point apparatus was used for the melting points measurements.

### b. α-Glucosidase inhibition assay

The α-glucosidase inhibitory activity was measured by the method described by Schmidt et al(Schmidt, Lauridsen, Dragsted, Nielsen, & Staerk, 2012). In brief, 90 µL of 0.1 M phosphate buffer (pH 7.5, 0.02% NaN3), 10 µL test sample dissolved in DMSO, and 80 µL of enzyme solution (well concentration 0.05

U/mL) were added to each well. The mixture was incubated at 28 °C for 10 min before adding PNPG to a final volume of 200 µL (final well concentration 1.0 mM). A blank was used consisting of enzyme, substrate, and test solvent instead of sample. Absorbance was measured at 405 nm every 40 s for 35 min. BioTek Power Wave XS microplate photometer with built-in incubator, controlled by GEN5 ver. 2.05.2005 software was used for incubation and absorbance measurements. The α-glucosidase inhibitory activity was expressed as percentage inhibition and was calculated using the following formula:
% inhibition = ($Slope_{blank}$– $Slope_{sample}$) / $Slope_{blank}$ *100
Acarbose was used as positive control and all measurements were performed in triplicate (Student's t-test $p<0.05$).

**c. Molecular modelling**

*Receptor preparation*

The crystallographic structure of Glucosidase was obtained from Protein Data Bank (PDB) (Berman et al., 2000). Crystal structure was selected for Glucosidase. The crystal structure was cleaned from all ingredients contained in pdb file except amino acid residues using BIOVA Ds Visualizer. MGL Tools was used to add missing residues, hydrogen atoms, charges and to remove non-polar hydrogen atoms.

*Ligand preparation*

Two-dimensional structures of selected ligands **3a** and **6b** was drawn by Marvinsketch (Marvin 17.24.0, ChemAxon, 2017) program (Figure 1) and converted to three-dimensional structure by Biovia DS Visualizer (Biovia, Discovery Visualizer Studio, v19.1.0.18287).

**d. Chemical synthesis**

*General Procedure for the Preparation of Indole-based thiosemicarbazones **3a-d***

The synthetic procedure was discussed in the previous work. The spectroscopic data were also outlined in the previous work (Bingul, 2019).

*General Procedure for the Preparation of Quinoline-based thiosemicarbazones **6a-d***

2-Chloro-3-Formylquinolines: Vilsmeier reagent was prepared by the treatment of *N,N*-dimethylformamide (0.025 mol) and phosphoryl chloride (0.070 mol) under cool condition with an ice-salt bath for 20 min. The resulting mixture was added dropwise to a cooled solution of the acetanilide **4** (0.010 mol) in *N,N*-dimethylformamide (10 mL) with stirring. The mixture was stirred at 90 ″C for 12 h. A small amount of crushed ice was added, and the mixture was basified to pH 14 with 5 M NaOH. After stirring at ambient temperature for 1 h, the precipitate was filtered, washed with water, and dried to give the title compound **5**.
2-Chloroquinoline-3-carbaldehyde (**5**) (Shvo & Arisha, 1998). Yellow solid, yield: 60%; m.p. 150–152 ″C (Shvo & Arisha, 1998) m.p. 146–149 ″C); 1H-NMR ($CDCl_3$): δ 10.59 (1H, s, CHO), 8.79 (1H, s, H4), 8.11–7.65 (4H, m, H5, H6, H7, H8).

Quinoline-based thiosemicarbazones: Upon the preparation of quinoline carbaldehyde **5**, the Schiff base reaction was carried out between the appropriate thiosemicarbazides **2a-d** (1 eq.) and aldehyde **5** (1 eq.), under acidic condition (acetic acid 5 drops) in ethanol. The overnight stirring of the reaction mixture resulted the dark yellow product which is subsequently concentrated under vacuum to collect the yellow solid as targeted compounds.

(2E)-2-[(2-chloroquinolin-3-yl)methylidene]hydrazine-1-carbothioamide **6a**
The title compound was synthesized following the general procedure using 2-Chloroquinoline-3-carbaldehyde **5** (200mg, 1.39 mmol) and thiosemicarbazide **2a** (124 mg, 1.39 mmol) in EtOH (25 mL) with 5 drops of acetic acid. The compound **6a** was obtained as a white powder; yield: 81%; m.p. 180 °C; 1H-NMR (d6 -DMSO): δ 11.80 (1H, s, NH), 9.32 (1H, s, H4), 8.51 (1H, s, CH), 8.28 (2H, d, NH2 J = 8 Hz) 8.01–7.70 (4H, m, H5, H6, H7, H8), IR: vmax 2967 C–H, 1525 C=N 1595 C=S 3138 N–H 3257 and 3391 cm-1 NH2

2E)-2-[(2-chloroquinolin-3-yl)methylidene]-N-methylhydrazine-1-carbothioamide **6b**
The title compound was synthesized following the general procedure using 2-Chloroquinoline-3-carbaldehyde **5** (200mg, 1.39 mmol) and 4-methylthiosemicarbazide **2b** (142 mg, 1.39 mmol) in EtOH (25 mL) with 5 drops of acetic acid. The compound **6b** was obtained as a white powder; yield: 84%; m.p. 197 °C; 1H-NMR (d6 -DMSO): δ 11.88 (1H, s, NH), 9.21 (1H, s, H4), 8.76 (1H, d, NH J = 8 Hz), 8.51 (1H, s, CH), 8.02–7.71 (4H, m, H5, H6, H7, H8), 3.07 (3H, d, CH3, J =3.6 Hz) IR: vmax 2944 C–H, 1524 C=N, 1535 C=S, 3131 N–H and 3377 cm-1 N–H

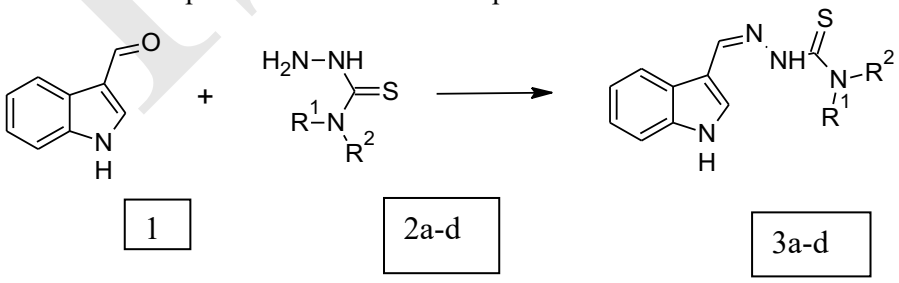
2E)-2-[(2-chloroquinolin-3-yl)methylidene]-N,N-dimethylhydrazine-1-carbothioamide **6c**
The title compound was synthesized following the general procedure using 2-Chloroquinoline-3-carbaldehyde **5** (200mg, 1,39 mmol) and 4,4-dimethylthiosemicazrbazide **2c** (164 mg, 1,39 mmol) in EtOH (25 mL) with 5 drops of acetic acid. The compound **6c** was obtained as a yellow powder; yield: 89%; 186 °C; 1H-NMR (d6 -DMSO): δ 11.80 (1H, s, NH), 9.05 (1H, s, H4), 8.75 (1H, s, CH), 8.08–7.60 (4H, m, H5, H6, H7, H8), 3.63 (3H, s, CH3), 3.24 (3H, s, CH3); IR: vmax 2933 C–H, 1520 C=N, 1617 C=S, 3350 cm-1 N–H

