

# **First International Conference on Bio-antioxidants ICBA 2017**

"Natural bio-antioxidants – as a base for new  
synthetic drugs and food additives/supplements"

## **BOOK OF ABSTRACTS**

### **Young Scientists School on Bio-antioxidants YSSBA 2017**

25-29 June 2017

Bulgarian Academy of Sciences - Presidium  
Sofia, BULGARIA

<http://icba2017.orgchm.bas.bg>

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Dear Colleagues,

On behalf of the Scientific Committee and the Organizing Committee, we are pleased to announce the **First International Conference on Bio-antioxidants (ICBA 2017)** as well as the **Young Scientists School on Bio-antioxidants (YSSBA 2017)**, both dedicated to the “*Natural bio-antioxidants – as a base for new synthetic drugs and food additives/supplements*”. The two events will be held at the Bulgarian Academy of Sciences – Presidium in Sofia, Bulgaria, from the 25th to the 29th June 2017.

The ICBA 2017 / YSSBA 2017 will highlight the following strategic topics:

- A. Oxidative Stress and Human Health**
- B. Natural Bio-antioxidants**
- C. Synthetic Analogues of Natural Bio-antioxidants**
- D. Homogeneous and Heterogeneous Lipid Oxidation**
- E. Food Analysis, Food Additives and Food Supplements**
- F. Advanced Methods for Analysis of Bio-antioxidants**

Most recently, special attention is turned not only to the therapy of some difficult to treat diseases, but also to the prevention of these diseases, which has an important social effect. By these means, the theme and the topics of the conference are undoubtedly current during the last decade.

Many leading scientist, but also several young investigators from all over the world (Bulgaria, Russian Federation, Italy, Spain, Poland, Brazil, Austria etc.), whose research and practice are closely related and dedicated to the development and application of novel bio-antioxidants for prevention and therapy of clinically and socially important diseases related to the oxidative stress, are going to attend the conference and share their knowledge, both in scientific and clinical aspect. Approximately 120 participants and 30 young scientists, students and pupils will take part of this prestigious international conference.

We look forward to welcoming you in lovely Sofia for this special event. Please visit the website of Visit Sofia to enjoy an extraordinary moment where sightseeing, gastronomy, art and history meet.

Yours sincerely,



**Prof. Dr. Vessela D. KANCHEVA**  
Chairman of ICBA 2017



**Mag.pharm. Dr.rer.nat. Stefan A. DOCHEV**  
Coordinator of YSSBA 2017



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# **ICBA 2017**

## **Plenary Lectures**

## **Oxidative stress, lipid oxidation and bio-antioxidants**

Vessela D. Kancheva

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*Oxidative stress* is associated with increased production of so called reactive oxygen species (ROS), i.e. different active radicals and peroxides, or with a significant decrease in the effectiveness of antioxidant defenses, such as antioxidant enzymes, glutathione, antioxidant vitamins. Free radicals are responsible for the pathogenesis of a wide range of diseases: cancer and cardiovascular diseases, asthma, arthritis, inflammations, neurodegenerative disorders, Parkinson's disease and dementia. *Lipid oxidation* - free radicals formation in the hydrophobic parts of the biological membranes first initiates radical disintegration of the hydrocarbon "tails" of the lipids. This process is known as "lipid peroxidation". *Bio-antioxidants* - i.e. biologically active compounds with antioxidant potential - natural and synthetic analogues - have a wide range of applications. Nowadays they play an important role in human health and diseases prevention. They are components of food additives and are used for treatment of different diseases as monotherapy (in low concentration) or in complex therapy (in high concentration) with drugs. Many of the drugs today are synthetic modifications of naturally obtained substances. This report presents information about structure-activity and beneficial effects on human health of known and new bio-antioxidants. The following bio-antioxidants are considered: Flavonoids, Chalcones, Cinnamic acids, Simple coumarins, Bis-coumarins, Curcumin related compounds and Lignans. Design of new molecules with known antioxidant activity is based on structure antioxidant activity relationship. Creation of new *bio-antioxidant compositions* of two and three components and different effects: stronger (synergism), summary (additivism) or negative (antagonism) are also discussed.

## **The impact of free radicals and bioantioxidants on Neurodegenerative Diseases**

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Oxidative and nitrosative stresses may be defined as the imbalance between the production of reactive oxygen species (ROS), reactive nitrogen species (RNS) and the ability to detoxify the reactive intermediates or to repair damage induced by them. ROS and RNS generated in biological systems by several processes initiate and promote degenerative diseases including neurodegeneration in Central Nervous System (CNS). In the CNS, reactive species are responsible of the so-called auto-oxidation of important neurochemicals (i.e. dopamine) and can directly oxidize macromolecules such as proteins, lipids and nucleic acids (DNA, RNA) often resulting in apoptotic cell death. ROS and RNS can also indirectly damage neurons by activating specific cellular pathways and inducing the expression of stress-sensitive genes responsible to amplify the oxidative injury. In particular, glia-mediated inflammation with overproduction of ROS and RNS is often responsible of neuronal damage. The uncontrolled overproduction of reactive species is known to actively contribute to aging and neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Biologically active compounds with antioxidant properties such as natural bio-antioxidants (and their synthetic analogues) play a key role on the inhibition of neuroinflammation and the correction of oxidant/antioxidant imbalance in patients suffering from neurodegenerative diseases. The neuroprotective activity of bio-antioxidants is not only related to their ROS/RNS-trapping properties, but also to their anti-inflammatory effect and the ability to suppress genes induced by pro-inflammatory mediators (i.e. cytokines) released by activated glial cells. Therefore, it appears extremely important to clarify the chemical basis of antioxidant activities and multiple beneficial effects of bio-antioxidants, as phenolic compounds, tocopherols and ascorbic acid contained in plants, spices and food. Moreover, recent scientific evidences suggest that mixtures of bio-antioxidants may be more active in the prevention of neurodegenerative disease. Having made these considerations, it appears critical to monitor, preserve and protect bio-antioxidants during industrial processes (i.e. food preparation, storage and distribution) in order to ensure their biological activity. The development of nanostructured biosensors for monitoring the bio-antioxidant content in agrifood matrices, but also in biological fluids, is an emerging approach in this direction.



## **Propolis: a rich source of natural antioxidants**

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Propolis (bee glue) is a sticky material collected by bees from plants, and serves dual purposes in the hive: as building material and as defensive substance. Its most important feature is the antimicrobial activity, a vital part of bees' social immunity, helping them to prevent infections. In addition, propolis has the potential to increase bee longevity via antioxidant-related pathways. Because of these valuable properties, it has been a traditional constituent of remedies for more than 2000 years, and nowadays, it is applied in food additives, cosmetics and functional foods. Recently, new promising aspects of propolis application emerge in food preservation and food packaging, based on the favorable combination of antioxidant and antimicrobial properties, and low toxicity.

As bee glue is a plant derived product, its chemical composition is highly variable and depends on the local flora. For this reason it offers a significant chemical diversity and the study of propolis from different locations often reveals new constituents and new antioxidant molecules.

Our studies on propolis from different regions of the world have revealed numerous compounds with high radical scavenging and antioxidant potential. The importance of the concentration of caffeic acid phenethyl ester in European type propolis was demonstrated, detecting a statistically significant correlation between them. This fact strongly suggests that concentration of CAPE in propolis and propolis extracts may be used to characterize its antiradical potential and used in standardization. Bioactive prenylated *p*-coumaric acid derivatives were isolated from Brazilian green propolis, and their effect on kinetics and mechanism of lipid bulk phase oxidation was studied. Compounds with significant antiradical activity were isolated from Brazilian red propolis (prenylated benzophenones), Kenyan propolis (prenylated flavonoids), Indonesian propolis (alkylresorcinols). Thus, further studies of propolis from unexplored region have the potential to lead to the isolation of new natural antioxidants.



## Design and synthesis of natural-like phenols and hydroxylated biphenyls to be applied in biomedical and agricultural fields.

