

## ABSTRACTS

# ROLE OF NATURAL ANTIOXIDANTS (VITAMIN C, E, AND POLYPHENOLS) IN SKIN AGING PREVENTION

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### ABSTRACT

**INTRODUCTION:** Skin aging is a complex biological process influenced by intrinsic factors, such as chronological aging, and extrinsic factors, notably ultraviolet (UV) radiation. Excessive reactive oxygen species (ROS) generated by environmental and metabolic stress lead to oxidative damage, collagen degradation, elastin fragmentation, and cellular senescence, resulting in wrinkles, loss of elasticity, and pigmentation changes. Natural antioxidants, including vitamin C, vitamin E, and polyphenols, have been shown to counteract oxidative stress and maintain skin integrity, but their combined preventive potential remains under-explored.

**AIM:** The aim of this work is to evaluate the role of vitamin C, vitamin E, and polyphenols as natural antioxidants in preventing skin aging and promoting skin health.

**MATERIALS AND METHODS:** This review summarizes findings from experimental and clinical studies examining the effects of vitamin C, vitamin E, and polyphenols on skin aging. Data were collected from peer-reviewed journals, including dermatology, pharmacology, and nutrition studies, focusing on antioxidant activity, collagen synthesis, inhibition of matrix metalloproteinases (MMPs), and photoprotection. Mechanistic pathways and translational applications were analyzed qualitatively.

**RESULTS AND CONCLUSION:** Vitamin C neutralizes ROS, supports collagen synthesis, and regenerates oxidized vitamin E. Vitamin E protects membrane lipids and synergizes with vitamin C to reduce UV-induced damage. Polyphenols exhibit antioxidant and anti-inflammatory effects and inhibit MMP-mediated collagen breakdown. Combined administration—topically or via diet—enhances skin elasticity, reduces oxidative damage, and prevents photoaging. These findings highlight the preventive and translational potential of natural antioxidants in dermatology and aesthetic medicine. Further research is recommended to optimize formulations and delivery systems for maximal efficacy.

**Keywords:** *skin aging, antioxidants, vitamin C, vitamin E, polyphenols, photoaging*

# MOLECULAR AND CLINICOBIOLOGICAL INSIGHTS FROM JAK2 V617F QUANTIFICATION BY DIGITAL DROPLET PCR IN BULGARIAN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS

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## ABSTRACT

**INTRODUCTION:** The *JAK2 V617F* mutation is the most common molecular driver in Philadelphia chromosome-negative myeloproliferative neoplasms (MPN), contributing to disease pathogenesis, progression and thrombotic risk. Quantitative evaluation of allele burden has emerging value for diagnosis, risk stratification and monitoring under therapy. Digital droplet PCR (ddPCR) offers high analytical sensitivity and precision for mutant allele quantification.

**AIM:** The aim of this work is to determine the *JAK2 V617F* variant allele fraction (VAF) using ddPCR in a real-world Bulgarian MPN cohort and to analyze correlations with hematological parameters and diagnosis.

**Materials and Methods:** This retrospective analysis included 479 unique patients tested between 2022 and 2025. DNA was extracted from whole blood and *JAK2 V617F* was quantified using ddPCR (Bio-Rad). Variant allele fraction (%) was calculated as mutant copies/total allele copies ×100. Clinical data were collected, including hemoglobin, leukocytes, platelets and diagnosis. Pearson correlation coefficients were used for clinicobiological associations.

**RESULTS AND CONCLUSION:** A total of 129/479 patients (26.9%) were *JAK2*-positive (VAF > 0.1%). Mean VAF values were highest in primary myelofibrosis (0.36%), followed by polycythemia vera (0.25%), MPN-NOS (0.23%), and essential thrombocythemia (0.07%). *JAK2* VAF correlated positively with leukocyte count ( $r = 0.50$ ) and negatively with hemoglobin ( $r = -0.22$ ) and platelets ( $r = -0.30$ ), consistent with published biological behavior of mutant clones. These findings support the clinical relevance of quantitative *JAK2* analysis and confirm ddPCR as a sensitive platform suitable for future longitudinal molecular monitoring and therapeutic response assessment.

**Keywords:** *JAK2 V617F*, ddPCR, myeloproliferative neoplasms, allele burden, molecular diagnostics, hematology

**Funding:** This research was financed by the Research, Innovation and Digitalisation for Smart Transformation program 2021–2027, funded by the European Union, Project BG16RFPR002-1.014-0007 Center for Competence PERIMED-2.

# CARDIOPROTECTIVE EFFECTS OF DIETARY POLYPHENOLS: MOLECULAR MECHANISMS AND PREVENTIVE POTENTIAL

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## ABSTRACT

**INTRODUCTION:** Cardiovascular diseases (CVDs) remain the leading cause of morbidity and mortality worldwide, largely driven by oxidative stress, inflammation, and endothelial dysfunction. Dietary polyphenols, naturally occurring compounds found in fruits, vegetables, tea, and red wine, have gained increasing attention for their potential cardioprotective properties. These bioactive molecules exhibit antioxidant and anti-inflammatory activities that may counteract the molecular mechanisms underlying cardiovascular disease progression.

**AIM:** The aim of this work is to evaluate the cardioprotective effects of dietary polyphenols, focusing on their molecular mechanisms, clinical evidence, and preventive potential in cardiovascular health.

**MATERIALS AND METHODS:** This review summarizes data from experimental, epidemiological, and clinical studies examining the effects of polyphenol-rich diets on cardiovascular function. Literature was collected from peer-reviewed journals in cardiology, nutrition, and pharmacology. Key parameters analyzed included oxidative stress modulation, endothelial function, lipid metabolism, and inflammatory pathway regulation.

**RESULTS AND CONCLUSION:** Dietary polyphenols demonstrate significant cardioprotective effects by scavenging reactive oxygen species (ROS), enhancing endothelial nitric oxide production, and inhibiting lipid peroxidation. They improve vascular function, reduce LDL oxidation, and lower blood pressure, thereby contributing to the prevention of atherosclerosis and other cardiovascular disorders. These findings highlight the translational potential of dietary polyphenols as accessible, natural agents in preventive cardiovascular medicine. Future studies should explore bioavailability optimization and long-term clinical efficacy.

**Keywords:** *polyphenols, cardiovascular health, antioxidants, endothelial function, oxidative stress, preventive medicine*

# EFFECT OF A NUTRACEUTICAL SUPPLEMENT ON SERUM ARACHIDONIC ACID AND OXIDATIVE STRESS MARKERS IN RELATION TO LIFESTYLE HABITS

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## ABSTRACT

**INTRODUCTION:** Lifestyle factors are a major cause of stroke, making prevention essential. A key stroke mechanism is oxidative stress, which, due to the brain's high metabolism, results in lipid peroxidation. This process releases arachidonic acid (AA) from neural membranes, worsening damage. As a supplement, citicoline is used to protect these phospholipids from such harm.

**AIM:** The aim of this work is to investigate citicoline and plant extract supplement's effect on serum AA, malondialdehyde (MDA), and total thiols (TT) in healthy volunteers, in relation to lifestyle habits.

**MATERIALS AND METHODS:** In a six-month, single-arm study, 43 healthy volunteers (aged 40–65) received a daily supplement (Cytodeox™). Serum AA levels (UPLC-MS), MDA, and TT (colourimetric analysis) were evaluated pre- and post-intervention. Lifestyle factors (smoking, BMI, physical activity) were assessed through questionnaires for subgroup analysis.

**RESULTS AND CONCLUSION:** Post-intervention, overall serum AA and MDA levels significantly decreased. Subgroup analysis revealed that this beneficial effect was absent in smokers. Supplementation induced a significant negative correlation between the change (delta) in AA and delta TT ( $r = -0.3176$ ,  $p < 0.05$ ). This relationship, an indicator of systemic stress, was significant only in groups with high baseline oxidative stress (smokers, BMI > 25, physically active). This suggests that low-stress individuals (non-smokers, normal weight) have sufficient antioxidant buffering capacity to obscure this interaction.

We conclude that the supplement's efficacy and its interaction with thiol homeostasis are primarily influenced by lifestyle.

**Keywords:** *arachidonic acid, citicoline, plant extracts, oxidative stress, MDA, total thiols, lifestyle factors*

**Acknowledgement/Funding:** *This study was supported by the European Union-NextGenerationEU, project No BGRRP-2.004-0009-C02 and by the Medical University of Varna, Science Fund, Project No. 21015.*

## UNDERSTANDING THE BIOCHEMICAL BASIS OF LAZINESS

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### ABSTRACT

**INTRODUCTION:** Laziness is considered a biochemical disorder as a result of comfort, lack of activity, brain, hormonal imbalances, and genetic prepositions, which negatively affects our quality of life.

**AIM:** The aim of this work to review the current understanding of laziness at a molecular level, its negative effects on the human health, and its possible control.

**MATERIALS AND METHODS:** Publications from peer-reviewed journals, indexed in ScienceDirect, PubMed, Taylor and Francis, Springer, and Frontiers, were analyzed.