(2E)-2-[(2-chloroquinolin-3-yl)methylidene]-N-ethylhydrazine-1-carbothioamide **6d**
The title compound was synthesized following the general procedure using 2-Chloroquinoline-3-carbaldehyde **5** (200mg, 1,39 mmol) and 4-ethylthiosemicarbazide **2d** (164 mg, 1,39 mmol) in EtOH (25 mL) with 5 drops of acetic acid. The compound **6d** was obtained as a pale yellow powder; yield 87%; 197°C; 1H-NMR (d6 - DMSO): δ ): δ 11.82 (1H, s, NH), 9.19 (1H, s, H4), 8.79 (1H, s, CH), 8.06–7.69 (4H, m, H5, H6, H7, H8), 3.65 (3H, t, J= 2.4 Hz, CH3), 1.19 (2H, d, J= 2.4 Hz, CH2); IR: vmax 2974 C–H 1535 C=N 1599 C=S 3138 N–H and 3343 cm-1 N–H

## 3. Results and Discussion

### a. Chemistry

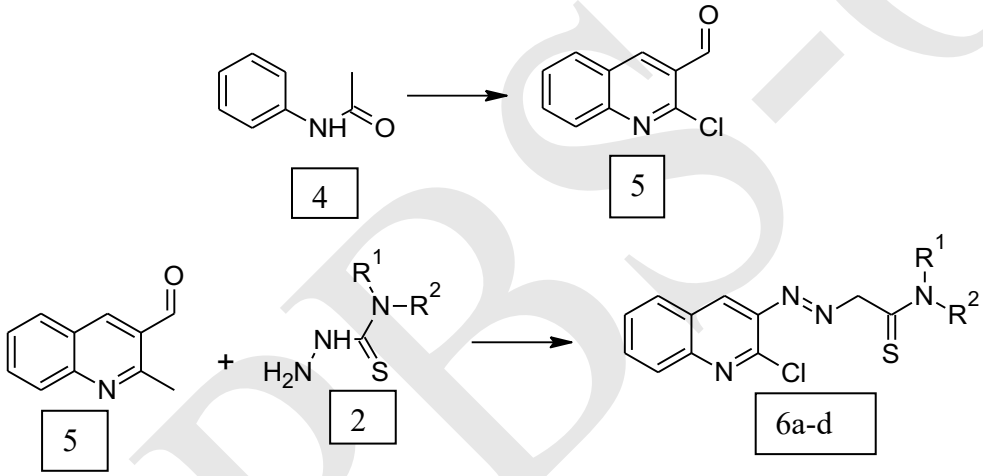
The synthesis of targeted indole based thiosemicarbazones **3a-d** was achieved by the treatment of indole-3-carboxyaldehyde with the corresponding thiosemicarbazides via Schiff base reaction using acetic acid in ethanol at room in yields of 67%–82% (Scheme 1). The characteristic analysis (FT-IR, NMR and HRMS) of the final compounds were discussed in previous work.




**Scheme1.** Reagents and conditions:EtOH,a few drops AcOH, overnight rt

| Thiosemicarbazide | $R^1$ | $R^2$ | Product |
|---|---|---|---|
| **2a** | H | H | **3a** |
| **2b** | H | Me | **3b** |
| **2c** | Me | Me | **3c** |
| **2d** | H | Et | **3d** |

The synthetic pathway for the preparation of final quinoline based thiosemicarbazones **6a-d** was designed in two step reaction sequences. The first step was the yield of 2-chloro- quinoline-3-carbaldehyde **5**. The Vilsmeier cyclisation of the acetanilide **4** resulted the targeted quinoline carbaldehyde **5** in high yield. (Scheme 2) (French & Wirtel, 1926; Meth-Cohn, Rhouati, Tarnowski, & Robinson, 1981). Following aqueous work-up, carbaldehyde was then treated with corresponding thiosemicarbazides **2a-d** at room temperature in ethanol under acidic condition to generate the targeted quinoline thiosemicarbazones **6a-d** in 81%–89% yields (Scheme 2).




**Scheme 2:** Reaction condition: $POCl_3$, 90 °C 24h EtOH, AcOH, rt overnight

| Thiosemicarbazide | $R^1$ | $R^2$ | Product |
|---|---|---|---|
| **2a** | H | H | **6a** |
| **2b** | H | Me | **6b** |
| **2c** | Me | Me | **6c** |
| **2d** | H | Et | **6d** |

The condensation of aldehyde with the amino group of thiosemicarbazides was proved by the detection of characteristic singlet CH proton at 8.51-8.79 ppm, whereas the corresponding thioamide NH proton appeared at 11,80–11,81 ppm for all the quinoline-based thiosemicarbazones. The $^1$H NMR spectra of compound **6a** revealed the free $NH_2$ group at 8.28 ppm. The mono and di methyl substitutions in the case of compounds **6b** and **6c** resonated at 3,07 and 3,63-3.24 ppm respectively. In the case of compound **6d**, the methylene and methyl groups at the thiosemicarbazone N end resonated as triplet and doublet signals around 1.19 ppm and 3.65 ppm, respectively. The aromatic protons raised from the quinoline ring appeared at the range of 7,69-8,06 ppm as multiplet for H5, H6, H7 and H8 and singlet signals for H4 protons.

**b. Biological studies**

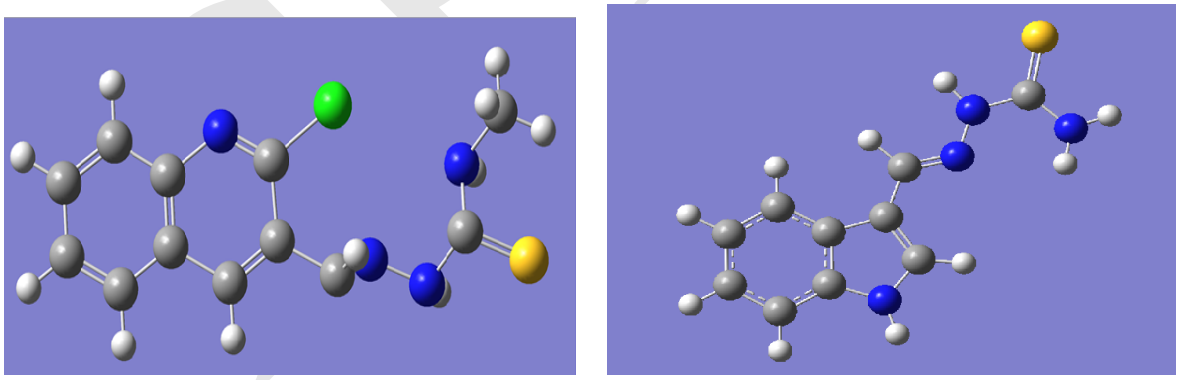
*α-Glucosidase inhibition assay*

The Table 1 shows the α-glucosidase inhibition potency of the eight **3a-d** and **6a-d** at the 800µM concentration and Acarbose has been used as standard. The synthesized compounds were found to be less effective compared to the standard. The similar pattern of inhibitions was detected in the case of compounds **3a** and **3c**, members of indole based thiosemicarbazones and the value of %28,10 and %22,38 inhibition were detected as the most promising results among the tested compounds. In the case of quinoline based thiosemicarbazones, the compound **6b** with methyl substitution at the thiosemicabazone N end showed the highest inhibition with the value of around 10,75%, whereas the rest of the compound demonstrated either lower or no activity against the designated enzyme. It was concluded that indole heterocyclic systems have been detected more sensitive towards the enzyme compared to quinoline counterparts.