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Flexible structures in natural and synthetic biologically active dimeric molecule have attracted considerable attention as effective ligands for receptor molecules involved in the etiology of many diseases in virtue of the possibility to enhance binding affinity in a wide variety of proteins. Hydroxylated biphenyls are widely present in nature and conversely their natural monomeric phenols, they represent an important source of bioactive compounds not yet well investigated. Structurally, they are C<sub>2</sub>-symmetry dimers of phenols where two aromatic rings are C-C single bond bridged. The presence of hydroxylated functionalities in the biphenyl structure provides interesting features in term of bioavailability, interactions with proteins, pharmacological and antioxidant activities and stereochemical control. Compared to phenols, often, hydroxylated biphenyls are less toxic than the corresponding phenolic monomer.

Biological activity of naturally occurring biphenyls can be enhanced by light modifications of substituents in the ring and lateral aliphatic chains where the new molecule still features a hydroxylated biphenyl unit.

Our interest in that class of compounds is high and during several years we have designed and prepared small collections of hydroxylated biphenyls. By virtue of close cooperations with research groups expert in different fields, biological activity of hydroxylated biphenyls have been studied in comparison with that of the corresponding monomers.

Herein we describe our recent research in designing, synthesis and studies of natural-like phenols and hydroxylated biphenyls starting from natural phenols as eugenol, vanillin, curcumin, apocynin, creosol, zingerone and dehydrozingerone. Their biological activity in biomedical and agricultural fields will be presented.

**References:** Marchiani, A. et al., *Amino Acids* **2013**, *45*, 327-338; Kancheva, V. et al., *Food Chem.* **2014**, *157*, 263-274. Dettori, M.A. et al., *Lett. Drug Des.&Discov.* **2015**, *12*, 131-139. Pedotti, S. et al., *Polyhedron* **2016**, *117*, 80-89. Pani, G. et al., *J. Agric.Food. Chem.* **2014**, *62*, 4969-4978. Profumo, E. et al., *Oxid. Med. Cell. Longev.* **2016** doi.org/10.1155/2016/1246485. Pani, G. et al., *PLOSone* **2016**, DOI:10.1371/journal.pone.0157316. Ruzza, P. et al., *Eur.J.Med.Chem.* **2017**, *126*, 1034-1038. Monti, P. et al., *Talanta* **2017**, *162*, 151-156.

## The antioxidant activity of catecholamines in dispersed lipid systems

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Some cellular mechanisms are common to neurodegenerative diseases: protein misfolding, aggregation, and formation of deposits in the nervous tissue. Such proteins can bind the transition metal ions to make them prone to initiate redox cycles and to overproduce the Reactive Oxygen Species. The oxidative stress can lead to damage or even death of neurons. Results of *in vitro* experiments showed that catecholamines, besides their neurotransmitting function, can protect the cells against oxidative damage, however, the mechanism of this activity remained unclear.

We employed advanced kinetic and calorimetric techniques (Differential Scanning Calorimetry, Isothermal Titration Calorimetry) to study the mechanisms of interaction of liposomes with catecholamines and their localization at water/lipid interface. As a result, we proposed a new model of interactions of dopamine with phospholipid membranes: electrostatic forces dominate in these interactions and dopamine interacts superficially with phospholipid membranes without penetrating into the bilayer hydrocarbon core. Our results are the first quantitative description of such interactions of catecholamine neurotransmitters.<sup>1</sup> We also presented a mechanism of synergistic effect observed for catecholamines (L-DOPA and dopamine) and analogue of  $\alpha$ -tocopherol (PMHC).<sup>2</sup> PMHC reacts with peroxy radicals and its phenoxyl radical is recovered/reduced to phenol form at water/lipid interface by semiquinone radicals formed during reaction of catecholamine with peroxy radicals. Final products are quinone and PMHC. Mechanism described by us can be potentially important in biological systems involving various classes of natural antioxidants.

Last part of this talk will be devoted to a reaction of PMHC with superoxide radical anion – recently we described an unusual stoichiometry of this process carried out in homogeneous solutions and we proposed a possible mechanism.<sup>3</sup>

<sup>1</sup> Jodko-Piórecka, K., Litwinienko, G. *ACS Chemical Neuroscience* **2013**, 4, 1114-1122.

<sup>2</sup> Jodko-Piórecka, K., Litwinienko, G. *Free Radical Biology and Medicine* **2015**, 83, 1-11.

<sup>3</sup> Cedrowski J., Litwinienko G., Baschieri A., Amorati R. *Chemistry -A European Journal* **2016**, 22, 16441 – 16445.

## **Antioxidant activity of natural compounds in food model systems**

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Antioxidants of natural origin play an important role in preventing oxidant damage both *in vivo* and *in vitro*. In the body, dietary antioxidants neutralize free radicals and therefore control age-related and chronic disease. In foods, antioxidants play a crucial role in preventing or delaying autoxidation and formation of off-flavors as well as loss of food quality and nutritional value. To measure antioxidant activity, a variety of methods are available and so far no uniform procedure has been found to be applicable to all model systems. In Food Science, from practical point of view most important are the methods with directly additions of the extracts or pure natural antioxidants to bulk oil, emulsion, meat, fish. After storage time, the oxidative damage of lipids or proteins are measured.

The aim of this lecture is to provide the findings and perspective related to evaluation of antioxidant activity in selected food models. The protective effect will be discussion in terms of the used plant material, natural antioxidant added to food, and analytical method used for measure oxidation process.

## Widening the Chemiluminescence Bioantioxidant Assays

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Since reactive oxygen species (ROS) responsible for an oxidative degradation of organic and biomaterials cause the excited-state generation manifested by the light emission, measuring the chemiluminescence derived from oxidation processes provides an efficient tool to monitor the ROS as well as the pertinent antioxidative species and their reactivity. Such a chemiluminescence approach is most straightforward in cases of structurally simple bioantioxidants (foremost, of monophenolic origin). However, increasing the structural complexity of bioantioxidants molecules entails new intricate features of their behavior in oxidation processes. Besides, considering the metabolic transformations of bioantioxidants under physiological conditions is of prime importance for a rational design of the adequate and reliable bioantioxidant assays. Herein, we provide a detailed analysis of the mechanistic and kinetic intricacies of the chemiluminescence in oxidation reactions in the presence of bioantioxidants and the addressed issues refer to (i) changing the chemiluminescence kinetics upon consuming complex bioantioxidants in oxidation of hydrocarbon substrates with respect to what is observed in cases of structurally simple chain-breaking antioxidants, (ii) appearance of the "nonclassical" channels of the excited-state generation in the presence of polyphenolic reactants in the oxidation system (referred to the formation of dioxetane radicals), (iii) possibility of emerging more than one light emitters and involving the electronically excited species in the reaction sequence in cases of metal-catalyzed processes and (iv) metabolic transformation of bioantioxidants in the presence of biomolecules (most prominently, amino acids) with the formation of stronger endogenous bioantioxidants. To address the latter issue, we have recently designed a novel assay based on the peroxidase-catalyzed luminol chemiluminescence process in the presence of H<sub>2</sub>O<sub>2</sub> involving polyphenolic bioantioxidants and amino acids. In case of glycine, oxidation of polyphenolic species in such a system under physiological conditions affords new products (most likely, polyphenol-glycine adducts), whose antioxidant activity is much higher than that of unoxidized bioantioxidant molecules.

# **ICBA 2017**

## **Keynote Lectures**

## **OXIDATIVE STRESS AND RELATED DISEASES**

Prof. DSci. Veselina Gadjeva, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

Oxidative stress plays a significant role in the pathogenesis of a number of human diseases such as ischemia/reperfusion injury, atherosclerosis, cancer, neurodegenerative diseases and allergy. We have a big experience on the investigations the level of some oxidative stress parameters for 160 normal healthy volunteers aged between 14 to 55 years of either sex and over 700 patients with different diseases.

Herein we present our results on investigation the influence of chemotherapy on the plasma level of lipid peroxidation and antioxidant status of patients with malignant hematological diseases.

The results showed that there is an increased oxidative stress and imbalance in the antioxidant defense in non-treated patients as a result of accumulation of free radicals due to the cancer process. After polychemotherapy, the oxidative stress increased and the imbalance of antioxidant enzyme system significantly progresses. So, administration of antineoplastic agents during cancer chemotherapy results in a much greater degree of oxidative stress than is induced by cancer itself. Patients with polychemotherapy with the combination of vincristine, melphalan, cyclophosphamide, lomustine CCNU (M-2 ) are in the biggest risk of oxidative injury.