**RESULTS AND CONCLUSION:** Laziness affects the prefrontal cortex in the brain, which is responsible for planning, problem-solving, and emotions. While the role of serotonin in laziness is dual, low thyroid, sex hormones, GABA, dopamine and high cortisol levels from chronic stress can cause fatigue, reduced ability to motivate, focus, and make decisions. A role in laziness is played by deficiencies in vitamins B<sub>12</sub>, D, and C, Fe, Mg, and Zn, because they are important for the energy production, erythrocyte function in the oxygen transport and muscle contraction. Poor sleep, diet rich in refined carbohydrates, white bread, saturated fats lead to lethargy and oxidative stress that damages mitochondria, resulting in fatigue. Laziness can result in obesity, cardiovascular disease, hypertension, diabetes, cancer, immune, bone and muscle disorders, depression, and anxiety. Enough sleep to recover the dopamine receptors, balanced diet with vitamins and essential amino acids as precursors of hormones, outdoor physical activities to increase serotonin and dopamine, and self-motivation with small achievements are examples of approaches to control laziness.

**Keywords:** *laziness, lack of motivation, mood-regulating hormones, stress hormones, brain metabolism, mitochondrial dysfunction*

**Acknowledgement/Funding:** *Academic Flight Program, the Student Council at the Prof. Dr Assen Zlatarov State University of Burgas.*

# OMEGA-3 FATTY ACIDS IN BRAIN AGING AND NEUROINFLAMMATION

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## ABSTRACT

**INTRODUCTION:** Brain aging is associated with cognitive decline, neuroinflammation, and oxidative stress, which contribute to neurodegenerative diseases such as Alzheimer's and Parkinson's. Among dietary factors influencing brain health, omega-3 polyunsaturated fatty acids (PUFAs), particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have emerged as essential nutrients for neuronal function and protection. These fatty acids play critical roles in maintaining membrane integrity, modulating inflammatory responses, and supporting synaptic plasticity.

**AIM:** The aim of this work is to evaluate the role of omega-3 fatty acids in preventing neuroinflammation and age-related cognitive decline, highlighting their mechanisms of action and translational potential in preventive neurology.

**MATERIALS AND METHODS:** This review summarizes experimental, clinical, and epidemiological data investigating the neuroprotective effects of omega-3 fatty acids. Literature was sourced from peer-reviewed journals in neuroscience, nutrition, and pharmacology, focusing on molecular pathways, inflammatory mediators, and cognitive outcomes.

**RESULTS AND CONCLUSION:** Omega-3 fatty acids reduce neuroinflammation by downregulating pro-inflammatory cytokines (IL-1 $\beta$ , TNF- $\alpha$ ) and inhibiting microglial overactivation. They enhance neuronal survival through antioxidant effects, improved mitochondrial function, and increased production of specialized pro-resolving mediators (SPMs). Clinical studies associate higher omega-3 intake with slower cognitive decline and reduced risk of neurodegenerative disorders. These findings underscore the preventive potential of omega-3 fatty acids in promoting healthy brain aging and reducing inflammation-related neurodegeneration.

**Keywords:** *omega-3 fatty acids, DHA, EPA, neuroinflammation, brain aging, preventive neurology*

# STUDY OF THE MECHANISM OF MOLECULAR ACTION OF SYNTHETIC ANGIOTENSIN AND DERIVATIVES ON MODEL MEMBRANE SYSTEMS

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## ABSTRACT

**INTRODUCTION:** Angiotensin converting enzyme 2 (ACE2) is the main membrane receptor for the entry of the SARS-CoV-2 virus into cells. ACE2 converts the octapeptide angiotensin II (Ang II) into the heptapeptide angiotensin 1-7 (Ang 1-7). Treatment with analogs of Ang 1-7 would compensate for its deficiency during the virus induced ACE2 blockade, and would restore the system's balance both peripherally and at the central nervous system level.

**AIM:** The interaction between Ang 1-7 and derivatives, and model membranes, composed of palmitoyl-oleoyl phosphatidylcholine (POPC), a component of the lipid rafts, in which the peptides receptor is located, was studied. This study is a first step towards elucidating the mechanisms of peptides action, as the membrane lipid composition is a key factor for acquiring the appropriate peptide conformation.

**MATERIALS AND METHODS:** The interaction between synthetic Ang 1-7 and derivatives and POPC monolayers, before and after the addition of the peptides, was analyzed by Langmuir monolayer technique, and Brewster angle microscopy (BAM).

**RESULTS AND CONCLUSION:** The results obtained showed a shift of the  $\pi/A$ -isotherms towards a larger area per lipid molecule, indicating the penetration of the peptides into the monolayers, especially for the amidated analogs. These results were confirmed by the compression moduli, calculated from the isotherms, which exhibited higher maximum values after addition of the peptides, corresponding to a reduced elasticity of the monolayers. In addition, BAM images showed a densification of the lipid monolayers in presence of the peptides with an appearance of aggregates, which was more pronounced for Ang 1-7 analogs.

**Keywords:** *angiotensin 1-7 and derivatives, lipid rafts, Langmuir monolayer technique, Brewster angle microscopy*

**Acknowledgement/Funding:** *This study is financed by the Scientific Research Fund of the Ministry of Education and Science, Project No. KII-06-H71/9/08.12.2023, and by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, Project No. BG-RRP-2.004-0008-C01.*

## VOLATILE PROFILE OF *AGRIMONIA EUPATORIA* ESSENTIAL OIL AND INFUSION

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### ABSTRACT

**INTRODUCTION:** *Agrimonia eupatoria* L. is a perennial herb widely used in European folk medicine for treating diarrhea, skin and oral inflammations, liver or gallbladder disorders, etc. The aerial parts are applied externally (gargles, compresses, baths) and internally (tea). Its bioactive compounds, including phenolic acids, flavonoids, tannins, triterpenoids, and vitamins, exhibit antioxidant, anti-inflammatory, antimicrobial, and other therapeutic effects, while data on its volatile constituents remain limited.

**AIM:** The aim of this study is to determine the phytochemical composition of essential oil (EO) isolated from the dried aerial parts and infusion of *Agrimonia eupatoria*.

**MATERIALS AND METHODS:** The plant material was purchased from a retail outlet and collected during the flowering period in July–August from the Eastern Rhodopes region. Essential oil was obtained by steam distillation. To quantitatively separate EOs from floral waters, liquid-liquid extraction using pentane as an organic solvent was employed. Pentane was used to isolate and analyze the volatile constituents contained in the specially prepared tea. Samples were taken from the final solutions for qualitative and quantitative analyses using GC–MS and GC–FID.

**RESULTS AND CONCLUSION:** The results contribute to our understanding of the phytochemical profile of the plant. *Agrimonia eupatoria* EO mainly contained mono- and sesquiterpenes, aldehydes, ketones, and terpene alcohols. In contrast, herbal infusion showed lower concentrations of these compounds due to volatility, with linalool being dominant. Linalool and phytol, the key bioactive components in tea and oil respectively, share anti-inflammatory, antioxidant, and antimicrobial properties that may explain the herb's beneficial effects.

**Keywords:** *Agrimonia eupatoria* L., folk medicine, essential oil, tea, aerial parts, common agrimony

**Funding:** This study is financed by The European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, Project № BG-RRP-2.004-0009-C02

## COULD ADIPOKINES BE THE ANSWER FOR OBESITY-ASSOCIATED COLORECTAL CANCER?

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### ABSTRACT

**INTRODUCTION:** The global prevalence of obesity and overweight is increasing significantly and possesses a serious health challenge. Epidemiological studies reported in the past decade have shown that obesity is a risk factor for numerous malignant diseases, including colorectal cancer (CRC). The precise molecular mechanisms linking abundant adipose tissue to CRC are complex and still under investigation. The imbalance in the production of hormones secreted by adipocytes could represent one of the major links underlying the association between obesity and CRC.

**AIM:** The main goal of this review was to synthesize current knowledge on the role of adipokine dysregulation in the initiation and progression of obesity-associated CRC.

**MATERIALS AND METHODS:** A literature review was performed in PubMed, Scopus, and Web of Science for studies (January 2005–September 2025) investigating the association between obesity and CRC. Search terms included “obesity,” “adipokines,” “adipokine signalling,” and “colorectal cancer.” Studies were selected based on their focus on adipokine signaling pathways, *in vitro* and *in vivo* models of CRC, epidemiological data linking adipokines to CRC risk, and their relevance to the obesity-cancer axis.

**RESULTS AND CONCLUSION:** The obesity-driven dysregulation of adipokine production provides a favorable microenvironment for colorectal carcinogenesis. The activation of multiple oncogenic signaling pathways like JAK/STAT, AMPK, PI3K/Akt, NF- $\kappa$ B, etc. is induced by imbalance in adipokine secretion. Elevated levels of pro-inflammatory adipokines (leptin, resistin, visfatin, omentin) and reduced levels of adiponectin promote tumorigenesis. Further studies into adipokine modulation on CRC progression could provide better patient stratification and improve therapeutic strategies.