**Table 1.** Glucosidase Inhibition percentage of tested compounds

| **Compound 800µM** | **Glucosidase % inhibition** |
|---|---|
| **3a** | 28,10297 |
| **3b** | NA |
| **3c** | 22,38363 |
| **3d** | NA |
| **6a** | 2,491682 |
| **6b** | 10,7526 |
| **6c** | 4,653099 |
| **6d** | NA |
| **Acarbose** | $IC_{50}$ µM 1033,8 |

***Molecular docking***

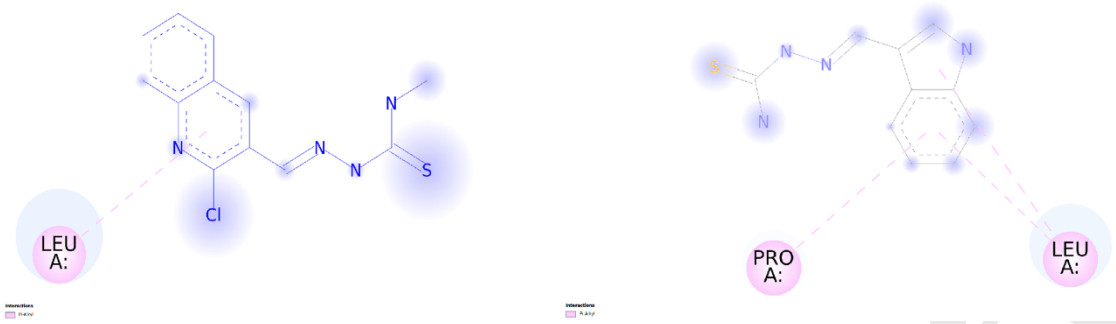


(a) (b)

**Figure 1.** Two-dimensional structures of selected ligands (a) **3a** and (b) **6b**

Two selected compounds with the highest inhibition efficiencies obtained from biological study were successfully docked to binding sites of Glucosidase. The targeted compounds displayed mainly hydrophobic interactions with the residues LeuA and ProA and LeuA (Figure 2). The binding interactions raised from the aromatic benzene rings of indole and quinoline systems and the weak

interactions were found to be the possible reason for the low inhibition efficiency obtained from biological assay.




(a) (b)

**Figure 2.** 2D representations of (a) **3a** and (b) **6b** to binding site of Glucosidase.

## 4. Conclusion

The indole and quinoline heterocyclic systems have been condensed with the thiosemicarbazides to generate eight thiosemicarbazones via Schiff base reaction. The final compounds were tested against the Glucosidase enzyme to identify the inhibition potency and molecular docking was carried out to understand the binding patterns of selected compounds on the catalytic site of the enzyme. Biological potency was found to be lower and binding interactions were detected as weaker for both indole and quinoline based thiosemicarbazones and the highest efficiency was determined in the case of indole heterocyclic systems **3a** with the %28 inhibition value. It was also confirmed that the designated compound revealed more hydrophobic interactions on the catalytic site of the enzyme.

## Conflict of Interest

No potential conflict of interest was reported by the authors.

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**FULL PAPER – ORAL PRESENTATION**

# EVALUATION OF ANTIOXIDANT AND ENZYME INHIBITORY ACTIVITY OF THREE DIFFERENT TEAS

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## Abstract

Tea has been one of the widely consumed beverages all over the world for thousands of years. In this study, three different types of tea (black, green, and white tea) obtained from the *Camellia sinensis* plant were investigated in terms of antioxidant and enzyme inhibition activities. Total phenol and flavonoids were investigated by Folin-Ciocalteu and aluminium chloride colorimetric method respectively. The antioxidant activity was assessed with DPPH and ABTS radical scavenging assay. Extracts prepared from three different types of tea were investigated by the 96-well plate method for their inhibitory effect against important enzymes in the treatment of human pathologies such as: diabetes ($\alpha$-amylase and $\alpha$-glucosidase), neurodegenerative disorders (acetylcholinesterase and butyrylcholinesterase) and hyperpigmentation (tyrosinase). According to results, thegreen tea extract showed strong DPPH radical scavenging and tyrosinase inhibitory activity than the black and white tea extracts. The green tea extract contains higher amount of phenolic compounds (185.98±0.48 mgGAE/g) while black tea extract contains highest total flavonoid contents (80.23±6.51 mgQE/g). Green tea extract was found to have the highest inhibition effect on acetylcholinesterase and butyrylcholinesterase enzymes used in Alzheimer's disease therapeutic strategy. The results suggests that differet tea types ara a valuable source of polyphenolic compounds and functional dietary supplements and green tea has a potential use in antioxidant and anti-alzherimer drug formulations as well as food supplements.
**Key Words**: Tea, *Camelia sinensis*, antioxidant activity, enzyme inhibitory

## 1. Introduction

Tea is the second most consumed beverage after water, used by many consumers around the world, and often attracts attention for the health benefits that come with regular use. Different tea products such as black tea, oolong tea, green tea and white tea are produced from the leaves of the Camellia sinensis plant with different methods applied during harvesting and processing. This difference is related to the degree of oxidation of polyphenols in fresh leaves, depending on the fermentation process during processing (Damiani et al., 2014). In black and oolong teas, during fermentation, the polyphenols in the tea leaf are transformed into theaflavins and thearubigins, which are responsible for its characteristic aroma and color, as a result of enzymatic oxidation by endogenous polyphenol oxidases and peroxidases (Obanda et al., 2004). To avoid enzymatic oxidation, green teas are steamed, roasted, and perhaps oven fired. In white teas, 1-2 very young leaves and buds covered with fine white hairs are used, processed through sunshine withering, and drying. The chemical composition of tea varies a lot according to the type of variety, growing conditions such as season, climate, soil, horticultural practices

such as mechanical or hand picking, leaf age, and different technologies of tea producing factories (Carloni et al., 2013).

Tea mainly contain phytochemicals such as polyphenols, caffeine, minerals and trace quantities of vitamins, amino acids and carbondydrates (Prasanth et al., 2019). Fresh tea leaves contain a wide range of phenolic compounds such as flavonoids, catechins, flavonols, proanthocyanidins and phenolic acids. While there are more catechins in green tea, these catechins are replaced by theaflavins and thearubigins by the fermentation process in black tea. The most abundant phenolic component in green tea is epigallocatechin gallate (EGCG). It is followed by epicatechin gallate (ECG), epigallocatechin (EGC) and epicatechin (EC) (Kumar & Goel, 2019). In addition, strictinin, an important another phenolic acid derivative, is especially effective in allergic diseases(Maeda-Yamamoto et al., 2007).

Reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical, hydrogen peroxide and lipid peroxide, are synthesized in our body by various biochemical means. Since ROS are very reactive, they damage the cell by attacking protein, nucleic acid, amines and cell membrane, which are the most important elements of biological systems, so many chronic diseases occur over time (Riley & Behrman, 1991). For this reason, the natural antioxidants that present in herbal products, vegetables and fruits has always been the focus of research thanks to their protecting effect on human health against oxidative stress (Pandey & Rizvi, 2009). Because tea leaves are processed differently to produce black, green, and white tea, it's important to know which tea may be potentially more beneficial in terms of antioxidant activity. Therefore, the present study was carried out to explore antioxidant and enzyme inhibitory activity of green, white and black teas. The total phenol and flavonoid contents were also determined.

## 2. Material and Methods

### a. Plant materials

The green, white, and black tea samples were purchased from herb markets in Sivas, Turkey, in September 2017. The dried powder of teas (10 g) was mixed with 100 mL hot water. Each mixture was macerated with intermittent shaking at room temperature for 24 h. After filteration with whatmann filter paper, the supernatant was concentrated at 40°C in a rotary evaporator (Buchi, Swiss). The extracts were keeped in refergrator until use.

### b. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol and dimethyl sulfoxide (DMSO) were obtained from Merck (Darmstadt, Germany). Folin-Ciocalteu's phenol reagent were purchased from Fluka Chemie GmbH (Buchs, Switzerland).

### c. GC-MS analysis of Tea extracts

GC–MS analysis of the ethanol extracts of M. alba and M. nigra was performed using an Agilent GC 7890A - (5975C inert MSD) system equipped with an Agilent HP-5MS fused a capillary column (30 × 250 µm ID × 0.25 µm df). For GC–MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas was used as a carrier gas at a constant flow rate of 1.5 ml/min, and an injection volume of 1 µl was employed. The injector temperature was maintained at 250°C, the ion source temperature was 200°C, the oven temperature was programmed at 120°C, with an increase of 10°C/min–160°C, then 4°C/min–200°C for 1 min, then 8°C/min–300°C for 1 min, ending with a 20 min isothermal at 300°C. The total GC–MS running time was 51.5 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Identification of the phytocomponents presented in the extracts was conducted by comparing the spectrum of unknown compounds with the spectrum of known components

stored in library of National Institute Standard and Technology NIST-05a.L, W9N11.L, and WILEY7n.l.

**d. Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)**

The spectrophotometric Folin-Ciocalteu (F-C) technique was used to measure the TPC in the tea extracts (Clarke et al., 2013). The TFC was evaluated by aluminium chloride colorimetric assay based on previous method (Yang et al., 2011), quercetin was used for creating calibration curve and results expressed as mg QE/g extract.

**e. Antioxidant activity**

The *in vitro* DPPH radical scavenging activity of tea extracts was performed according to the method of Clarke et al (2013). The ABTS radical scavenging activity was conducted according to the method (Re et al., 1999). Iron chelating property of the extracts was determined using the method.

**f. Enzyme inhibitory activity**

Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE) inhibitory activity were measured using Ellman's method as previously reported (Ellman et al., 1961). The α-amylase inhibitory activity of the extracts was assayed according to the procedure described before(Özek et al., 2019). The effect of tea extract on α-glucosidase inhibition was determined by spectrophotometric method as previously reported (Telagari & Hullatti, 2015). The $IC_{50}$ concentration required for inhibition of 50% of α-amylase and α-glucosidase was determined graphically and Acarbose was used as a positive control. The tyrosinase enzyme inhibitory activity of the tea extracts was carried out as described previously (Jeong et al., 2009).

**g. Statistical analysis**

Data were expressed as means means ± standard deviations of three parallel measurements. $IC_{50}$ values were calculated using linear regression analysis by Microsoft Excel programme for Windows. Data analyses were performed using Graphpad (Version 9.0, USA) software. Statistical differences between three groups were compared using the Mann-Whitney U-test and statistical significance was considered at $p < 0.05$ level.

## 3. Results and Discussion

**a. GC-MS analysis**

GC-MS chromatogram analysis of three different tea extracts showed different peaks which indicates that certain major compounds are the same, they differ in some substances (Figure 1-3). On comparison of the mass spectra of the constituents with the NIST, Wiley library, the unknown compounds were characterized and identified. The mass spectra of all the identified compounds contained in the tea extracts were given in Table 1. Among the identified compounds, the most dominant compounds were caffein, 2-propenoic acid, cyclododecane, and 9-octadecenamide.