Having in mind that newly synthesized spin labeled nitrosourea SLENU exhibited in vivo high antitumor effects against different tumor models and possessed antioxidant properties like those of the typical antioxidants such as vitamin E, we consider that new combination chemotherapy schemes containing lower dose of the high toxic antineoplastic agent plus proper spin labeled nitrosourea such as SLENU could decrease the toxic side effects of chemotherapy scavenging  $\cdot\text{O}_2^-$ .

## **Modulation of Oxidative Stress as a Pharmacological Strategy**

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Oxidative stress (OS) is linked with many pathologies ranging from cancer to neurodegenerative diseases and modulators of oxidative stress can be of value in some of these diseases. Different direct and indirect mechanisms by which OS can be modulated will be presented, including scavenging and metal chelating effects, mimicking the antioxidant enzymes or upregulation of their expression, activation of nuclear factor erythroid 2-related factor 2 (Nrf2) and inhibition of pro-oxidant enzymes among others.

The reasons that may explain why redox therapies failed in many instances will be illustrated. Novel approaches to redox therapies are necessary and the availability of biomarkers capable to predict the relative clinical response is crucial.

### **Selected references**

1. Saso L, Firuzi O. Pharmacological applications of antioxidants: lights and shadows. *Curr Drug Targets*. 2014;15:1177-99.
2. Firuzi O, Miri R, Tavakkoli M, Saso L. Antioxidant therapy: current status and future prospects. *Curr Med Chem*. 2011;18:3871-88.



## Natural antioxidants from herbs and shrubs of Mediterranean grasslands

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We started our research with an ethno-botany survey on plant species utilized by farmers to treat ailments of domestic animals. We identified the species that had high farmers' consensus for efficacy. We collected such species from natural grasslands and analyzed them for their antioxidant capacity (TEAC) by ABTS and DPPH methods, total phenolics, total flavonoids, and extractable condensed tannin content. We also determined their antioxidant activity in human cell-lines by using a cell-permeant indicator, 2',7'-dichlorofluorescein diacetate (DCF) and examined the cell viability after the treatment with plant extracts, by using a tetrazolium-based colorimetric test ([3-(4,5 dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide] MTT assay). We observed wide variation of antioxidant capacities, ranging from 3.91 to 131.14 TEAC/100 g DW. We found high antioxidant capacities in the same set of species in both chemical assays (ABTS, DPPH). The detection of antioxidant activity did not always translate into biologically active compounds when tested in cells. Some of the plant species showed antioxidant activity in cells and in the chemical assays, although further investigations are needed to assess benefits to animals, effective natural sources of antioxidants might be uncovered from traditional ethno-veterinary knowledge.

## **Interfacial concentrations of antioxidants in oil-in-water emulsions and cut-off effects: role of the HLB of the antioxidant on their efficiencies.**

Carlos Bravo-Díaz<sup>1</sup>, Marlene Costas<sup>2</sup>, Sonia Losada-Barreiro<sup>1,2</sup>, Fátima Paiva-Martins<sup>2</sup>, Laurence S. Romsted<sup>3</sup>.

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Key aspects relating the structural features of antioxidants (AOs) to their activities are not understood, in part, because structural similarities of AOs do not always mean similarities in their activity. We employed the pseudophase kinetic model to determine distributions and interfacial concentrations of series of AOs with the same reactive group but of different hydrophobicity in intact oil/water/tween 20 emulsions. We also determined the effects of the AOs on the oxidative stability of the emulsions by monitoring the formation of conjugated dienes (CDs) at early stages of oxidation and evaluated their relative effects as the time required to increase the amount of conjugated dienes, CDs, after the propagation step of the radical reaction is reached.

Results show that AO efficiency does not increase linearly with increasing the number of C atoms in their alkyl chain but is parabolic-like, with a maxima at an intermediate (C4 – C12) chain length. This parabolic dependence of AO efficiency upon chain length for both chemical and biological activities of series of homologous AOs up to a critical point after which their activity decreases is known as the “cut-off” effect. Results provide physical evidence that the variations in their efficiency are due to differences in their interfacial concentrations, confirming that there is a direct relationship between the interfacial AO concentrations and their efficiency. Application of the pseudophase kinetic model furnishes, therefore, a natural explanation, based on molecular properties, to the cut-off effect.

## On the Design of Synthetic Analogues of Natural Products

Vladimir Dimitrov

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The interest towards utilization of natural compounds is steadily growing in the industrial countries applying them for naturally based products for different purposes (therapeutic and preventive formulations, cosmetics, functional foods, etc.), but also as inspiration for development of drugs. Between 25% and 50% of currently marketed drugs owe their origin to natural products. Compounds isolated from natural sources are used in recent years as “templates” for design of synthetic drugs by applying of different kind of approaches and technologies. There are billions of possible combinations of small organic fragments providing the diversity of naturally available compounds. Less than 1% of the space containing natural compounds has been explored with the purpose to create pharmaceutical products. However, the identification of pharmaceutically relevant natural compound is not enough for creation of a synthetic drug, which is in general the requirement of the pharmaceutical industry. For the identification of a drug candidate is necessary to synthesize a variety of new structures for optimization of the bioactivity. The main tool in the optimization campaign is the synthetic chemistry. In the present contribution several modern synthetic approaches will be presented on the examples of bioactive natural compounds and newly approved drugs.

# MUTUAL INFLUENCE OF LIPIDS-ANTIOXIDANTS-SURFACTANTS IN MICROHETEROGENEOUS SYSTEMS

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In recent years, there has been a steady trend in the use of natural products in innovative technologies for the creation of healthy and healing foods, cosmetics, medicines containing essential unsaturated lipids and natural antioxidants (AO). Multicomponent microheterogeneous systems usually contain surface-active substances (surfactants), which may have an impact not only on the structure and on distribution of components in the system, but also on the reactivity and chemical behavior of key components. This topic reviews the current understanding concerning the role of surfactants themselves in oxidation preventing and inhibiting, and the influence of surfactants on behavior of known natural and synthetic antioxidants. The mutual influence of the components and the conditions of occurrence of synergism and antagonism in a complex system of {Surfactants-AO-Lipids} is discussed as well.

Phospholipids are common natural nonionic (zwitterionic) surfactants. Egg and soy phosphatidylcholine (PC, lecithin) are widely used in food, cosmetic and medical industries. The various mechanisms of the PC influence on lipid oxidation are considered such as synergistic enhancement of the inhibiting effects in the combination PC with phenolic antioxidants (tocopherol, catecholamines, quercetin and its derivatives); the protective and antioxidant role of PC due to the obstruction of the exit of radicals from the mixed reversed micelles with hydroperoxides and metal compounds.

Cationic surfactants ( $S^+$ ), which are used as antibacterial additives in detergent formulations, or as antibacterial drugs, were found to catalyze lipid oxidation [1-3]. The key reaction responsible for catalytic lipid oxidation is accelerated decomposition of hydroperoxides (LOOH), primary oxidation products, into the radicals in mixed micelles:  $m\text{LOOH} + nS^+ \rightleftharpoons \{nS^+..m\text{LOOH}\} \rightarrow \text{LO}_2^\bullet$ . A faster consumption of AO in the reactions with  $\text{LO}_2^\bullet$  and LOOH activated in the mixed micelles determines the antagonism of cationic surfactants and AO.

Special attention is paid to the peculiar pro- and antioxidant properties of thiols such as endogenous glutathione, cysteine and homocysteine, as well as a number of SH-containing drugs included in the list of vital and essential medicines. Recent data show that the thiols are very different from other AO in reactions with reactive forms of oxygen (ROS) and other oxidants (iodine, quinones, quinone-amines,  $\text{Fe}^{3+}$ ). In combination with phenolic AO, thiols can show synergism of the antioxidant action, whereas with the aromatic amines, carotenoids, and some other AO thiols may exhibit antagonism.