**Keywords:** *obesity, colorectal cancer, adipokines, adipokine signaling*

**Acknowledgement:** *This study is financed by the European Union–NextGenerationEU through the National Recovery and Resilience Plan of the Republic of Bulgaria, Project № BG-RRP-2.004-0009-C02, Research group 3.1.1 Natura4Health. In addition, resources from the Science Fund, Medical University of Varna, Bulgaria, grant number 24011, have been used.*

# EFFECT OF A 15-MONTH ANTI-TNF- $\alpha$ THERAPY COMBINED WITH METHOTREXATE ON PLASMA LEVELS OF VISFATIN IN WOMEN WITH RHEUMATOID ARTHRITIS—PRELIMINARY STUDIES

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## ABSTRACT

**INTRODUCTION:** Rheumatoid arthritis (RA) is a chronic autoimmune disease, characterized by joint inflammation, swelling, progressive cartilage destruction, bone erosion, and increased systemic complications. Visfatin is a pro-inflammatory adipocytokine that plays an important role in RA. Some studies indicate that visfatin levels are elevated in RA and may be associated with the degree of inflammation, clinical disease activity, and radiographic joint damage. However, these results are inconsistent.

**AIM:** The aim of this study was to evaluate the association between plasma visfatin levels and disease activity in RA, and to assess the effect of a 15-month anti-tumor necrosis factor  $\alpha$  (anti-TNF- $\alpha$ ) therapy in combination with methotrexate (MTX) on the visfatin levels.

**MATERIALS AND METHODS:** Thirty-one RA patients treated with either etanercept, adalimumab or certolizumab pegol in combination with MTX were included in this study. The clinical response to the therapy was evaluated by a 28-joint disease activity score (DAS28). Plasma levels of visfatin were evaluated using immunoassay kit at baseline and 15 months after the start of anti-TNF- $\alpha$  therapy.

**RESULTS AND CONCLUSIONS:** Baseline visfatin levels were higher in women with RA compared with healthy subjects ( $2.39 \pm 0.63$  vs.  $1.12 \pm 0.46$  ng/mL,  $p < 0.001$ ). After 15-month anti-TNF- $\alpha$  therapy, visfatin levels were significantly reduced compared with the baseline values ( $2.39 \pm 0.63$  vs.  $1.97 \pm 0.21$  ng/mL,  $p < 0.01$ ). Baseline visfatin levels correlated with patients' age ( $r = 0.743$ ;  $p < 0.001$ ), swollen joint count ( $r = 0.593$ ;  $p < 0.01$ ), and DAS28 ( $r = 0.589$ ;  $p < 0.01$ ). In conclusion, this study demonstrates an association between an elevated visfatin levels and disease activity in RA. The beneficial effect of long-term anti-TNF- $\alpha$  therapy may, at least in part, be mediated by decreases in plasma visfatin levels.

**Keywords:** Rheumatoid arthritis, visfatin, anti-TNF- $\alpha$  therapy, adalimumab, etanercept, disease activity

**Acknowledgement/Funding:** This study is financed by the Medical University of Silesia, Katowice, Poland (Grant No. BNW-2-039/N/3/F).

# TNF- $\alpha$ INHIBITORS COMBINED WITH METHOTREXATE MODULATES PLASMA LEVELS OF CHEMERIN IN WOMEN WITH RHEUMATOID ARTHRITIS—PRELIMINARY STUDIES

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## ABSTRACT

**INTRODUCTION:** Rheumatoid arthritis (RA) is an autoimmune disease associated with a chronic inflammation, which gradually leads to cartilage destruction, intra-articular bone erosion, and increased cardiovascular risk. Among the mechanisms involved in the development of synovitis and premature atherosclerotic events, adipocytokines, notably chemerin, seem to play an important role. Chemerin is a hormone-like chemoattractant molecule synthesized by adipose tissue and immune cells. It enhances endothelial dysfunction by inducing the expression of adhesion molecules in endothelial cells, which contributes to atherosclerotic plaque formation and destabilization. Chemerin is also involved in the enhancement of pro-inflammatory cytokines and cartilage matrix-degrading enzymes synthesis by fibroblast-like synoviocytes, leading to persistent inflammation and joint destruction.

**AIM:** Given the pivotal role of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in chemerin's synthesis, we aimed to investigate the effects of TNF- $\alpha$  inhibitors (TNFaI) on plasma chemerin levels in female RA patients.

**MATERIALS AND METHODS:** Plasma chemerin levels were determined by immunoassay at baseline, and after 15 months of anti-TNF- $\alpha$  treatment.

**RESULTS AND CONCLUSION:** Chemerin levels were significantly elevated in RA patients before the implementation of therapy compared to controls. A 15-month anti-TNF- $\alpha$  therapy reduced plasma chemerin levels. Moreover, before the first dose of TNFaI, a positive correlation was found between the chemerin and the 28-joint disease activity score based on the erythrocyte sedimentation rate (DAS28-ESR,  $r = 0.48$ ;  $p < 0.01$ ) and the age of the patients ( $r = 0.50$ ;  $p < 0.01$ ). In summary, long-term treatment with TNFaI decreases circulating chemerin levels, which may represent an important mechanism for preventing cartilage damage and cardiovascular events in RA.

**Keywords:** chemerin, TNFaI, rheumatoid arthritis

**Acknowledgement/Funding:** This study was financed by the Medical University of Silesia, Katowice, Poland (Grant No. BNW-2-039/N/3/F).

# HEPARANASE-1: A MULTITASKING PROTEIN IN CANCER AND VIRAL INFECTIONS WITH THERAPEUTIC POTENTIAL

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## ABSTRACT

**INTRODUCTION:** Heparanase-1 (HPSE), a heparan sulfate–degrading enzyme involved in extracellular matrix remodeling and tumor progression, is increasingly being recognized as a critical host factor in viral infections. We previously showed that during hepatitis C virus (HCV) infection, HPSE expression is up-regulated *in vitro* and in *in vivo*—in patient liver biopsies through oxidative stress and NF- $\kappa$ B activation. Functionally, HPSE facilitates HCV dissemination by enhancing virus release via CD63-dependent exosomal pathways, without affecting viral entry or replication. Its inhibition markedly reduces viral production, positioning HPSE as a promising target for host-directed antiviral therapy.

**AIM:** Here, we investigated the role of HPSE in Human Immunodeficiency Virus type 1 (HIV-1) infection.

**MATERIALS AND METHODS:** To this end, we modulated HPSE expression in cell lines and evaluated the effect of PI-88 (muparfostat), a sulfated oligosaccharide and clinical HPSE inhibitor, on HIV-1 infection in primary human cells and humanized mouse models.

**RESULTS AND CONCLUSION:** PI-88 potently inhibited HIV-1 infection across all models tested. Notably, its antiviral effect was independent of HPSE expression. Mechanistic analyses revealed that PI-88 acts at the viral attachment stage; however, this activity was unrelated to its HPSE-inhibitory function. Unexpectedly, HPSE upregulation rather reduced HIV-1 infection, whereas HPSE downregulation enhanced infection, contrasting with reports in other viral systems where HPSE promotes viral spread. These findings challenge the conventional understanding of HPSE in viral pathogenesis and suggest that it may play virus-specific roles. Overall, our findings highlight heparanase-1 as a relevant therapeutic target in virology and suggest that PI-88 could serve as an effective anti-HIV-1 inhibitor, with potential for broad-spectrum antiviral therapy and drug repurposing.

**Keywords:** *heparanase-1, HCV, HIV-1, antivirals, PI-88*

**Acknowledgement/Funding:** *This study is financed by ANRS MIE.*

## PHYTOTHERAPEUTIC POTENTIAL OF BULGARIAN FLORA IN STRESS MODULATION

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### ABSTRACT

**INTRODUCTION:** Bulgaria's rich medicinal flora has a long tradition of use in treating disorders of the nervous system, particularly distress and anxiety. Contemporary ethnobotanical surveys confirm that local communities continue to rely on herbal remedies for psychological well-being. Many of these plants contain adaptogens and other phytochemicals that modulate neuroendocrine and immune pathways involved in stress responses.

**AIM:** This study aims to synthesize clinical and preclinical evidence on Bulgarian medicinal plants pertinent to psychological stress, with emphasis on mechanisms, efficacy, and safety.

**MATERIALS AND METHODS:** This narrative review examined studies from major databases such as PubMed/NCBI, Web of Science, Scopus on Bulgarian herbs linked to stress, mood, sleep, or neuroinflammation. Priority was given to human trials.

**RESULTS AND CONCLUSION:** Evidence supports anxiolytic, mood, and sleep benefits of *Melissa officinalis* (lemon balm), attributed to rosmarinic acid, citral, and related constituents. Human data suggest calming and antidepressant effects with good tolerability, though larger RCTs are needed. *Lavandula angustifolia* products rich in linalool/linalyl acetate are widely used for anxiety and stress, with Bulgarian chemotypes well characterized chemically, supporting quality standardization. *Satureja montana* polyphenols show preclinical anxiolytic/antidepressant and neuroprotective actions relevant to stress-related cognitive decline. Iridoid-rich Bulgarian plants exhibit anti-inflammatory, immunomodulatory, and metabolic effects across NF- $\kappa$ B, MAPK, and AMPK pathways—mechanisms central to stress pathophysiology and comorbid depression—supporting adjunctive potential. Psychoneuroimmunology data further justify immunoregulatory botanicals as complements to standard therapy. Overall, promising signals exist, but heterogeneity of extracts, small samples, and limited clinical trials warrant standardized preparations and dose-response studies.