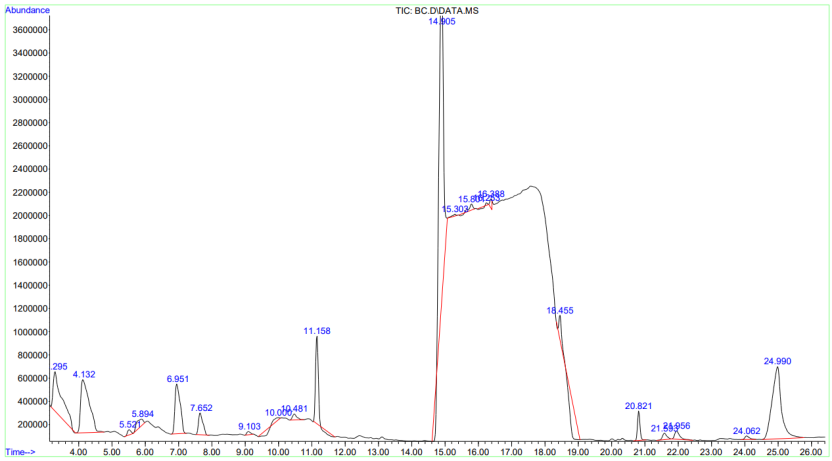


**Figure 1.** GC–MS chromatogram of white tea extract

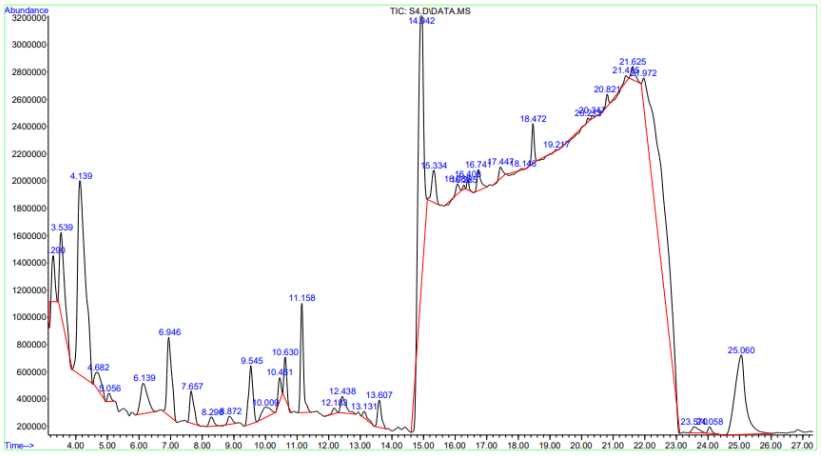


**Figure 2**. GC–MS chromatogram of black tea extract

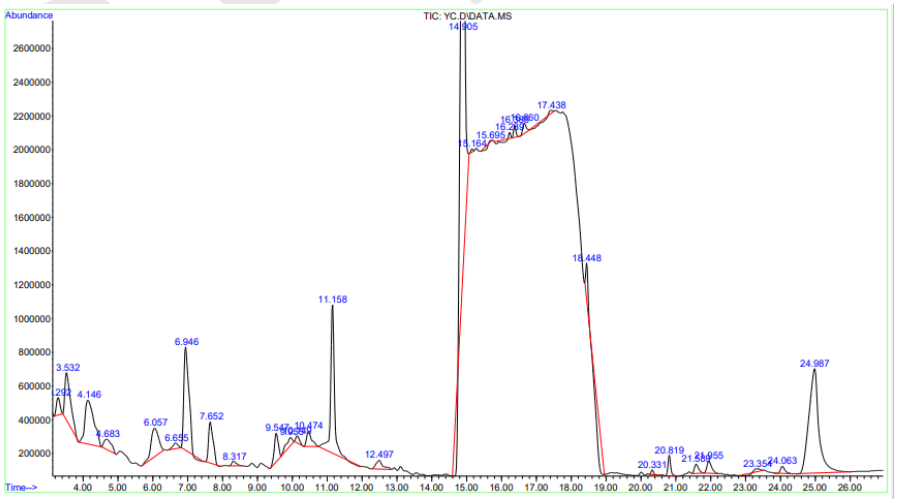


**Figure 3**. GC–MS chromatogram of green tea extract

**Table 1.** Phytocomponents identified in the extract of white, black and green tea by GC–MS

| No. | Rt | Name of the compound | White tea | Black tea | Green tea |
|---|---|---|---|---|---|
| 1. | 3.290 | 2-Ethyl-5-methylthiophene | | | 1.11 |
| 2. | 3.295 | 2-Methyl-tetrahydropyridin-4-one | 10.27 | 1.27 | |
| 3. | 3.534 | 4H-Pyran-4-one | | 4.92 | 4.96 |
| 4. | 4.122 | 4H-1,2,4-Triazole, 3-amino-4-ethyl-Phenol | 13.11 | | |
| 5. | 4.142 | N-(Cyanomethyl)-perhydroazepine | | 17.09 | |
| 6. | 4.142 | 3-Cyano-3-methyl-4-oxopentanamide | | | 7.01 |
| 7. | 4.689 | 3-methoxy-Benzenethiol | | 1.36 | 1.41 |
| 8. | 5.054 | Cyclohexane | | 0.30 | |
| 9. | 5.521 | 2-Methoxy-3,4,4-trimethylazetine | 0.48 | | |
| 10. | 5.886 | Glutamic acid | 1.39 | | |
| 11. | 6.129 | Pyrogallol | | 2.46 | 4.25 |
| 12. | 6.960 | Cyclododecane | 7.53 | 4.22 | 9.14 |
| 13. | 7.649 | 2,4-di-tert-butyl-Phenol | 2.71 | 1.54 | 3.32 |
| 14. | 8.319 | Pyrazole-5-carboxylic acid | | | 0.36 |
| 15. | 8.866 | 2-Ethyl-5-methylthiazole | | 0.39 | |
| 16. | 9.109 | 1-Naphthalenemethanamine | 0.32 | | |
| 17. | 9.555 | Lactone of 5-Acetyl-1,3,3,4,5-pentamethylbicyclo[2.1.0]pentan-2-one | | | 1.96 |
| 18. | 10.001 | Quinic acid | 1.19 | 0.23 | 2.71 |
| 19. | 10.002 | 2-Nonen-1-o | | 0.97 | |
| 20. | 10.468 | dihydro - coniferyl alcoho | | 0.72 | |
| 21. | 10.630 | Bicyclo[3.1.0]hex-2-ene | | 1.33 | |
| 22. | 11.157 | 2-Propenoic acid | 6.30 | 3.72 | 9.97 |
| 23. | 12.435 | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol | | 1.02 | |
| 24. | 12.495 | 2(4H)-Benzofuranone | | | 1.06 |
| 25. | 13.124 | trans-4-(3-Acetylaminopropyl)cyclohexanol | | 0.36 | |
| 26. | 13.611 | 1-(6-Methyl-2-pyrazinyl)-3-methyl-1-butanol | | 1.20 | |
| 27. | 14.908 | Caffeine | 37.42 | 42.31 | 31.03 |
| 28. | 20.828 | Methyl stearate | 2.04 | | 0.98 |
| 29. | 21.599 | 9-Octadecenamide | 0.78 | | 1.68 |
| 30. | 21.964 | Hexadecanamide | 1.19 | | |
| 31. | 23.363 | Propanedinitrile | | | 0.05 |
| 32. | 23.566 | 3-phenyl-2-methylindole | | 0.48 | |
| 33. | 24.052 | 2(1H)-Naphthalenone | 0.45 | 0.28 | |
| 34. | 24.072 | 3-Ethoxy-7-(2-propenyl)-2-cyclohepten-1-one | | | 0.53 |
| 35. | 24.985 | 9-Octadecenamide | 19.15 | 9.65 | 18.23 |

**b. TPC & TFC**

In this study, the total bioactive compounds of three different type of teas were investigated in terms of TPC and TFC with spectrophotometric method (Table 2). We found that TPC was highest in the green tea extract (185.98 mg GAE/g extract), it followed by white tea extract (136.20 mg GAE/g extract) while the black tea extract showed the least TPC (102.84 mg GAE/g extract). As for TFC, the order was as follows: black tea extract (88.23 mg QE/g extract) > green tea extract (31.19 mg QE/g extract) > white tea extract (13.35 mg QE/g extract).

**Table 2.** Total phenolic and flavonoid content of the white, black and green tea extracts*

| Extracts | Total phenolic content (mg GAE/g extract) | Total flavonoid tontent (mg QE/g extract) |
|---|---|---|
| White tea | 136.20 ± 4.16 | 13.35 ± 1.57 |
| Black tea | 102.84 ± 2.95 | 88.23 ± 6.51 |
| Green tea | 185.98 ± 0.48 | 31.19 ± 4.44 |

* means ± Standar Deviation (SD, n=3), GAE: Gallic acid equivalent; QE: quercetin equivalent. Different letter superscript in the same columns indicates significant differences between the extracts ($p \leq 0.05$)

### c. *In Vitro* Antioxidant Activity

In this study, the antioxidant potential of tea extracts was tested concerning their radical scavenging (DPPH and ABTS) activity (Figure 4-5). We found that the green tea extract was the most effective DPPH scavenger ($IC_{50}$: 959.4 µg/ml) and also ABTS scavenger ($IC_{50}$: 1.14 µg/ml), it may be attributed to the highest TPC detected in the green tea extract. In a study, the antioxidant activity and radical scavenging effects of different tea extracts was reported in the order of semifermented tea > nonfermentea tea > fermented tea (Yen & Chen, 1995). In another study, the antioxidant profile of different teas was determined in terms of ABTS, ORAC and LDL assay and found as the order of green tea > white tea > black tea (Carloni et al., 2013). Compared with these results, the results obtained in our study show compatibility.