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## References

- [1] O. T. Kasaikina, V. D. Kancheva, T.V.Maximova et al. // Oxid.Comm 2006, N3, 574.
- [2] V. D. Kancheva and O. T. Kasaikina //Current Medicinal Chemistry, 2013, **20** 4784-4805
- [3] O.T.Kasaikina, L.M.Pisarenko // Russ. Chem. Bull. (Int. Ed.), 2015, **64**, 2319.

## Preservative properties of lipophilic extracts of a by-product resulting from jumbo squid (*Dosidicus gigas*) commercialisation

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### ABSTRACT

By-products of marine species are body parts that are usually removed before commercialization and can include heads, blood, bellies, viscera, skin, trimmings, bones and fins. Among them, squid skin by-product has received considerable attention as being an important source of coloured pigments with potential healthy compounds. Jumbo squid (*Dosidicus gigas*) is one of the biggest known cephalopods and has captivated an increasingly technological and composition interest in a great number of countries. During its processing, up to 60% of its weight is treated as by-products that nevertheless may contain biologically active compounds related to antioxidant and antimicrobial activities.

In this context, the present research aimed to analyse the potential preservative behaviour of a 0.5% acetic acid-ethanol extract obtained from jumbo squid skin (JSS). The study was developed in three complementary steps. In a first one, JSS extracts were characterised with respect to solubility in different solvents, absorption UV-Vis and FT-IR spectra, and tested for its radical scavenging activity against ABTS and oxygen radical absorbance capacity (ORAC assay) and potential toxicity. Secondly, the potential ability of JSS extracts for inhibiting lipid oxidation (assessment of conjugated dienes and trienes, peroxides, thiobarbituric acid value and polyene index) was analysed in an accelerated model system including a heated (50°C for 12 days) marine oil (i.e., cod liver oil). Finally, aqueous solutions containing acetic acid-ethanol extracts of JSS were included into the icing media employed for the chilling storage of Atlantic hake (*Merluccius merluccius*) and Atlantic mackerel (*Scomber scombrus*); in both storage studies, the chemical (lipid oxidation development and hydrolysis; volatile amines formation), microbial (development of microbial groups) and sensory (skin and mucus development; eyes appearance; external odour; gills appearance and odour; consistency; raw and cooked flesh odour; cooked flesh taste) quality of fish was evaluated throughout a 13-day storage.

As a result, the whole study demonstrated that acetic acid-ethanol extracts from JSS can be considered a valuable antioxidant and antimicrobial source. In agreement with previous research, this preservative activity could be explained on the basis of the presence of lipophilic-type molecules (i.e., ommochromes compounds). Consequently, squid skin may be successfully employed to obtain new products related to food processing and pharmaceutical industries with environmental sustainability benefits and leading to a more effective use for jumbo squid by-products.

## **Essentiality of dietary non-essential amino acids for antioxidative defense**

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The amino acids are not only a building blocks of proteins, but confer many other functions: regulation of cell signaling pathways (Arg, Gln, Gly); regulation of acid-base balance (Gln); regulation of antioxidative reactions (Glu, Cys, Gly); regulation of gene expression (Gln, Arg, Gly); regulation of immune function (Arg, Gln, Pro); regulation of energy and nutrient metabolism (Arg, Glu, Gln, Pro, Gly), and others.

The aim of current study is to present scientific data concerning the essentiality of dietary non-essential amino acids (NEAA) for antioxidative defense in the body, and to present data about their composition and quantity in Bulgarian foods.

There are several proposed mechanisms for explanation the antioxidant activity of amino acids. The role of the amino acids that participate in glutathione synthesis (Cys, Glu, Gly) is clarified. Experimental dietary supplementation with those amino acids showed increases hepatic GST synthesis. Glutathione plays an important role in maintaining cellular redox balance and reducing oxidative stress. L-cysteine activate the generation of hydrogen sulfide (H<sub>2</sub>S), cysteine-sulfinate, taurine and inorganic sulfur. Endogenous H<sub>2</sub>S is an anti-inflammatory, neuroprotective and antioxidant agent. It is demonstrated that H<sub>2</sub>S has therapeutic activity for oxidative stress, associated with neurodegenerative diseases.

NEAA are critical for antioxidative defenses also through antioxidative enzymes: glutathione peroxidase, superoxide dismutase, and H<sub>2</sub>O<sub>2</sub>- peroxidase.

Arginine has a multifaceted roles as a precursor for nitric oxide (NO) including vasodilation, antioxidation and antiplatelet effects. Some of arginine's effects as antioxidant and anti-inflammatory effects are independent of NO-production and arginine intake is directly related to serum levels of NO.

The current data clearly showed, that non-essential amino acids, especially that containing extra thiol, thioether, or extra amine group, had strong antioxidant activities. On the basis of the essentiality of dietary non-essential amino acids for antioxidative defence of the human body, it is recommended their broad participation in preventive and healthy diets.

## Naturally derived products as nutraceuticals for human health

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The nutraceuticals market is one of the most promising ones in the world.

However the nutraproducts need to be studied scientifically by showing which are the specific ingredients derived from plants, animals, insects, foods and so on.

Only by such approach clinical data could be produced to support the efficacy of the nutraproducts.

Irrespective of their source all medicinal chemical compounds should be accounted to similar standards of quality (identity purity stability), clinical effectiveness and safety.

This presentation aims to describe some basic principles and to provide some examples for naturally derived products which could be preventive and helpful for normalizing some human health disorders.



**ICBA 2017**

**Oral Presentations**

## **Antioxidant activity of lanthanide(III) complexes with biologically active ligands**

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Lanthanide complexes have recently received considerable attention in the field of medicine. The oxidative behavior of Ln(III) ions is of great importance for their medicinal applications. These studies highlighted the potential of developing novel lanthanide complexes with improved biological potency. As a part of our continuing work on the synthesis and pharmacological application of newly synthesised lanthanide(III) complexes, it was observed that changes in the structure of the ligands, using different metals as well as reaction conditions had profound effects on selectivity and activity of the products obtained. In the present work lanthanide(III) complexes with biologically active coumarins have been synthesized and their structures have been determined using theoretical, analytical and spectral investigations. The oxidative behavior of the lanthanide(III) complexes was estimated. The inhibitory effects of the complexes compared to that of the ligands proved their cytotoxic/cytostatic properties. The results have shown that the complexes reveal promising pharmacological properties which in all the cases are more pronounced for the metal complexes than for the respective ligands.

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## ***In vitro/in vivo* evaluation of the antioxidant potential of DicaFFEoylquinic acid isolated from *Geigeria alata***

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*Geigeria alata* (DC) Oliv. & Hiern. (Asteraceae), commonly known as 'Gud-gad' is a traditional aromatic medicinal plant with a number of biological activities, used in traditional medicine in Sudan. The aim of our study was to isolate and identify the major biologically active compound from *Geigeria alata* roots and to investigate its antioxidant activity both *in vitro* and *in vivo*. The main compound of *G. alata* roots was isolated using low-bar liquid chromatography and identified by liquid chromatography-mass spectrometry (LC-MS) as 3,5-dicaFFEoylquinic acid (diCQA). *In vitro* antioxidant activity of diCQA was performed by colorimetric methods. Compared to the crude extracts and pure chlorogenic acid, diCQA showed stronger radical scavenging activity and reducing power. On the basis of these *in vitro* data we suggested a good *in vivo* antioxidant activity of diCQA which was traced in male Wistar rats with streptozotocin (STZ) induced diabetes. Diabetes results in severe organ pathology which main pathophysiological mechanisms are related to oxidative stress, discerned by increased production of malondialdehyde (MDA) and disturbance in both non-enzymatic (GSH) and enzymatic (GPx, GR, GSH) antioxidant defense. diCQA (5 mg/kg/po) administered for 21 days to control and diabetic Wistar rats ameliorated the activity of the antioxidant enzymes and the levels of the cellular protector GSH as well as reduced the production of MDA. It also exerts antidiabetic effect in diabetic rats. On the basis of these results as well as knowing that formation of ROS is considered to be one of the mechanisms in the pathogenesis of diabetes we conclude that diCQA isolated from *Geigeria alata* possesses antioxidant properties which most probably determined its antidiabetic activity *in vivo*.