**Keywords:** *Bulgarian medicinal plants, adaptogens, lemon balm, lavender, iridoids, psychoneuroimmunology*

# ISOLATION AND SCREENING OF HALOPHILIC BACTERIA FOR BIOSURFACTANT PRODUCTION

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## ABSTRACT

**INTRODUCTION:** Biosurfactants produced by halophilic bacteria are gaining attention as eco-friendly and biocompatible alternatives to synthetic surfactants due to their high surface activity, stability under extreme conditions, and intrinsic antimicrobial properties. These amphiphilic biomolecules hold great promise for bioremediation, biomedical and pharmaceutical applications, including antimicrobial formulations, biofilm inhibition, wound healing, and drug delivery systems.

**AIM:** In this study, moderately halophilic bacteria capable of biosurfactant production were isolated and screened from saline mud collected at the Burgas solar salterns (Bulgaria).

**Materials and Methods:** The halophilic microbiota was enriched in Bushnell–Haas (BH) medium containing 10% NaCl and different hydrocarbons as sole carbon and energy sources. Primary screening in BH liquid medium evaluated the isolates' ability to degrade hexadecane while producing biosurfactants. Biosurfactant synthesis was assessed using haemolytic agar, oil displacement, Parafilm M, and emulsifying index ( $E_{24}$ ) assays.

**RESULTS AND CONCLUSION:** Thirty halophilic bacterial strains were isolated on BH agar supplemented with 2% hexadecane, 2% olive oil, or 2% glycerol. Four isolates—BS7OL, BS8OL, BS9GL, and BS10HD—exhibited strong emulsifying activity ( $E_{24} = 56\%$ ) and reduced surface tension to 27.5–45 mN/m after 7 days of batch fermentation, confirming biosurfactant production. The observed emulsifying activity and surface tension reduction suggest that the biosurfactants produced by these halophilic strains may possess broad potential and could serve as natural, non-toxic agents for bioremediation and biomedical formulations. This study provides the first evidence of biosurfactant-producing halophilic bacteria from the Burgas salterns and their potential role in the development of novel bio-based antimicrobial products.

**Keywords:** halophilic bacteria, hexadecane, biosurfactants

**Acknowledgement/Funding:** This study is financed by the BNSF under project KP-06-H87/2.

## ANTICANCER ACTIVITY OF *ACHILLEA THRACICA* VELEN

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### ABSTRACT

**INTRODUCTION:** Since the beginning of human civilization, medicinal plants have been part of the social life. Many endemic medicinal plants are introduced into the global herbal market. A team from Sofia University provided a system for *ex situ* conservation through *in vitro* cultivation and *ex vitro* adaptation of the Bulgarian endemic species *Achillea thracica* Velen.

**AIM:** The aim of this study was to evaluate the *in vitro* anticancer activity of the Bulgarian endemic species *Achillea thracica* Velen. and its applied potential in clinical practice.

**MATERIALS AND METHODS:** *In vitro* cytotoxicity and proapoptotic activity of *A. thracica* extracts upon HT1080 and SK-HEP-1 cell lines were estimated. The SK-HEP-1 cells were treated with methanolic extracts at dose 50 µg/mL for 24 hours. The HT1080 cells were treated with hot water extracts at dose range from 50 to 600 µg/mL for 48 h. 5-fluorouracil was used as a positive control. Student's t-test was performed.

**RESULTS AND CONCLUSION:** The methanolic extracts of *Achillea thracica* grown under different conditions significantly inhibited SK-HEP-1 cell line proliferation/viability. The hot water extract significantly inhibited the HT 1080 cell line vitality. After treatment with 200, 400, and 600 µg/mL, the value of the growth rate constant of the cell population decreased. The percentage of HT 1080 cells with nuclear morphology changes was significantly increased. The pyknosis was established after treatment with all water extracts. Karyolysis and a sign of karyorrhexis were observed also.

The *Achillea thracica* extracts inhibit of HT1080 and SK-HEP-1 cell line proliferation/viability. Our results demonstrate the applied potential of Bulgarian endemic species *Achillea thracica* as a functional food during clinical anticancer treatment.

**Keywords:** *Achillea thracica* Velen., *in vitro* cytotoxicity, proapoptotic activity, functional food

**Acknowledgement/Funding:** This study is financed by the Bulgarian Ministry of Education and Science, Grant No. RD-08-113/05.02.2025.

# AMYLOID-B (WITH SEQUENCES 1-42 AND 42-1) AND OXIDIZED PHOSPHOLIPIDS COOPERATIVELY DRIVE MEMBRANE REMODELLING IN ALZHEIMER'S DISEASE PATHOLOGY

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## ABSTRACT

**INTRODUCTION:** The generation of large unilamellar vesicles (LUVs), characterized by a lipid profile like that of neuronal membranes in both norm and pathology (in the current case, Alzheimer's disease), along with investigation the influence of amyloid- $\beta$  oligomers, plays a crucial role for establishing changes in the bilayer order (through the Generalized Polarization data) and membrane surface charge (i.e.,  $\zeta$ -potential), which are directly determined by intra- and intermolecular reorganization events among the lipophilic molecules.

**AIM:** The aim of this work is consideration of the impact of biophysical and biochemical alterations on the membrane order and the surface charge, when comparing LUVs with a lipid profile characteristic of a physiological state (POVPC : Ch : SM) with one containing: 1. oxidized lipid(s) POVPC and/or PGPC; 2. amyloid- $\beta$  oligomers formed by  $A\beta_{1-42}$  or  $A\beta_{42-1}$  (rA $\beta$ ) aggregated peptides with AA sequences 1-42 and 42-1, respectively.

**MATERIALS AND METHODS:** The materials applied to this study include: DMEM; POPC (16:0-18:1 PC); POVPC (16:0-5:0 PC (CHO)); PGPC (17:0-5:0 PC (COOH)); Cholesterol; Sphingomyelin; 1-42 and 42-1 AA sequenced amyloid- $\beta$  peptides; Laurdan.

The methods used are as follows:

- ◆ formation of liposomal structures using the Bangham method;
- ◆ GP sensing by fluorescence spectroscopy;
- ◆ measurement of  $\zeta$ -potential through electrophoretic light scattering.

**RESULTS AND CONCLUSION:** According to the GP values, there is an influence of the:

- ◆ oxidized lipids, reflecting the more pronounced ordering effect;
- ◆ 1-42 and 42-1-composed amyloid- $\beta$  oligomeric forms, further reducing membrane fluidity;
- ◆ A $\beta$  in the presence of oxidized lipids, the latter of which:
  1. significantly enhancing the effect of 1-42-assembled oligomers,
  2. having a weaker impact on the reversed A $\beta$  in the presence of ONLY one of the two oxidized lipophilic molecules, considering the synergistic effect of both coexisting derivatives.

Regarding the  $\zeta$ -potentials, significantly more electronegative values of the samples with oxidized lipids due to potential exposure of their polar group (aldehyde and / or carboxyl one) on the membrane surface are observed. The higher  $\zeta$ -potential values of the PGPC-containing mixtures stand for the deprotonated carboxyl group under physiological conditions.

**Keywords:** liposomes, LUVs, (oxidized) lipids, amyloid- $\beta$  peptides/oligomers, lipid order,  $\zeta$ -potential

**Acknowledgements:** *The authors thank the: 1. Bulgarian Ministry of Education and Science for support: The Scientific Infrastructure on Cell Technologies in Biomedicine (SICTB) D01-361/2023; 2. National Centre for Biomedical Photonics D01-352/2023—part of the Bulgarian National Roadmap for Scientific Infrastructures 2020–2027, and 3. COST Action CA22153 EuroCurvoBioNet.*

## MUTATIONAL SPECTRUM OF KRAS IN PATIENTS WITH METASTATIC COLORECTAL CANCER

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### ABSTRACT

**INTRODUCTION:** KRAS mutations are common oncogenic drivers in mCRC and predict resistance to anti-EGFR therapy. The prognostic significance of specific subtypes and co-occurring variants remains under investigation.

**AIM:** The aim of this work is to evaluate the frequency, spectrum, and clinical relevance of KRAS mutations in patients with mCRC and assess their association with disease progression.

**MATERIALS AND METHODS:** A retrospective cohort of 100 patients with histologically confirmed mCRC, treated with radical surgery and systemic chemotherapy, was analyzed. KRAS mutations were assessed in FFPE tumor DNA by allele-specific real-time PCR (analytical sensitivity 1–5% VAF). Samples with  $\geq 20\%$  tumor cell content were included. Patients were stratified as KRAS-mutant or wild-type. Disease progression was evaluated radiologically and clinically; statistical analysis used the chi-square test.