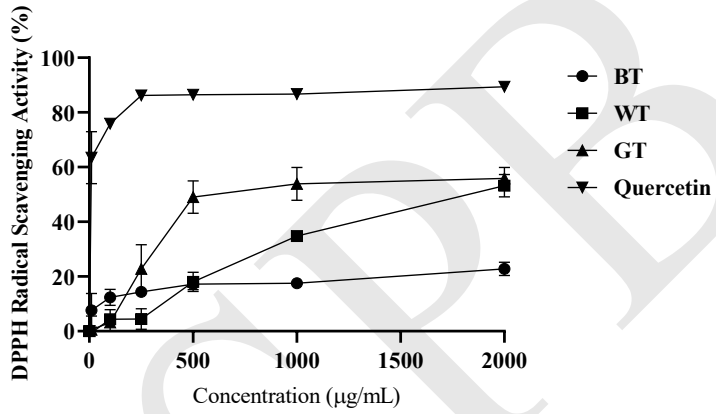


**Figure 4.** DPPH radical scavenging activity of tea extracts and standard compound quercetin

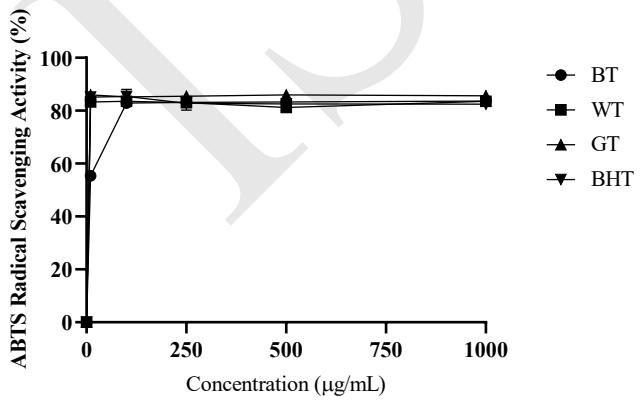


**Figure 5.** ABTS radical scavenging activity of tea extracts and standard compound BHT

**d. Enzyme inhibition activity**

Inhibition of enzymes responsible for the destruction of important substances that play a role in the pathology of some diseases has been the target mechanism in the treatment of some chronic diseases. Acetylcholine is an important neurotransmitter found at central and peripheral synapses, regulating learning and memory functions. but this substance is destroyed by acetylcholinesterase in the brain. For this reason, inhibition of the enzyme responsible for the degradation of acetylcholine is an important treatment target in the treatment of diseases such as Alzheimer's, which are caused by the decrease of this substance. Among the different tea extracts, it was found that the green tea was most effective in inhibiting AChE ($IC_{50}$: 760.9 µg/mL) and BChE ($IC_{50}$: 1501 µg/mL), respectively. It is important to note that the high anticholinesterase inhibitory activity was observed in green tea extract, it was also found as the most abundant with phenolic content, this could be explained by the relationship between phenolic compounds and anticholinesterase activity. The methanol extract of tea pericarp showed highest AChE inhibitory activity than seed extract with the $IC_{50}$ value of 336.88±5.52 µg/mL(Jo et al., 2012).

Tyrosinase enzyme is the most important limiting enzyme in melaogenesis. The first step during this production is the hydroxylation of L tyrosine to DOPA. The enzyme tyrosinase plays an important role in the production of melanin, which is responsible for skin color, and therefore inhibitors of this enzyme are used in cosmetic preparations due to its skin whitening activity. As for tyrosinase, the white tea and green tea extract exhibited weak inhibitory effect while black tea has no effect. In a study, ten kinds of green tea were screened for their tyrosinase inhibitory activity, and epicatechin gallat, gallocatechin gallat and epigallocatechin gallate were identified as the major active constituents in the tea(No et al., 1999). It has been reported that the methanol extract of Camelia sinensis pericarp showed tyrosinase inhibitory activity with $IC_{50}$ value of 735.58 µg/mL(Jo et al., 2012).

Diabetes is a metabolic disease characterized by high blood glucose levels. Amylase and glucosidase enzymes break down the polysaccharides ingested into glucose. For this reason, these two enzyme inhibitors are used in the treatment of diabetes in order to prevent the sugar spike that occurs after a meal. As for the enzyme that related with diabetes mellitus, black tea was most effective against the glucosidase ($IC_{50}$: 1501 µg/mL), while white tea extract was most active against amylase ($IC_{50}$: 776.1 µg/mL) (Table 3). In a previous study, it was reported that the green tea and black tea were showed α-glucosidase inhibitory activity with $IC_{50}$ values of 2.82 ± 0.23 and 2.25 ± 0.06 µg/mL, respectively (Yang & Kong, 2016). Compared to our results, their activity was found to be high, which may be due to the difference in chemical content of the tea extract we used in our study, due to the extract preparation method.

**Table 3.** Enzyme inhibitory effects of tea extracts*

| Extracts/ reference drug | AChE | BChE | Tyrosinase | Amylase | Glucosidase |
|---|---|---|---|---|---|
| **White tea** | 1031 ± 1.99 | 5150 ± 1.34 | 67684 ± 0.11$_a$ | 776.1 ± 0.91$_c$ | 1094 ± 1.49$_{ab}$ |
| **Black tea** | 902.2 ± 0.97 | 11437 ± 0.66 | N.A. | 708.6 ± 0.87$_{ab}$ | 248.2 ± 2.03$_a$ |
| **Green tea** | 760.6 ± 1.81 | 1501 ± 0.89 | 18605 ± 0.15$_b$ | 1574 ± 0.50$_{ac}$ | 609.3 ± 1.85$_b$ |
| **Galanthamine** | 24.40 ± 0.69 | 22.20 ± 1.27 | - | - | - |
| **Kojic acid** | - | - | 51.07 ± 0.39$_{ab}$ | - | - |
| **Acarbose** | - | - | - | 215.9 ± 2.01$_{bc}$ | 866.0 ± 0.98$_{ab}$ |

* means ± Standar Deviation (SD, n=3). Different letter superscript in the same columns indicates significant differences between the extracts ($p \leq 0.05$)

## 4. Conclusion

When the results obtained are evaluated, it can be said that tea has strong antioxidant and enzyme inhibition activity at different levels. As can be seen from the enzyme inhibitory results, black tea and green tea have a stronger inhibition on the glucosidase enzyme, which is the target mechanism in the treatment of diabetes, than the reference substance acarbose. Therefore, we can summarize that the tea we drink not only prevents the rise in sugar after meals, but also helps to protect health for our body due to its potent antioxidant activity.

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**Conflict of Interest**

I have no conflict of interest to disclose.

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FULL PAPER – ORAL PRESENTATION

# PREPARATION AND CHARACTERIZATION OF COMBINED DRUG CONTAINING TOPICAL NANOEMULGELS FOR SKIN DISEASES: A PRELIMINARY STUDY

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## Abstract

Today, there is a severe increase in skin diseases. Among the reasons that cause this increase, environmental factors and malnutrition types/resources are common. Along with the increase in skin diseases, new ways of treatment and new dosage forms continue to be sought. In recent years, nanoemulsions, one of the new generation nano-sized drug delivery systems, have attracted much attention. Nanoemulsions are oil-in-water (O/W) or water-in-oil (W/O) dispersions of two immiscible liquids stabilized using a suitable surfactant. Nanoemulsions have the potential to overcome many disadvantages of conventional drug formulations. Nanoemulgels are emulsion-based topical gel formulations in which nano-sized emulsion droplets can be prepared with the help of high-energy or low-energy methods and converted into nanoemulsion by adding a suitable gelling agent. The aim of this study is to prepare and characterize nanoemulgel formulations containing salicylic acid and povidone-iodine in combination. Combined drug containing nanoemulgels have been successfully prepared. Some characterization studies have been carried out on these nanoemulgels. However, additional characterization studies will be done in the future. In this study, salicylic acid and povidone iodine were combined for the first time. Combining the therapeutic properties of both salicylic acid and povidone-iodine would provide many advantages for the treatment of many skin diseases. Nanoemulgels containing this drug combination can be developed further and used in the treatment of skin diseases.
**Key Words**: Nanoemulsion, nanoemulgel, salicylic acid, povidone-iodine, characterization.