**Acknowledgements:** The study was carried out with the financial support by the Medical University of Sofia (Council of Medical Science, Project № 408, Contract 44/2016)

## **Role of Some Neosynthesized Styryl Heterocycles in ROS and mycotoxin control.**

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Ochratoxin A (OTA) is a mycotoxin whose dangers have been sufficient for many countries to regulate its presence in various foods. In different countries, the black Aspergilli group, in particular *Aspergillus carbonarius*, causes the highest OTA contamination in fruit. Here we describe the effect of three novel styryl heterocyclic compounds on (1) the prevention of OTA biosynthesis by *A. carbonarius* cultured in a conducive liquid medium, (2) Reactive Oxygen Species (ROS) formation, and (3) lipoxygenase (LOX) activity. The most effective control of OTA biosynthesis was achieved with (E)-3-(3,4-dimethoxystyryl)-thiophene. In fungal cultures treated with this compound at 5, 25, 50, and 100 ppm, OTA biosynthesis decreased by 45% to 95% until the experiment's conclusion (8 days).

(E)-3-(3-methoxy-4-acetoxy-styryl)-thiophene significantly inhibited OTA biosynthesis (by about 50%). However, this effect lasted for only few days when used at 5 or 25 ppm and up to 8 days when used at 50 or 100 ppm; (E)-3-(3-methoxy-4-hydroxy-styryl)-thiophene did not exert any control on OTA biosynthesis. The compounds able to control OTA biosynthesis also significantly inhibited ROS formation and LOX activity. The nature of the substituent in para position strongly affects the molecule's reactivity. Lower reactivity enhances the inhibition of OTA biosynthesis, in particular long-term. Natural compounds present in edible plants having a styryl heterocyclic scaffold may be effective inhibitors of OTA biosynthesis.

## Effects of orotic and 5-amino orotic acids on the free radicals accumulation in rat blood serum.

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Orotic (HOA) and 5-aminoorotic (HAOA) acids are ligands in metal complexes with *in vitro* antioxidant and anticancer activities. Dietary orotic acid *in vivo* increases the free radicals formation in the liver by diminishing both activity and mRNA level of Cu,Zn-SOD. It seems that HAOA may act as antioxidant by scavenging free radicals, but as prooxidant by diminishing the efficacy of Cu,Zn-SOD. The effect of orotic acid on the accumulation of free radicals in the blood serum is still not assessed. In this investigation free radicals formation in rat blood serum was achieved by adding small amount of xanthine, thus superoxide production was increased. The effects of HOA and HAOA (within concentrations of  $10^{-4}$  and  $10^{-6}$  M) on the accumulation of free radicals in the blood serum was monitored using spectrophotometric method. Much less free radicals were detected in the blood plasma in the presence, than in absence of these compounds, the effect being stronger for HAOA. The weaker antioxidant effect of HOA compared to HAOA on the free radicals accumulation in rat blood serum might be result of negative influence on Cu,Zn-SOD, along with radicals scavenging activity of HAOA. More detailed investigations are under way. It was assumed that at concentrations below 0.1mM, the antioxidant effect of HOA and HAOA in the blood serum prevailed.

## **Radical scavenging activity toward 2,2-diphenyl-1-picrylhydrazyl and hydroxyl radicals of 5-aminoorotic acid and its Ga(III) complex**

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Along with antitumor activity, flexible control over oxidative stress (OS) levels is a desirable quality of any anticancer drug. Radicals scavenging activity (RSA) toward 2,2-diphenyl-1-picrylhydrazyl radical (DPPH<sup>•</sup>) is widely used to evaluate the ability to eliminate free radicals by donating hydrogen. 5-aminoorotic acid (HAOA) has antioxidant properties and has been used as a ligand in lanthanide(III) complexes possessing anticancer activity in cell cultures. Ga(III) salts are known for their anticancer activity. Thus, the Ga(III) complex with HAOA (GaAOA) might be a promising anticancer agent with antioxidant properties, that have not been explored so far. In the present work, the UV spectra and RSA of HAOA and GaAOA toward DPPH<sup>•</sup> and OH<sup>•</sup> were evaluated and discussed. The stereochemistry of HAOA and its Ga(III) complex was evaluated, and compared with spectral data. Two factors affected the UV spectra of the molecules: their arrangement and their interaction with the solvent. As far as the RSA was determined in absolute ethanol (for DPPH<sup>•</sup>) and in water (for OH<sup>•</sup>), the UV spectra of the molecules in water and ethanol were discussed. The hypochromicity in the UV spectra of GaAOA, compared to the expected intensities, indicated an arrangement of the ligands that diminished the dipole moment. The RSA of HAOA and GaAOA towards both radicals was concentration-dependent. GaAOA, at the lowest concentration, in ethanol, exhibits signs of dissociation, manifested in an anomalous RSA increase. That demonstrates the potential of GaAOA for a controlled release of the antioxidant ligands.

## **Antioxidant Activity of Black Chokeberry (*Aronia melanocarpa*) Polyphenols: *in vitro* and *in vivo* Evidences and Possible Mechanisms of Action.**

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Black chokeberry (*Aronia melanocarpa*) is a distinctive berry with a high content of polyphenol compounds and possesses one of the highest *in vitro* antioxidant activities among fruits. The bioavailability of aronia polyphenols seems to be low, but there is ample evidence for chokeberry health benefits including antidiabetic, cardioprotective, hepatoprotective, antimutagenic, and anticarcinogenic effects. The lecture presents the available information for the bioavailability and antioxidant activity of chokeberry polyphenols and explains the possible mechanisms of action *in vivo* in the prevention and treatment of oxidative stress-related diseases. It reviews the available data for chokeberry antioxidant activity *in vitro*, in isolated cells and cell lines, and *in vivo*, in both human subjects and animals. It is evident that *in vivo* antioxidant action of chokeberry extends far beyond radical scavenging and includes suppression of reactive oxygen and nitrogen species formation, inhibition of prooxidant enzymes, restoration of antioxidant enzymes, and probably cellular signaling to regulate the level of antioxidant compounds and enzymes.

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## Study on the *in vitro* activity of Praseodymium complex of 5-aminoorotic acid

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The aim of our study was to investigate the possible cytoprotective and antioxidant potential of Praseodymium complex of 5-aminoorotic acid (PrAOA) on sub-cellular (isolated rat liver microsomes) and cellular (isolated rat hepatocytes) level.

*In vitro* hepatoprotective and antioxidant activity of PrAOA were evaluated on non-enzyme lipid peroxidation (on isolated rat liver microsomes) and *tert*-butyl hydroperoxide (t-BuOOH) (on isolated rat hepatocytes) toxicity models. The effects of PrAOA, were compared to those of 5-aminoorotic acid and Quercetin.

Isolated rat liver microsomes are suitable *in vitro* model of lipid membrane, which is convenient for the investigation of lipid peroxidation processes. On this model, we determined the production of MDA, product of the lipid peroxidation. In conditions of non-enzyme lipid peroxidation, Praseodymium complex of 5-aminoorotic acid (at concentration 100  $\mu$ M) revealed good statistically significant antioxidant activity (decreasing MDA production), closer to those of Quercetin and stronger than those of 5-aminoorotic acid (AOA).

Isolated rat hepatocytes are a suitable model for hepatotoxicity studies. We determined the main parameters of the functional and metabolic status of rat hepatocytes: cell viability (measured by trypan blue exclusion), and the levels of lactate dehydrogenase (LDH), reduced glutathione (GSH) and malondialdehyde (MDA). In t-BuOOH-induced oxidative stress, Praseodymium complex of 5-aminoorotic acid (at concentration 100  $\mu$ M) showed statistically significant cytoprotective and antioxidant activities, closer to those of Quercetin and stronger than those of AOA. The complex prevented the loss of cell viability and GSH depletion, decreased LDH leakage and MDA production.

The stronger hepatoprotective and antioxidant activity of PrAOA on both *in vitro* toxicity models than those of AOA, might be due to the presence of Praseodymium in the complex.

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## The Flavonoids Composition of Bulgarian Foods – Comparison with USDA Database

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Flavonoids are polyphenolic antioxidants of plant origin, comprising more than 5000 individual compounds. Their protective role to human health is associated with reduced risk of development of series of degenerative diseases. Therefore the quantitative knowledge of the flavonoids composition is of crucial importance for elaboration of healthy nutrition diets.