**RESULTS AND CONCLUSION:** KRAS mutations were identified in 50% of patients ( $n = 50/100$ ). The most frequent variants were G12X (52%), followed by G13D (18%), A146X (16%), Q61X (6%), and A59X (4%). Compound KRAS mutations ( $\geq 2$  distinct pathogenic variants per tumor) were detected in 9% of all cases. Disease progression was significantly more frequent in the KRAS-mutant group compared to the WT group (82% vs. 10%,  $\chi^2 p < 0.001$ ). All patients harboring compound KRAS mutations exhibited progressive disease. KRAS mutations, particularly compound alterations and substitutions at codons 12, 61, and 59, are associated with a more aggressive clinical course in mCRC. Expanded KRAS genotyping may support improved prognostic stratification. Limitations: retrospective design and lack of time-to-event analysis (PFS/OS) preclude definitive prognostic conclusions. Prospective studies are warranted.

**Keywords:** *colorectal cancer, KRAS*

**Acknowledgement/Funding:** *This study is financed by Project № BG-RRP-2.004-0007-C01 Strategic Research and Innovation Program for the Development of MU - PLOVDIV-(SRIPD-MUP)*

# SUBSTITUTING SODIUM WITH CHARGED AMINO ACID ESTER CATIONS IN BETULINIC ACID FORMULATIONS ENHANCES ANTICANCER POTENTIAL

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## ABSTRACT

**INTRODUCTION:** Worldwide, breast cancer (BC) is the most common invasive cancer among women. Its treatment is complex and involves drugs acting through various mechanisms, including DNA-damaging agents, mitotic inhibitors, receptor blockers that interfere with hormone or growth factor signaling, and monoclonal antibodies targeting specific molecular pathways involved in tumor growth and survival. Despite their therapeutic success, these treatments often face challenges related to toxicity, drug resistance, and limited selectivity. Natural compounds represent a promising source of new anticancer agents due to their structural diversity and multi-target potential; however, their clinical application is frequently limited by poor solubility, stability, and bioavailability.

**AIM:** This research aims to improve the physicochemical properties and biological activity of betulinic acid (BA), a naturally occurring pentacyclic triterpenoid with strong anticancer potential against BC.

**MATERIALS AND METHODS:** The study involved the synthesis of BA organic salts using the neutralization method and the evaluation of their cytotoxicity against two BC cell lines using the MTT assay. For the selected compounds, additional assays were performed to assess their effects on reactive oxygen species (ROS) generation, MMP-2/MMP-9 activity, and clonogenic potential in BC cells, as well as cytokine production in peripheral blood mononuclear cells (PBMCs).

**RESULTS AND CONCLUSION:** The most active betulinates against the two BC cell lines were those containing positively charged amino acids such as Arg and Lys. These compounds also reduced the metastatic potential and clonogenicity of tumor cells and induced intrinsic ROS production. Interestingly, the ProOEt-containing betulinate showed the highest immunostimulatory potential in PBMCs. Overall, substituting sodium with charged amino acid ester cations enhances both the anticancer and immunomodulatory properties of BA formulations.

**Keywords:** *betulinic acid, anti-breast cancer activity, ROS, immunostimulatory activity*

**Acknowledgement/Funding:** *This research is funded by the Bulgarian National Science Fund, project number KP-06-H69/2.*

## MICROPLASTIC CONTAMINATION OF RICE: A HIDDEN FOOD SAFETY RISK. A REVIEW

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### ABSTRACT

**INTRODUCTION:** Microplastics (MPs) and nanoplastics (NPs) have become ubiquitous contaminants in terrestrial and aquatic ecosystems, posing an emerging threat to global food security. Recent evidence indicates that rice (*Oryza sativa* L.), a staple food for over half of the world's population, may serve as a main pathway for human microplastic ingestion. Plastic residues can infiltrate rice at multiple stages—cultivation, processing, packaging, and storage—raising concerns regarding chronic exposure and potential health impact.

**AIM:** This review aims to critically summarize current knowledge on MP contamination in rice, focusing on sources and pathways of exposure, analytical detection methods, and implications for food safety and human health.

**MATERIALS AND METHODS:** A literature review was conducted using Scopus, ResearchGate, PubMed, and several other databases analyzing studies published between 2000 and September 2025. Keywords included “microplastics in rice,” “food contamination,” “toxicological effects,” “pollution in rice,” “microplastic contamination.” Most studies originated from China, India, and Australia.

**RESULTS AND CONCLUSION:** Findings reveal that MPs enter rice through agricultural soils contaminated by plastic mulches and irrigation water, as well as via industrial processing and packaging materials. Reported concentrations range from 3 to 13 mg of plastic per 100 g of rice, depending on geographical origin and processing method. Despite growing concern, standardized analytical protocols and toxicological assessments remain limited. Further interdisciplinary research is essential to quantify exposure risks and guide regulatory frameworks for plastic use in food production.

**Keywords:** *microplastics, food contamination, toxicological effects, pollution in rice, microplastic contamination*

# BEYOND THE GLOBAL DATABASE: DISTINCT FREQUENCIES OF CHEMOTHERAPY-RELATED PHARMACOGENETIC VARIANTS IN THE BULGARIAN POPULATION

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## ABSTRACT

**INTRODUCTION:** Pharmacogenetic variants affect both chemotherapy efficacy and toxicity. However, global databases underrepresent Eastern European populations, limiting the applicability of existing data. Bulgaria has a unique genetic background, and understanding population-specific pharmacogenetic profiles is crucial for optimizing personalized cancer therapy.

**AIM:** The objective of this study was to investigate germline pharmacogenetic variants associated with drug metabolism in cancer patients, characterize their clinical significance, and compare their frequency in the Bulgarian population with global data.

**MATERIALS AND METHODS:** A total of 140 individuals were enrolled, and 90 completed the study, including 50 cancer patients (26 colorectal, 13 non-small cell lung, and 11 breast cancer) and 40 healthy individuals. Genomic DNA was isolated from 50 samples and cell-free DNA (cfDNA) from 40 patients. All samples underwent next-generation sequencing (NGS), followed by the interpretation of germline variants in genes involved in the metabolism of chemotherapeutic agents.

**RESULTS AND CONCLUSION:** A two-step comparative analysis was performed. First, variant frequencies were compared between patients and healthy controls. Then, the combined cohort of 90 participants was compared with global reference data from gnomAD. Among the 23 germline pharmacogenetic variants analyzed, six showed higher and four lower allele frequencies in the Bulgarian population compared to global data. These results highlight population-specific genetic features that may influence chemotherapy metabolism and toxicity, emphasizing the importance of incorporating Bulgarian pharmacogenetic data into global databases to improve personalized cancer treatment.

**Keywords:** *pharmacogenetics, germline variants, chemotherapy, allele frequency, Bulgarian population, personalized medicine*

**Acknowledgement/Funding:** *This study is financed by project No. BG-RRP-2.004-0007-C01 Strategic Research and Innovation Program for the Development of MU-PLOVDIV-(SRIPD-MUP).*

## THE SCIENCE BEHIND TOURNIQUET USE

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### ABSTRACT

**INTRODUCTION:** Tourniquets are medical devices used to control blood flow during surgery and in emergency trauma. In surgery, they create a bloodless field with improved visibility and reduced intraoperative blood loss, with specific utility in orthopedic and trauma surgery.

**AIM:** The aim of this work is to provide practical information on the safe and effective use of tourniquets across a range of medical scenarios by bringing attention to important concerns and side effects.

**MATERIALS AND METHODS:** Scientific articles, published in the period from 1999 to 2025, in peer-reviewed journals included in the specialized databases ScienceDirect, PubMed, Taylor and Francis, Springer, and Frontiers, were analyzed.

**RESULTS AND CONCLUSION:** Our survey provides an overview of both emergency and surgical tourniquets, discussing their mechanism, types, and correct application. The critical decision-making points in the application of tourniquets include when to apply and when to remove them, and how to assess for potential complications following application. Special attention has to be drawn to the complications associated with tourniquet use in the form of ischemic injuries and damage due to pressure, and the best practices in minimizing risks. Inappropriate tourniquet applications can cause compartment syndrome, skin, vascular, and muscle damages, nerve palsy, and even exacerbated hemorrhage. The modern tourniquet use includes supplementation of patients with antioxidants to diminish the provoked oxidative stress through the ischemia-reperfusion injury. Medical professionals must possess a good understanding of tourniquet application so that benefits can be maximized and injuries minimized.

**Keywords:** *tourniquet, emergency, surgery, trauma, oxidative stress, compartment syndrome*

**Acknowledgement/Funding:** *Academic Flight Program, the Student Council at the Prof. Dr. Assen Zlatarov State University of Burgas.*

## 3D OSTEOLOGICAL LIBRARY. FROM SCAN TO PRINT

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### ABSTRACT

**INTRODUCTION:** Anatomical education currently relies on classroom experience to allow students to understand the complex 3D relationships of bones and associated structures. The supplementary material for individual study lacks the spatial depth needed to fully grasp the concepts which we would like to teach and makes preparation for students difficult.

**AIM:** 3D online models and physical 3D prints of the osteological collection of the university will allow students to access the bones for their preparation at home and allow for better teaching tools in the university. Our goal is to create a low-cost, reproducible workflow for the scanning, uploading, and printing of 3D osteology models.