## 1. Introduction

Today, there is a severe increase in skin diseases. Among the reasons that cause this increase, environmental factors and malnutrition types/resources are common. Along with the increase in skin diseases, new ways of treatment and new dosage forms continue to be sought. In recent years, nanoemulsions, one of the new generation nano-sized drug delivery systems, have attracted much attention.

Nanoemulsions are O/W or W/O dispersions of two immiscible liquids stabilized using a suitable surfactant. Nanoemulsions can typically be formed with less surfactant than other colloidal dispersions and have more excellent kinetic stability properties than coarse emulsions. Nanoemulsions can be made into various dosage forms, such as liquids, creams, sprays, gels, aerosols, and foams, and administered by oral, intravenous, intranasal, pulmonary, ocular, and topical routes.[1] Nanoemulsions

have the potential to overcome many disadvantages of conventional drug formulations. Loading poorly water-soluble drugs into nanoemulsions increases their wettability and/or solubility, improving their pharmacokinetics and pharmacodynamics by different routes of administration. The nanoemulsion droplets act as a drug reservoir, making the nanoemulsions a multifunctional platform for treating various diseases. The advantages of nanoemulsions such as optimum drug release, long-term efficacy, drug intake control, low side effects, and drug protection from enzymatic or oxidative processes have been reported in recent years.[2, 3]

Nanoemulgels are emulsion-based topical gel formulations in which nano-sized emulsion droplets can be prepared with the help of high-energy or low-energy methods and converted into nanoemulsion by adding a suitable gelling agent. Nanoemulgels are composed of various polymers, surfactants, and oily substances of natural, synthetic, and semi-synthetic nature, and the droplet sizes range from 5 to 500 nm. Because nanoemulsions contain both nanoemulsion and gel base (dual characters), they are among the suitable options as drug delivery systems. The nanoemulsion component of the nanoemulgel protects the active substance from enzymatic degradation and reactions such as hydrolysis, and the gel base provides thermodynamic stability to the emulsion by increasing the viscosity of the aqueous phase by reducing the interface and surface tension.[4, 5]

In this study, salicylic acid and povidone-iodine were used as active ingredients. Salicylic acid is a natural ingredient derived from the bark of the willow tree (Salix alba). It has been used worldwide for centuries for its analgesic, antipyretic and anti-inflammatory properties. Salicylic acid is highly irritating to the gastric mucosa when taken orally, and therefore topical use is preferred. The absorption of salicylic acid in the topical application is variable. The systemic effects of salicylic acid in topical applications are minimal when applied in low to moderate doses to intact skin. However, if there is deterioration in the structure of the stratum corneum, measurable levels of salicylic acid may be present in the body. Salicylic acid can be used topically as a keratolytic, bacteriostatic, fungicide, and photoprotective. Today, it is frequently used to treat warts, calluses, localized hyperkeratosis, plaque psoriasis, actinic keratosis, ichthyosis, and comedonal acne.[6] Povidone-iodine is a complex formed with iodine with antiseptic properties and povidone, a synthetic carrier polymer that does not have microbicidal activity. In an aqueous medium, free iodine is released from the povidone-iodine complex into the solution. The antiseptic activity increases, and iodine release continues until an equilibrium is established.[7] Povidone-iodine is also a broad-spectrum antiviral agent against enveloped and non-enveloped viruses such as adenovirus, rotavirus, rhinovirus, human immunodeficiency virus, herpes virus, and measles, polio, rubella, measles, and influenza viruses.[8] The aim of this study is to prepare and characterize nanoemulgel formulations containing salicylic acid and povidone iodine in combination.

## 2. Material and Methods

### a. Materials

Salicylic acid, povidone-iodine, and linseed oil were purchased from Riedel-de-Haën, BASF, and Sigma, respectively. Ethanol, Tween, and Span were purchased from Merck. HPMC E15 was kindly received as a gift from Santa Farma İlaç A.Ş.

### b. Solubility of Salicylic Acid in Different Oils

Concentrated suspensions of salicylic acid in different oils were prepared and stirred for 72 hours on a magnetic stirrer at room temperature. Afterward, the samples were centrifuged, the supernatants were diluted at certain ratios, and the amounts of dissolved salicylic acid were determined by the validated UV-VIS spectrophotometric method.

### c. Preparation of Salicylic Acid Nanoemulsions

Nanoemulsions containing salicylic acid were prepared by the sonication method. First, salicylic acid was dissolved in oil, and appropriate surfactants were added and sonicated. After homogenization,

pure water was added, and nanoemulsions were formed.

**d. Preparation of Nanoemulgels with Povidone-Iodine**

HPMC E15 polymer was swollen in distilled water, and a specific concentration of povidone-iodine was added. Then, nanoemulsions containing salicylic acid were added to these prepared gel bases and mixed until homogeneous.

**e. Determination of Droplet Size Distribution Nanoemulsions**

Droplet size distribution, zeta potential, polydispersity index, and conductivity of nanoemulsions were determined with the zetasizer device in DAYTAM.

**f. Type Determination of Nanoemulsions**

Type determination of nanoemulsions was made according to the dilution method. Nanoemulsions were diluted with distilled water at a ratio of 1:9, and a homogeneous mixture was obtained.

**g. pH Determination of Nanoemulgels**

The pH of the nanoemulgels was measured with a pH meter.

**h. FT-IR Analysis of Nanoemulgels**

FT-IR spectra were taken to evaluate whether there is an interaction between the active ingredients and the excipients that make up the nanoemulgels.

## 3. Results and Discussion

**a. Solubility of Salicylic Acid in Different Oils**

The solubility study results are given in Table 2 below. As a result of the study, the highest solubility value was found in linseed oil. For this reason, linseed oil was used as the oil phase in the preparation of emulsions.

**Table 2.** Solubility results of salicylic acid (n=3, mean±standard deviation).

| **Oil** | Sesame oil | Olive oil | Linseed oil | Sunflower oil | Mineral oil |
|---|---|---|---|---|---|
| **Solubility (mg/mL)** | 10.62±0.36 | 10.65±1.30 | 13.66±0.66 | 11.52±0.85 | 0.56±0.03 |

**b. Preparation of Salicylic Acid Nanoemulsions**

Many modifications have been made while preparing nanoemulsions. The ratios of the formulation components are given in the Table 3 below. In addition, the optical microscope images of the prepared nanoemulsions are given in Figure 2 below. As can be seen from the images, nanoemulsions with very homogeneous size distribution have been successfully prepared by the ultrasonication method.