The aim of the study is to estimate differences between Flavonoids Content of Bulgarian foods and the USDA Flavonoids Database in order to establish the main factors leading to data variability.

For achieving the set aim, the Bulgarian Flavonoids Food composition Database have been translated and transferred in Excel format, according the unified Access scheme of the USDA Database. The fast track and comparison of the data was made with automotive program written on VBA.

In the present study original data for representatives of 3 major classes of flavonoids in Bulgarian foods are presented. The data for the flavonols – myricetin, quercetin and kaempferol, the catechins – (+)-catechin and (-)-epicatechin and flavones – luteolin and apigenin are measured by validated HPLC methods. The food samples are collected according precise sampling plan and the origin of all foods has been documented. In the current study data for 21 Bulgarian wines, 15 fruits, 30 vegetables and vegetable products and 3 leafy green condiments are reported. Our data are compared with the pool of results, summarized in USDA Database for the Flavonoids Content of Foods, where data for 506 foods are presented, covering 308 scientific literature papers.

The results show that for most of the foods our data are in tune with USDA database, however we could point out that data for (+)-catechins in sour cherry are reported only in our study and this how our data enrich the flavonoids content pool. One of the major defenses was found for Parsley, where data for apigenin in Bulgarian leafy condiments are with mean value of 74.79 mg/100g fresh weight (n=3, min=63,99 mg/100g, max=84,20 mg/100g), while in USDA Database the mean value for apigenin is 215.46 mg/100g (n=26, min= 0 mg/100g, max = 630.00 mg/100g).

Conclusion: the current study presents the defenses between one Regional and one International Flavonoids Food Composition Database and emphasizes the importance of precise data from different origin for establishing the specificity and biovariability of foods in order to provide correct nutrition guidelines and healthy dietitian recommendations.

## Evaluation of the antioxidant potential of defatted extract from *Astragalus spruneri* (Fabaceae) in spontaneously hypertensive rats (SHRs)

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*Astragalus spruneri* Boiss. (Fabaceae) is a clump-forming perennial plant. It is distributed in the Balkan Peninsula and Turkey and belongs to the section Incani DC of subgenus *Cercidothrix*. This section of genus *Astragalus* L. is known to have issues with its taxonomic structure and morphological similarities are often present. Only a few of the species from the section have been studied, as many being rare or endangered.

The aim of this study was to evaluate the antioxidant potential of extract from *A. spruneri* in spontaneously hypertensive rats (SHR). Hypertension is a socially significant disorder and oxidative stress is regarded as one of the main pathophysiological mechanisms. Defatted extract of *A. spruneri* (AS) was administered at a dose of 100 mg/kg bw (1/20 LD<sub>50</sub>) for 14 days. At the end of the treatment period the animals were euthanized and the activities of catalase (CAT), superoxiddismutase (SOD) and glutathionperoxidase (GPx) as well as the levels of non-enzyme cell protector reduced glutathione (GSH) were assessed in the brain, liver, kidney and spleen of SHR. In comparison to normotensive Wistar rats, in control, non-treated SHRs the GSH level and the activity of GPx were decreased in all organs, while the activity of CAT and SOD was decreased in brain, liver and kidney, and unchanged in spleen. Compared to the control SHRs *A. spruneri* extract exerted antioxidant activity, discerned by statistically significant increased activities of CAT and SOD in liver and kidney, of GPx and GSH - in liver, kidney and spleen. It is worth to be noted that the extract did not exert any effect in the brain. This might be due to the fact that it cannot penetrate the blood brain barrier. Based on the results of our study we could conclude that the lyophilized extract of *A. spruneri* showed antioxidant potential in spontaneously hypertensive rats – a model of essential hypertension in humans.

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## **Antioxidant properties of green synthesized silver nanoparticles and their effect on moderate local heat burn model.**

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The free radicals accumulation due to necrosis and oxidative burst contributes to the local and systemic effects of the thermal injury. If tightly controlled, the oxidative stress (OS) initiates wound healing, while if uncontrolled it results in local and distant tissues damage. The OS may be successfully diminished by administering scavengers of free radicals along with substances preventing bacterial superposition. Silver is common antimicrobial agent with no registered allergic reactions and high efficiency against antibiotic resistant bacterial strains. Recently, wound dressings, creams and ointments containing silver nanoparticles (AgNPs) are preferred in ulcerations treatment. There are two problems to be resolved in using wound dressing-coated AgNPs: the removal of potentially harmful byproducts of the AgNPs synthesis, and the risk of allergic reactions toward the carrier of AgNPs itself. The aims of this investigation were: 1- to perform AgNPs synthesis in a “skin friendly” reaction medium (glycerol) and natural antioxidants (green tea extract) as Ag<sup>+</sup>- reducing and capping agents; 2- to observe the effect of this product on the skin oxidative stress in the wound field in the acute phase of moderate local heat burn. AgNO<sub>3</sub> (1mM in glycerin) and green tea extract (100mg/ml, in glycerin) were used to prepare AgNPs suspension (Ag concentration of 0.02 wt%). The radicals scavenging activity for 30 minutes toward DPPH<sup>•</sup> radical (RSA) of the reactants and reaction product were evaluated. Moderate heat burn wounds (1 cm<sup>2</sup>) were created on the backs of 6 anaesthetized male Wistar rats. Three animals with burn wound were left untreated. The other three were topically treated ones per day with of 0.04 ml AgNPs suspension for three consecutive days. Three unwounded animals were used as controls. The lipid peroxidation (MDA) and RSA of both treated and untreated rats with burn were determined and compared with this for control animals. The AgNPs suspension showed slightly lower RSA than this of the green tea extract alone (86±3 and 90±2, respectively). After 72 hrs the injured skin RSA decreased almost twice, and the MDA increased three times, compared to the healthy control skin. Topical application of AgNPs containing suspension resulted in twice less MDA and almost 40% higher RSA in the wound field, in comparison with the untreated burned skin.

**ICBA 2017**

**Poster Presentations**

## **Antioxidant activity of naturally occurring phenols and their corresponding biphenols as individual compounds and in mixtures with anticancer drug 5-fluorouracil**

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The opportunity of combination therapies based on natural products and approved drugs, such as the combination of 5-fluorouracil (5-FU), an antineoplastic drug, with phenols has been successfully investigated. Although the growing interest in these mixtures and their potential use in medicinal applications, no information has been reported on their antioxidant activity in term of additivism or synergism that results from the combinations of these compounds. The present study aimed to investigate the antioxidant effect of 5-FU as individual compound and in equimolar mixture with some natural phenols and their corresponding biphenols. Electrochemical behavior of 5-FU and its antioxidant power on the oxidative environment produced by hydrogen peroxide (HP) have been assessed. Moreover, the eventual synergism produced by the co-presence of 5-FU and one of the most important physiological antioxidant compounds as ascorbic acid (AA) has been studied. Results showed that 5-FU did not show any antioxidant activity, not affecting the HP current, while AA was able to significantly reduce the signal produced by HP. The co-presence of 5-FU and AA determined a slightly higher decrease in the HP signal, if compared with AA alone. Protective effect of some naturally occurring phenols and their biphenols as individual compounds and as equimolar binary mixture with 5-FU were studied during bulk lipid autoxidation. Syntheses of biphenols were carried out under sustainable conditions by C-C coupling reaction of the corresponding monomer. The results of this study showed that the inhibitory effect of the mixtures was similar to those of the individual phenols and even weaker i.e. neither synergism, nor additivism were obtained.