**MATERIALS AND METHODS:** The workflow consists of five key steps: scanning, mesh computation, online uploading, slicing, and 3D printing. We assessed the anatomical accuracy of these models using the calcaneus as a reference due to its well-defined osteological measurements, which we cross-referenced and compared to the original bone. We found a less than 1% variation from the bone to the model to the print, the bottleneck currently still being the scanning.

**RESULTS AND CONCLUSION:** The workflow that we present is both affordable and accurate. This research sets the stage for wide-scale implementation of innovative 3D technology into the university's curriculum. As implementation progresses we will be able to quantify the effect that this will have on the quality of our teaching and allow other researchers and departments to improve their own approach to the technology.

**Keywords:** *anatomy, 3D scanning, 3D printing, education, osteology*

# ROLE OF ADENYLYL CYCLASES IN THE DEVELOPMENT OF VARIOUS DISEASES

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## ABSTRACT

**INTRODUCTION:** Adenylyl cyclases (ACs) catalyze the formation of the second messenger cyclic adenosine monophosphate (cAMP) from adenosine triphosphate. They are transducers that participate in signaling cascades, regulating metabolism, neurotransmission, cell growth and motility.

**AIM:** The aim of this work is to analyze the updated information on the structure, importance of ACs in norm and pathology, and therapeutic modulation of their activity.

**MATERIALS AND METHODS:** Articles, published in the period from 2011 to 2025, in peer-reviewed journals, included in the databases ScienceDirect, PubMed, Springer, Wiley, and Frontiers, were analyzed.

**RESULTS AND CONCLUSION:** Adenylyl cyclases are lyases divided into 6 classes that require as a cofactor  $Mg^{2+}$  ( $Mn^{2+}$ ). Human class III ACs are represented by 9 transmembrane and 1 soluble isoform. The transmembrane ACs are part of the G-protein-coupled receptors, via which act hormones and other signaling molecules. This leads to cAMP that activates protein kinase A (PKA). Disturbances in the cAMP signaling are found in infertility, bacterial infections, heart failure, erectile dysfunction, neurodegenerative disease, depression, schizophrenia, cystic fibrosis, diabetes, and cancer. However, cAMP-PKA signaling has a dual role in diabetes and oncogenesis. The excessive or insufficient AC activity reveals its role for understanding, preventing, and treatment of socially significant diseases. ACs are regulated by the G-proteins, calmodulin,  $Ca^{2+}$ , and bicarbonates, but they can be affected by bacterial toxins and drugs like colforsin daropate, sildenafil citrate, milrinone, and 2',5'-dideoxyadenosine. Phytochemicals, such as forskolin, curcumin, resveratrol, berberine, and epigallocatechin gallate, are beneficial modulators of ACs.

**Keywords:** *adenylyl cyclase, G-proteins, cAMP signaling, phytochemicals, socially significant diseases*

**Acknowledgement/Funding:** *Academic Flight Program, the Student Council at the Prof. Dr Assen Zlatarov State University of Burgas.*

# DROPLET DIGITAL PCR OUTPERFORMS RT-PCR BY REVEALING CLINICALLY RELEVANT LOW-FREQUENCY KRAS MUTATIONS IN COLORECTAL CANCER

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## ABSTRACT

**INTRODUCTION:** Mutations in the KRAS gene are key predictive biomarkers of resistance to anti-EGFR therapy in colorectal cancer (CRC). However, standard RT-PCR assays have limited sensitivity (~5% variant allele fraction, VAF), potentially missing low-frequency mutations that contribute to tumor heterogeneity and therapeutic resistance. Droplet digital PCR (ddPCR) offers improved analytical sensitivity (0.1–1% VAF), enabling detection of subclonal variants.

**AIM:** The aim of this work is to compare the analytical performance of RT-PCR and ddPCR for KRAS mutation detection in formalin-fixed paraffin-embedded (FFPE) CRC samples.

**MATERIALS AND METHODS:** Fifty FFPE samples from patients with colorectal adenocarcinoma were analyzed for KRAS hotspot mutations (codons G12, G13, A59, Q61, K117, A146). All samples were initially tested using RT-PCR as part of routine diagnostics, followed by reanalysis with ddPCR. Concordance and incremental detection rates were calculated.

**RESULTS AND CONCLUSION:** RT-PCR identified KRAS mutations in 25/50 cases (50.0%), whereas ddPCR increased the detection rate to 31/50 (62.0%). ddPCR revealed seven additional mutation-positive samples among RT-PCR wild-type cases, representing a 14% absolute gain in detection and preventing potential false-negative biomarker classification. These additional mutations showed low VAF (median 1.0%, range 0.5–3.4%), consistent with subclonal tumor populations. The mutation spectrum was dominated by codon 12 substitutions, aligning with published CRC mutation profiles. Beyond enhanced sensitivity, ddPCR enables longitudinal quantification of mutation burden, supporting its application in minimal residual disease monitoring and early detection of emerging resistance clones during anti-EGFR therapy. These findings support the integration of ddPCR as a complementary reflex assay to RT-PCR in precision oncology workflows for CRC.

**Keywords:** *droplet digital PCR, colorectal cancer, KRAS mutation*

# EXPLORATION OF NEW HALOMONAS STRAIN (BS10HD) WITH POTENTIAL FOR BIOSURFACTANT PRODUCTION

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## ABSTRACT

**INTRODUCTION:** Halophilic microorganisms are a promising source of biosurfactants—biodegradable, low-toxicity, and renewable surface-active compounds with diverse biotechnological, industrial and medical applications.

**AIM:** In this study, moderately halophilic microorganisms were isolated from saline mud collected at the Burgas solar salterns (Bulgaria) to investigate their capacity for biosurfactant production.

**MATERIALS AND METHODS:** A total of thirty bacterial isolates were recovered using Bushnell–Haas agar with 2% hexadecane, olive oil, or glycerol. The BS10HD strain showed good biosurfactant production, achieving strong emulsifying activity,  $E_{24} = 56\%$ , and lowering surface tension to 27.5 mN/m after seven days of batch fermentation. The cell-free supernatant was extracted with a variety of solvents to identify the most effective solvent for biosurfactant recovery. The extract material was analyzed by thin-layer chromatography (TLC) and Fourier transform infrared spectroscopy (FTIR). Upon spraying the TLC plate with ninhydrin reagent, the appearance of a pink spot indicated the presence of amine functional groups. FTIR analysis showed characteristic peaks for both lipid and peptide functional groups, including N–H ( $3,320\text{ cm}^{-1}$ ), C–H ( $2,920$  and  $2,850\text{ cm}^{-1}$ ), and amide I/II bands ( $1,650$  and  $1,540\text{ cm}^{-1}$ ).

**RESULTS AND CONCLUSION:** Based on the observed physicochemical properties and analytical data, it can be suggested that the biosurfactant produced by *Halomonas* species, BS10HD, is a lipopeptide compound. In this study, halophilic bacteria from the Burgas salterns were identified as a potent biosurfactant-producing microorganisms with promising potential for environmental, biotechnological, and medical applications.

**Keywords:** *biosurfactants, Halomonas species, lipopeptide, TLC*

**Acknowledgement:** *This work was supported by the BNSF under project KII-06-H 87/2.*

# VISUALIZING THE EFFECTS OF ANTIBACTERIAL DENDRIMERS ON THE MORPHOLOGY OF MODEL LIPID MEMBRANES

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## ABSTRACT

**INTRODUCTION:** In the view of the increasing antimicrobial resistance worldwide dendrimers have gained an increasing interest as an alternative of the widely used antibiotics. In this study, the surface interactions between two dendrimers with proven antibacterial activity, 4-N,N-dimethylamino-1,8-naphthalimide (Dab) and 3-bromo-Dab (Dab-Br), and model monolayer and bilayer membranes formed from POPC (1-palmitoyl-2-oleoylphosphatidylcholine) were analyzed.

**AIM:** The aim of the present study was to visualize the molecular mechanisms of interaction between nanoparticles of Dab and Dab-Br and POPC model membranes (monolayers and thin liquid films/TLFs).

**MATERIALS AND METHODS:** The two model systems used, Brewster-angle microscopy (BAM) of insoluble mixed POPC-dendrimers monolayers and the microinterferometric method for studying TLFs of Sheludko and Ekserowa, are highly informative methods for visualizing and analyzing surface interactions between model membranes and bioactive molecules.

**RESULTS AND CONCLUSION:** Brewster-angle microscopy images showed a significant change in the morphology and presence of surface aggregates of the mixed POPC+dendrimer monolayers as a result of the addition of the nanoparticles, with the effect being more pronounced for Dab-Br. Microscopic images of TLFs demonstrated that in presence of dendrimers the films were thinning more slowly and the time for black film formation increased, especially for Dab-Br. The film thicknesses measured showed that when Dab was added, the thickness did not change, while Dab-Br increased this parameter. The results obtained demonstrated the significance of the positive charge in the dendrimers studied on the antibacterial properties of novel antimicrobials in view of their destabilizing effect on bacterial membranes.