**Table 3.** Formulation components of nanoemulsions (mg).

| **Formulation Code** | **Salicylic Acid** | **Linseed Oil** | **Span 80** | **Tween 20** |
|---|---|---|---|---|
| **E1** | 50 | 1000 | 200 | 100 |
| **E2** | 50 | 1000 | 150 | 150 |
| **E3** | 50 | 1000 | 100 | 200 |

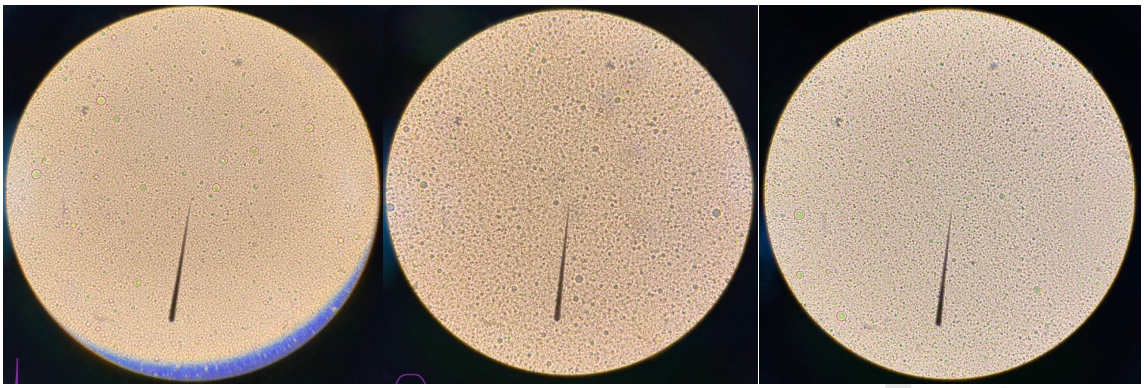

**Figure 2.** The optical microscope images of the nanoemulsions (left: E1, middle: E2, right: E3, 100x).

### c. Preparation of Nanoemulgels with Povidone-Iodine

Nanoemulgels containing both salicylic acid (50 mg) and povidone-iodine (100 mg) combined have been successfully prepared. The images of the prepared nanoemulgels are given in Figure 3 below.

**Figure 3.** The images of combined drug containing nanoemulgels (left: F1, middle: F2, right: F3).

### d. Determination of Droplet Size Distribution Nanoemulsions

The droplet sizes, zeta potentials, polydispersity indexes, and conductivity results of the prepared nanoemulsions are given in Table 4. Relatively low dimensions were obtained. The low polydispersity index indicates that the size distribution is in a narrow range. This result was also found to be compatible with optical microscope images. The high electrical conductivity values indicate that the outer phase of the prepared nanoemulsions is water.

**Table 4.** The droplet sizes, zeta potentials, polydispersity indexes, and conductivity results of nanoemulsions (mean±standard deviation).

| Formulation Code | Droplet Size (nm) | Zeta Potential (mV) | Polydispersity Index | Conductivity (mS/cm) |
|---|---|---|---|---|
| **E1** | 269.9±2.066 | -11.7±4.04 | 0.199±0.016 | 0.333±0 |
| **E2** | 308.2±1.044 | -17.5±5.47 | 0.207±0.005 | 0.283±0.002 |
| **E3** | 295.2±1.65 | -23.5±3.08 | 0.228±0.006 | 0.245±0.002 |

### e. Type Determination of Nanoemulsions

The images obtained by diluting the prepared nanoemulsions with water are given in Figure 4. The fact that they are immediately miscible with water shows that their outer phase is water. This result was also compatible with the electrical conductivity results.

**Figure 4.** The images obtained by diluting the nanoemulsions with water (left: E1, middle: E2, right: E3).

### f. pH Determination of Nanoemulsions and Nanoemulgels

The pH measurement results of the prepared nanoemulsions and nanoemulgels are given in Table 5. When the results are examined, it is seen that the pH's of both nanoemulsions and nanoemulgels are acidic. In addition, it was observed that the pH decreased more by gelling the nanoemulsions.

**Table 5.** The pH measurement results of the nanoemulsions and nanoemulgels.

| Formulation Code | pH | Formulation Code | pH |
|---|---|---|---|
| **E1** | 2.80 | **F1** | 2.14 |
| **E2** | 2.78 | **F2** | 2.01 |
| **E3** | 2.76 | **F3** | 2.12 |

### g. FT-IR Analysis of Nanoemulgels

The FT-IR spectra of the active substances, the nanoemulgels prepared and all the excipients used in the nanoemulgels are given in Figure 5 below. When the results are examined, it is seen that the active substances and excipients in the formulations do not interact, and there is no change in the spectrum of the active substances.

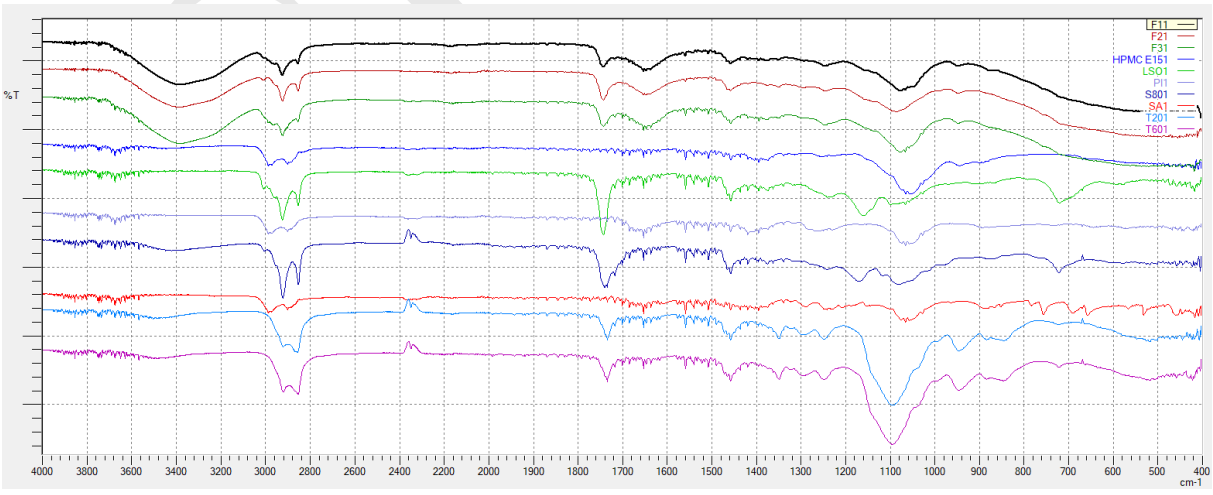


**Figure 5.** The FT-IR spectra of the active substances, the nanoemulgels and all the excipients used in the nanoemulgels.

## 4. Conclusion

Combined drug containing nanoemulgels have been successfully prepared. Some characterization studies have been carried out on these nanoemulgels. However, additional characterization studies will be done in the future. In this study, salicylic acid and povidone iodine were combined for the first time. Nanoemulgels containing this drug combination can be developed further and used in the treatment of skin diseases. Combining the therapeutic properties of both salicylic acid and povidone-iodine would provide many advantages for the treatment of many skin diseases.

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**Conflict of Interest**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this presentation.

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