## Chain breaking antioxidant activity of new synthetic analogues of curcumin as individuals and in mixtures with conventional antioxidants

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The effectiveness of development of new biologically active compounds with antioxidant potential has been proved in the treatment of various diseases. The design of new synthetic analogues which combine minimal toxicity, stability and improved biological properties compared to the parent compound has always been of interest for medicinal chemistry. Curcumin is one of the most powerful natural bio-antioxidants but unfortunately its poor bioavailability and instability at physiological level is a serious disadvantage. Previously, we observed high antiproliferative and apoptotic activity of C<sub>2</sub>-symmetry hydroxylated biphenyls curcumin-analogues against malignant melanoma [1]. We hypothesized that the presence of a biphenyl scaffold in a curcumin structure would control rigidity at the two aromatic rings and thus play an important role in enhancing antitumoral activity. Herein we have extended the synthesis of biphenyls-curcuminoids and their corresponding monomers. Chain-breaking antioxidant activity of the studied compounds is evaluated during bulk phase lipid autoxidation at 80 °C. Compounds that exhibit weak to moderate antioxidant activity were tested in mixture with stronger antioxidants. Synergism and antagonism of mixtures were assessed and discussed. All structures are optimized at UB3LYP/6-31+G(d,p) level in gas phase to explain the structure-activity relationships.

[1] Dettori, M. A. et al. *Lett. Drug Des. Discov.* **2015**, *12*, 131-139.



# SUSTAINABLE OXIDATIVE COUPLING OF NATURAL BIOACTIVE HYDROXYLATED PHENOLS

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Hydroxylated phenols like vanillin (4-hydroxy-3-methoxybenzaldehyde), apocynin (1-(4-hydroxy-3-methoxyphenyl)ethanone), iso-apocynin (1-(2-hydroxy-3-methoxyphenyl)ethanone), creosol (2-methoxy-4-methylphenol), zingerone (4-(4-hydroxy-3-methoxyphenyl)-2-butanone) and eugenol (2-methoxy-4-(prop-2-en-2-yl)phenol), are naturally occurring compounds. They are the main components of various plants like, *Vanilla planifolia*, *Picrorhiza kurroa*, *Guaiacum officinale*, *Zingiber officinalis* and *Eugenia carofillata*. These compounds show a broad spectrum of biological properties such as antiinflammatory, antiseptic, antioxidant and fungicide activities.<sup>a,b</sup> The corresponding C<sub>2</sub>-symmetric dimers, named hydroxylated biphenyls, generally show a higher bioactivity in comparison to the monomers.<sup>c</sup> Our interest in the field of hydroxylated biphenyls pushed us towards the optimization of oxidative coupling reactions of natural phenols with particular attention to aspects of sustainability of the synthesis. The reactions reported in the literature for symmetric dimerization of phenols deal with preparations mediated by chemical inorganic oxidants,<sup>d,e</sup> typically iron sulfate and potassium persulfate, or enzymatic reactions<sup>f</sup> (e.g. horseradish peroxidase). Surprisingly, to the best of our knowledge, there are not reported oxidative coupling reactions carried out with the aid of microwaves.

Our work is therefore aimed at optimizing, with the aid of microwaves, the synthetic process in terms of reduction of the amount of solvent, exclusive use of water as reaction medium and simplification of work-up operations. Dimers of hydroxylated natural phenols are important precursors of most of bioactive hydroxylated biphenyls recently prepared by our group.<sup>g-i</sup>

## References:

- a) Kim, J-H *et al. PLoS ONE*, **2014**, 9, 1-10. b) Lin, C-H. *et al. Mol. Plant Microbe. Interact.* **2009**, 22, 942-952. c) Klees, R. *et al. J. Biomed. Biotechnol.* **2006**, 2, 87246. d) Forsythe, W-G. *et al. Green Chem.* **2013**, 15, 3031-3038. e) Delomenede, M. *et al. J. Med. Chem.* **2008**, 51, 3171-3181. f) Antoniotti, S. *et al. Org. Lett.* **2004**, 6, 1975-1978. g) Dettori, M.A. *et al. Lett. Drug Des. Discov.* **2015**, 12, 131-139; h) Marchiani, A. *et al. Amino Acids*, **2013**, 45 (2), 327-338; i) Rozzo, C. *et al. BMC Cancer*, **2016**, 16:317.

# SUSTAINABLE SYNTHESIS OF NATURAL-LIKE HYDROXYLATED BIPHENYLS AS POTENTIAL BIOACTIVE COMPOUNDS

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Hydroxylated biphenyls with a  $C_2$ -symmetry axis have attracted considerable interest because of the biological activity exerted by a number of natural occurring compounds containing this moiety and because of their ability to transmit conformational information in stoichiometric and catalytic processes [1]. The geometrical possibilities offered by chemical transformation of the biphenyl skeleton make it a versatile building block in drug synthesis and catalysis [2]. With the aim to synthesize new biphenyl compounds, we have considered chalcones, an important class of bioactive compounds that has attracted much interest not only from the synthetic and biosynthetic perspectives but also due to its broad interesting biological activities [3]. Chalcone is chemically 1,3-diaryl-2-propen-1-one in which the two aromatic rings are bonded by a three-carbon  $\alpha,\beta$ -unsaturated carbonyl system [4]. Our study was aimed to prepare new chalcone compounds starting from commercial 1,1'-biphenyl-4,4'-diol, an exometabolite produced from cyanobacteria. For this purpose we prepared 4,4'-dihydroxy-[1,1'-biphenyl]-3,3'-dicarbaldehyde (*bis* salicylaldehyde) via formylation reaction of 1,1'-biphenyl-4,4'-diol with hexamethylenetetramine in the presence of trifluoroacetic acid [5]. Claisen-Schmidt condensation of *bis* salicylaldehyde with variously substituted acetophenones under microwaves technology provided the desired chalcone derivatives in good yields. Another class of compounds we have considered was a curcumin related biphenyls. In a previous study, we prepared dimer of OMe-dehydrozingerone **D6** [6]. We found that compound **D6** was more active in inhibiting malignant melanoma and neuroblastoma cells growth when compared to curcumin itself [7]. Starting from *bis* salicylaldehyde via Claisen-Schmidt condensation in the presence of LiOH and acetone we obtained an  $\alpha,\beta$ -unsaturated ketone analogue of **D6**. All synthesized compounds were obtained as air stable solids and easily purified by flash chromatography. Biological activity of all synthesized compounds is under evaluation.

## References

- [1] K. C. Nicolaou *et al.*, *Angew. Chem. Int. Ed.* **1999**, 38, 2096-2152. [2] G. Bringmann *et al.*, *Chem. Rev.* **2011**, 111, 563-639. [3] D. I. Batovska *et al.*, *Pharmacology* **2010**, 5, 1-29. [4] C. Zhuang *et al.*, *Chem. Rev.* **2017**, in press. [5] B. Karami *et al.*, *Helv. Chim. Acta* **2012**, 95, 455-460. [6] M. Pisano *et al.*, *Mol. Cancer* **2010**, 9:137. [7] M. Pisano *et al.*, *BMC Cancer* **2016**, 16:137.

## Phytochemical screening and antioxidant capacity of *R. damascena* Mill. flowers dry extract

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The by-products of agriculture industry have attracted the attention of scientists because of their potential antioxidant properties. *R. damascena* Mill is considered as one of the most important products for Bulgaria economy. Besides the rose essential oil, other famous rose products are concrete and absolute oils. The concrete oil is prepared by extraction of the fresh rose petals with n-hexane. What to do with this large amount of waste plant material?

The present study aimed a phytochemical characterization and assessment of the antioxidant capacity of the rose flowers after concrete oil production. Thus, the processed rose flowers were extracted with aqueous methanol followed by spray drying. Total phenolic content, total flavonoid content and total monomeric anthocyanins, determined spectrophotometrically, were found to be  $185.99 \pm 5.05$  mg GAE/g DE,  $154.90 \pm 3.41$  mg RE/g DE and  $22.71 \pm 1.77$  mg CGE/L extract. Rutin, hyperoside, isoquercitrin, quercitrin, astragalin were detected in the studied extract by TLC comparison with authentic standards. The presence of quercetin and kaempferol glycosides was confirmed by acid hydrolysis of the extract and TLC comparison with the corresponding aglycones. The antioxidant capacity of the rose flower dry extract was measured by its ability to scavenge the DPPH radical. Thus, the extract at concentration of 0.33 mg/ml was scavenging 64% of DPPH radicals. The antioxidant capacity expressed in Trolox equivalents (TE) was found to be  $1.58 \pm 0.05$  mM TE/g DE.