**Keywords:** dendrimers,; POPC, monolayers, thin liquid films

**Acknowledgement/Funding:** This study is financed by the Scientific Research Fund of the Ministry of Education and Science—Project KII-06-H51/15 dated 19 November 2021, by the Scientific Research Fund of the Sofia University, 80-10-137/03.06.2025, and by the European Union-NextGenerationEU through the National Recovery and Resilience Plan of the Republic of Bulgaria, Project № BG-RRP-2.004-0008-C01.

# POSTOPERATIVE CTDNA DYNAMICS AS AN EARLY INDICATOR OF MOLECULAR RESIDUAL DISEASE IN LUNG CANCER: PRELIMINARY RESULTS AND CORRELATION WITH NM STATUS, ADJUVANT THERAPY AND CLINICAL OUTCOME

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## ABSTRACT

**INTRODUCTION:** Circulating tumor DNA (ctDNA) reflects tumor burden and may detect molecular residual disease (MRD) after surgery. Early ctDNA kinetics could stratify recurrence risk before radiological progression. This study evaluates postoperative ctDNA dynamics in lung cancer and explores associations with clinicopathological characteristics.

**MATERIALS AND METHODS:** This preliminary study included 12 patients with histologically confirmed lung cancer treated with curative-intent surgery. Plasma was collected preoperatively, on postoperative day 10, and at 1 and 3 months. Total circulating DNA was quantified as a surrogate of ctDNA dynamics. Clinical parameters included tumor size, pathological stage, NM status, type of surgery, and adjuvant therapy. Longitudinal changes were assessed by paired statistics.

**RESULTS:** A consistent decline in ctDNA was observed after surgery in most patients. Two patients (16.7%) died during follow-up and showed minimal or absent ctDNA reduction, indicating persistent MRD. Slower ctDNA decline showed directional association with higher tumor burden and advanced NM status, while patients receiving adjuvant therapy tended to show a deeper ctDNA reduction by 3 months.

**DISCUSSION AND CONCLUSION:** These preliminary findings support the feasibility and prognostic relevance of ctDNA monitoring after lung cancer surgery. Lack of ctDNA decline appears associated with unfavorable early outcome, including mortality. ctDNA kinetics may therefore complement TNM staging in postoperative risk stratification and guide surveillance intensity and adjuvant treatment decisions. Future work will include mutation-specific ctDNA analysis (e.g., quantification of tumor driver variants) to improve molecular sensitivity and assess minimal residual disease with higher specificity. Larger prospective datasets and extended follow-up are in progress.

**Keywords:** lung cancer, ctDNA, molecular residual disease (MRD), prognosis, NM status, surgery, adjuvant therapy, liquid biopsy

**Funding:** Project No. 3.4.18 ctDNA as a Biomarker for Early Diagnosis and Monitoring of Patients with Lung Carcinoma—a project related to applied research aimed at innovation or intellectual property and implemented under Contract No. BG-RRP-2.004-0007-C01 Programme for Strategic Research and Innovation for the Development of MU–Plovdiv (PSRI-MUP), Pillar 2: Establishment of a Network of Research Universities of the Programme for Accelerating Economic Recovery and Transformation through Science and Innovation, from the Innovative Bulgaria Component of the National Recovery and Resilience Plan.

# A NEW FORMULATION CONTAINING CDP-CHOLINE AND PLANT EXTRACTS CAN MODULATE THE LIPID PROFILE AND REDOX STATUS OF HEALTHY VOLUNTEERS IN RELATION TO LIFESTYLE HABITS

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## ABSTRACT

**INTRODUCTION:** In recent decades, there has been increasing interest in developing natural-source supplements with high efficacy and fewer side effects for the prevention and treatment of dyslipidemia and redox imbalance. Recently, a new supplement containing CDP-choline, rosehip-derived vitamin C and extracts from green tea and aronia (Cytodeox™) was developed. Although the biological activities and health benefits of all ingredients are well documented, their potential to act cumulatively on lipid profile and oxidative stress remains unexplored. Additionally, the influence of lifestyle factors on these effects needs to be clarified.

**AIM:** The aim of this work is to evaluate the effects of Cytodeox™ on lipid profile and redox status in healthy volunteers after six months of supplementation, as well as to assess the influence of lifestyle factors on these effects.

**MATERIALS AND METHODS:** Forty-three volunteers aged 40–65 (F31/M12) were involved in the study with six months of Cytodeox™ supplementation. Their health status and lifestyle habits were obtained by a questionnaire. Fasting blood samples were collected before and after the Cytodeox™ supplementation to determine the lipid profile and oxidative stress parameters. Participants were grouped based on their gender and lifestyle habits.

**RESULTS AND CONCLUSION:** Cytodeox™ significantly reduced lipid profile parameters and malondialdehyde levels across the entire group. Smoking, but not alcohol consumption, had a negative impact on nutraceutical supplementation. Surprisingly, the beneficial effects were also manifested in the overweight and sedentary lifestyle groups. Cytodeox™ supplementation can improve the lipid profile and redox status, even in individuals with some poor lifestyle habits.

**Keywords:** *citicholine, plant extracts, lipid profile, oxidative stress, biomarkers, lifestyle factors*

**Acknowledgement/Funding:** *European Union-NextGenerationEU, project No. BG-RRP-2.004-0009-C02 and Medical University of Varna, Science Fund, Project 21015.*

## THERAPEUTIC POTENTIAL OF CHALCONES

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### ABSTRACT

**INTRODUCTION:** Chalcones represent a class of both natural and synthetically derived compounds characterized by an  $\alpha,\beta$ -unsaturated carbonyl system. They have been identified as promising scaffolds in medicinal chemistry owing to their structural simplicity and versatile reactivity. The growing interest of the scientific community in these compounds stems from the possibility of functionalizing their basic chemical structure at various positions, resulting in diverse pharmacological activities: antiarrhythmic, antiplatelet, antidiabetic, antineoplastic, anti-inflammatory, antioxidant, antiobesity, hypolipidemic, antituberculosis, antimalarial, antibacterial, antifungal, antiulcer, immunosuppressive, anxiolytic, and osteogenic. Despite promising *in vitro* data, subsequent *in vivo* studies are scarce for most of the compounds.

**AIM:** The aim of this study is to summarize the latest data on chalcones for which there is *in vitro* evidence and information on advanced-stage clinical trials.

**MATERIALS AND METHODS:** Literature review of peer-reviewed sources in global databases with a focus on chalcone derivatives in advanced phase of clinical trials was conducted.

**RESULTS AND CONCLUSION:** Chalcones continue to represent a rich and productive foundation in medicinal chemistry, particularly for anticancer and anti-infective applications.

Recent studies have focused on promising hybrid designs and SAR optimization. Priorities in future work should be to improve ADME, target specificity, *in vivo* validation, and clinical applications. The continued integration of synthetic chemistry, computational modeling, and biological evaluation of chalcones are the ways to accelerate their implementation into clinically viable therapeutics.

**Keywords:** *chalcones, structure–activity relationship, drug design*

# THE POTENTIAL OF SULFUR-CONTAINING MINERAL WATER AND SULFUR-CONTAINING BIOACTIVE COMPOUNDS TO MODULATE INFLAMMATORY MARKERS IN HUMAN INTESTINAL EPITHELIAL CELLS: A COMPARATIVE STUDY

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## ABSTRACT

**INTRODUCTION:** The therapeutic potential of sulfur-containing mineral water (SMW) has been recognised since ancient times. Given the substantial amount of data on the beneficial effects of sulfur-containing active ingredients in foods, interest in SMW has been increasing in recent years, particularly regarding its contribution to human health as part of the daily diet. The anti-inflammatory potential of sulforaphane (SFN), allicin (ALL), and taurine (TAU) is well documented.

**AIM:** This study aimed to analyse and compare the anti-inflammatory potential of SMW, SFN, ALL, and TAU on human intestinal epithelial cells (HIEC-6).

**MATERIALS AND METHODS:** A cell viability assay was performed, selecting three concentrations for each sulfur-containing compound. In a model of lipopolysaccharide-induced inflammation, total RNA was extracted, and the gene expressions of cyclooxygenase-2 (COX-2), tumour necrosis factor-alpha (TNF $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), and IL-1 $\beta$  receptor antagonist (IL1RN) were analysed.

**RESULTS AND CONCLUSION:** With the exception of ALL, all studied compounds significantly decreased the expression of COX-2 in a dose-dependent manner. No change in IL-1 $\beta$  expression was observed in SMW- and SFN-treated cells. All sulfur compounds, including SMW, significantly decreased TNF- $\alpha$  expression, and this effect was particularly pronounced in TAU-treated cells. SMW, SFN, and ALL, but not TAU, increased the mRNA levels of IL-1RN in unstimulated cells. In conclusion, SMW significantly modulated the expression of the studied markers, and these effects were comparable to those of SFN, TAU, and ALL. These results demonstrate the anti-inflammatory potential of SMW and elucidate the underlying mechanisms behind its therapeutic effects.

**Keywords:** *sulfur-containing mineral water, sulforaphane, taurine, allicin, HIEC-6, LPS-induced inflammation*

**Acknowledgement/Funding:** *This study is financed by European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, Project No BG-RRP-2.004-0009-C02 MUVE-TEAM; Medical University of Varna, Science Fund, Project No 22003.*

# PREVENTIVE BENEFITS AND ALLERGENIC RISKS OF *PLANTAGO LANCEOLATA* IN HUMAN HEALTH

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## ABSTRACT

**INTRODUCTION:** *Plantago lanceolata* (ribwort plantain) is traditionally used for respiratory and wound care and contains iridoids (aucubin, catalpol), phenylethanoid glycosides (acteoside/plantamajoside), and polysaccharides implicated in antioxidant, anti-inflammatory, antimicrobial, and barrier-support actions relevant to primary prevention.

**AIM:** This review aims to synthesize evidence on the preventive benefits and allergenic risks of *Plantago lanceolata* for human health and to outline the key research gaps.

**MATERIALS AND METHODS:** A narrative review was undertaken of studies indexed in PubMed/NCBI, Web of Science, Scopus, and MDPI journals concerning *Plantago lanceolata*—including ethnobotanical and phytochemical overviews, antimicrobial and antifungal evaluations, cytotoxicity investigations, allergenomics, and environmental/biomonitoring research—with emphasis on human relevance, mechanisms, safety, and standardization.

**Results and Conclusion:** Leaf extracts of *P. lanceolata* contain steroids, flavonoids, tannins, saponins, glycosides, and phenolics. Extracts inhibit Gram-negative and Gram-positive bacteria and show antifungal activity against *Candida albicans* and *Malassezia spp.*, supporting skin/mucosal prevention use cases. Methanolic extracts reduce A549 lung-cancer cell viability and potentiate methotrexate/cytarabine while better preserving macrophage viability—an adjunct-chemotherapy signal requiring *in vivo* confirmation and extract standardization. Immunoproteomics identifies multiple IgE-reactive pollen allergens, indicating a clinically relevant respiratory risk for sensitized individuals and favoring pollen-free oral/topical formulations. Overall, *P. lanceolata* shows credible preventive potential centered on barrier integrity, infection control, and inflammation modulation. Translation to practice will require chemically characterized preparations (e.g., acteoside/plantamajoside markers), dose-response and safety studies in at-risk groups, and clear allergy labeling with pharmacovigilance.

**Keywords:** iridoids, phenylethanoid glycosides, antimicrobial activity, wound healing, immunoproteomics

## PHYTOCHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF BILBERRY FRUIT JUICE (*VACCINIUM MYRTILLUS* L.) FROM BULGARIA

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### ABSTRACT

**INTRODUCTION:** Blueberries have been used for centuries in traditional medicine in Bulgaria and many countries worldwide.

**AIM:** This study examined polyphenol composition, antioxidant properties, and biological activity of bilberry (*Vaccinium myrtillus* L.) fruit juice from Bulgaria.

**MATERIALS AND METHODS:** Antioxidant potential was analyzed with ABTS, DPPH and FRAP methods. Total polyphenol, flavonoids and anthocyanin contents were measured with colorimetric methods. Specific anthocyanins content was measured by UPLC-MS/MS. Cytoprotective and superoxide anion radical scavenging activity were analysed in in vitro *Saccharomyces cerevisiae* experiments.

**RESULTS AND CONCLUSION:** UPLC-MS/MS analysis revealed total anthocyanin content of 338.5 mg/100 g fresh fruits and dominant compounds were malvidins (38.1%), delphinidins (33.2%), petunidins (18.6%), and cyanidins (10.1%). Total polyphenol content was  $8.40 \pm 0.29$  mg GAE/100 mL, and total flavonoid content— $7.26 \pm 0.11$  mg QE/100 mL. Antioxidant potential was as follows: ABTS ( $3.6 \pm 0.08$  mM UAE), DPPH ( $89 \pm 2.1$  %), and FRAP ( $2.4 \pm 0.09$  mM Fe(II)/L). Cell-based assays demonstrated significant cytoprotective activity and reduced methyl methanesulfonate-induced superoxide anions. These results demonstrate the value of Bulgarian bilberry juice as a source of bioactive compounds as a potential raw material for pharmaceutical and nutritional industry.

**Keywords:** anthocyanins, antioxidants, bilberry, cytoprotective, polyphenols, *Vaccinium myrtillus*

**Acknowledgement/Funding:** This study is financed by European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, Project No BG-RRP-2.004-0009-C02 MUVE-TEAM, Research Group 3.1.1. Natura4Health.

# FROM NATURAL HEALING TO FUTURE TECHNOLOGIES— SHAPING THE FUTURE OF PEDIATRIC CARE

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## ABSTRACT

**INTRODUCTION:** Pediatric care in the Children Department of Specialized Rehabilitation Hospital, Banja Koviljača is focused on rehabilitation through classical methods, integrating physical therapy, hydrotherapy and individualized exercise plans. It is supported by the unique natural factor—sulphuric mud and hydrogen sulfide-rich mineral water, but there are also perspectives of further technological development.

**AIM:** The aim of this paper is to present traditional and classical methods in the rehabilitation of children and to highlight new treatment possibilities that follow modern scientific and technological advancements.

**MATERIALS AND METHODS:** The presentation is based on structured overview of current therapeutic approaches in pediatric rehabilitation focusing on the use of natural agents. Indications and contraindications are presented in line with current clinical guidelines and literature. Emphasis is placed on integrating traditional approaches with recent scientific and technological advances.

**RESULTS AND CONCLUSION:** The therapeutic potential of sulphuric mud of Banja Koviljača in pediatric treatment derive from a well-known complex interplay of physical, chemical, biological and psychological factors along with traditionally acknowledged benefits. The future of pediatric rehabilitation in balneology can also benefit from emerging technologies. These tools do not replace our natural therapeutic factor but rather enhance its effectiveness and help us adapt to modern standards of care. In this way, we see the path forward as an integration of tradition, natural healing and accessible technological support, ensuring that pediatric patients receive the best of both worlds.

**Keywords:** *pediatric rehabilitation, hydrogen sulfide-rich mineral water, mud, modern technologies*

## BIOCHEMISTRY OF HYDROGEN SULFIDE IN THERMAL BATHS

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*Department of Radiology, Rehabilitation & Physiotherapy, Faculty of Medicine,  
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This lecture presents the biochemistry and physiology of hydrogen sulfide (H<sub>2</sub>S) in balneology, focusing on cutaneous delivery and clinical translation.

It begins with the properties of mineral-medicinal water and the H<sub>2</sub>S/HS<sup>-</sup> equilibrium at physiological pH, which determines bioavailability and effective dose in thermal practice, with activity as hydrogen sulfide and as polysulfides.

H<sub>2</sub>S will be reviewed as an active gasotransmitter without a specific receptor, acting on redox-sensitive targets, notably opening KATP ion channels with vasodilation, modulating mitochondrial hemoproteins, and affecting proteins with Fe-S or Zn clusters.

Protein cysteine persulfidation will be highlighted as a post-translational modification that tunes enzymes, ion channels, and transcription factors, embedding H<sub>2</sub>S within the reactive species interactome. Downstream effects through KEAP1/NRF2, PI3K/Akt/mTOR, Wnt/β-catenin, MAPK/ERK, and NF-κB pathways, together with influences on signal transduction and indirect effects on ribosomes, as well as activity on HDACs and DNMTs and activation of sirtuins, help explain cytoprotective, antioxidant, and anti-inflammatory outcomes observed with sulfurous mineral waters. Collectively, these mechanisms support H<sub>2</sub>S as a regulator of gene expression without altering DNA sequence and as a modulator of cellular epigenetics.

The talk will address how sulfurous mineral water acts in dermatology, otorhinolaryngology, musculoskeletal, and digestive conditions, and will compare balneation and peloids as delivery vehicles, considering the roles of temperature, exposure time, and water oxygenation.

**CONCLUSION:** H<sub>2</sub>S is a redox-signaling modulator that has already shown therapeutic potential in thermal medicine when rigorous, measurable protocols are applied.

**Keywords:** *sulfurous mineral water, hydrogen sulfide, balneology*

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Zhang M, Holman CD, Price SD, Sanfilippo FM, Preen DB, Bulsara MK. Comorbidity and repeat admission to hospital for adverse drug reactions in older adults: retrospective cohort study. *BMJ*. 2009 Jan 7;338:a2752. doi: 10.1136/bmj.a2752. PubMed PMID: 19129307; PubMed Central PMCID: PMC2615549

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## SCRIPTA SCIENTIFICA PHARMACEUTICA, volume 12, 2025, supplement 2

**Editor-in-Chief:** Prof. Dr. Svetlana Georgieva, MScBiotech, PhD

**Co-Editor-in-Chief:** Assoc. Prof. Veselina Panayotova, PhD

**Design and Layout Editing:** Emilia Yordanova

**Cover Design:** Hristina Hristova

**Proofreading:** Yordanka Peteva

Publ. Lit. group: III-3

Sent to print: December, 2025

Print sheets: 20

Approved for print: December, 2025

**Publisher:** Medical University of Varna

55 Marin Drinov Street, Varna 9002, Bulgaria

[www.mu-varna.bg](http://www.mu-varna.bg)

ISSN 2367-6000 (print)

ISSN 2367-5500 (online)

<http://press.mu-varna.bg/ojs/index.php/ssp>

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