The results of this laboratory scale study showed that the by-product obtained after concrete oil production could be regarded as a good inexpensive source of antioxidant polyphenolics and used as health-promoting ingredient of functional foods and dietary supplements.

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## Activity-guided extraction optimisation of highly efficient antioxidant plant species: Study of *Rhodiola rosea* L. (Golden root)

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*Rhodiola rosea* L. (Crassulaceae) is a perennial plant, known in the folk medicine as Golden root, Roseroot, Arctic root. It is used as adaptogen, to treat depression, anxiety, fatigue, for stabilisation of the remission in psychological disorders, for improvement of the memory, attention and cognitive functions of humans. Classical and ultrasonic extractions have been used in the determination of optimal conditions for isolation of bioactive compounds from *Rhodiola* L. species, such as salidroside, *p*-tyrosol, rosavin, rosarin, rosin, rosiridin, flavonoids etc. Microwave assisted extraction and supercritical fluid extraction have been employed also to optimise the content of bioactive compounds in *Rhodiola* species. However, the conclusions about the optimal extraction conditions in terms of the solvent composition, ratio of the volume of the solvent to the weight of the raw material, and extraction duration differ considerably in the literature, depending on the extraction method and on the experimental parameters (bioactive compounds, total polyphenol content, flavonoids etc.), selected for extraction optimisation.

Considering the increased interest in obtaining high quality plant extracts for the prevention of cancer, with neuro-, cardio-, hepato-protective properties, we studied a group of efficient antioxidant plant species: *Rhodiola rosea* L., *Sideritis scardica* Griseb., *Achillea collina* (Becker ex Rechb.) Heimerl, *Achillea thracica* Velen., *Inula helenium* L., *Clinopodium vulgare* L., *Thalictrum aquilegiifolium* L. etc. In this comparative analysis, the *Rhodiola rosea* root demonstrated superior antioxidant properties - half-maximum DPPH-inhibitory concentrations (IC<sub>50</sub>) of  $29.24 \pm 1.43$  microgram/mL, correlated with high total polyphenol content values of  $374.11 \pm 17.37$  mg GAE/g dry extract. Therefore, the Golden root was selected for antioxidant activity-guided extraction optimisation, using different experimental conditions: concentrations of aqueous ethanol (as an ecological solvent), temperatures, ratio of the volume of the solvent to the weight of the dried raw material and extraction duration. The analysis of the effects of the experimental variables on the DPPH-radical scavenging activity in correlation with the TPC and the processing yields, determined that extraction of *Rhodiola rosea* root in 25% ethanol for 3-5 min at 50°C, using solvent/solid ratio 15 (v/w), leads to extract with optimal antioxidant activity. Quantitative analysis of the secondary metabolites (salidroside, rosavin etc.), identified by UHPLC/HRMS in the *Rh. rosea* extract, obtained at the determined optimal antioxidant activity-guided conditions, are in progress.

## Antioxidant and antiproliferative activities of *Juniperus virginiana* “Grey Owl” cultivated in Bulgaria

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Cancer is a leading life-threatening disease, involving abnormal division of malignant cells with potential to metastasize in different organs of the living beings. The identification of new natural sources of substances for prevention and therapy of cancer is object of increasing scientific interest.

We studied the total extracts of more than 40 medicinal plants of genus *Achillea* L., *Artemisia* L., *Betula* L., *Cupressus* L., *Centaurea* L., *Clinopodium* L., *Inula* L., *Juniperus* L., *Ruta* L., *Stachys* L., *Tanacetum* L., *Verbascum* L., among others, and established a leading antiproliferative activity of *Juniperus virginiana* “Grey Owl”, cultivated in Bulgaria. Highly efficient half-maximum cell growth-inhibitory concentrations were determined by the MTT-dye reduction assay after treatment of a panel of cancer cells, including MDA-MB-231 (breast cancer, ER<sup>-</sup>, PR<sup>-</sup>, HER2<sup>-</sup>), MCF-7 (breast cancer, ER<sup>+</sup>, PR<sup>+</sup>, HER2<sup>-</sup>), HT-29 (human colorectal adenocarcinoma), HeLa (human cervical carcinoma), HepG2 (hepatocellular carcinoma), HL-60 (acute promyelocytic leukemia) cell lines, with the extract of *Juniperus virginiana* “Grey Owl” (galbula). The IC<sub>50</sub> values, calculated from the corresponding dose-response curves, were in the range of 0.6-1.4 microgram/mL of the total juniper extract after treatment of the selected cell lines for 72 hrs. Using the UHPLC/HRMS method, we identified plant secondary metabolites, including podophyllotoxin, in the studied juniper extract.

Considering the scientific interest to obtain plant extracts exhibiting cancer prevention properties aimed at the preservation of the living cells from the harmful free radicals, we determined also the antioxidant activity and the total polyphenol content of the studied juniper extract. Using the DPPH-radical scavenging activity assay, we found a half-maximum DPPH-inhibitory concentration (IC<sub>50</sub>) value of  $289.26 \pm 1.87$  microgram/mL of the *J. virginiana* “Grey Owl” dry extract, and a total polyphenol content value of  $72.78 \pm 3.84$  mg GAE/g dry extract.

In conclusion, the total extract of *Juniperus virginiana* “Grey Owl”, cultivated in Bulgaria, was characterized as exhibiting efficient antioxidant and antiproliferative properties that represent this plant species as having potential application for the prevention and therapy of cancer as well as a new natural source of podophyllotoxin for the pharmaceutical industry. Quantitative metabolite analysis by UHPLC/HRMS of the extracts obtained from Bulgarian and foreign representatives of genus *Juniperus* L. is envisaged in the near future.

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## EFFECT OF *M. OFFICINALIS* L. ON THE LEVEL OF INDUCED LIPID PEROXIDATION IN MOUSE LIVER

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The aim of this study was to evaluate the effect of *M. officinalis* L. aqueous extract (MAE) on the level of induced lipid peroxidation in mouse liver homogenate. Samples were prepared from homogenized BALB/c mice liver, and subsequently incubated with one of the following lipid peroxidation inducing agents: 0,5 mM H<sub>2</sub>O<sub>2</sub>; 0,1 mM FeCl<sub>3</sub>+ascorbate or H<sub>2</sub>O<sub>2</sub> + FeCl<sub>3</sub>+ascorbate, in the presence or absence of MAE. MAE was prepared by extraction with boiling deionized water in 1:10 ratio (w/v). Two-fold dilutions (1:8 to 1:128) from MAE were used for the experiment. The levels of lipid peroxidation in mouse liver homogenate, caused by all of the oxidative agents were significantly reduced by all tested dilutions MAE. *M. officinalis* aqueous extracts could be effective for protection of mouse liver from induced lipid peroxidation. **Acknowledgements:** Supported by Grant DN-03-13/2016, NSF-MES, Bulgaria and Grant DKOST01/10, NSF-MES, Bulgaria, COST Action FA1403.

**Semiquinone glucoside derivative (SQGD) isolated from *Bacillus sp. INM-1*: antioxidant activities and protection against radiation-induced oxidative changes**

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In the present investigation, antioxidant activities and radioprotective potential of a novel semiquinone glucoside derivative (SQGD) isolated from radio-resistant bacterium *Bacillus sp. INM-1* was evaluated. UV and  $\gamma$ -radiation induced changes in DPPH radical-scavenging capacity and **protection** by SQGD was assessed. Stable free-radical properties of SQGD in powder/ solution form were estimated by using Electron Paramagnetic Resonance (EPR) spectrometry. Strong EPR signal with g-value 2.00485 (crystalline form) and g-value 2.00520 (solution form), were an established characteristic of o-semiquinone radicals. To observe **antioxidant activities** of SQGD on body-stimulation, “real time” kinetic- blood experiments, organ biodistribution (10, 30, 60, 90 min, 4h, 24h), ascorbate radicals levels and ROS production *ex vivo* was analyzed in liver and serum (4h after i.p.) of SQGD treated mice (6 mice; injected once; dose- 40 mg/kg i.p.) by using EPR methods.

As a result of SQGD treatment, significantly stably localization ( $p < 0.05$ ) in the organs (liver, lung, kidneys, spleen, pancreas, brain) and blood was observed. The blood clearance showed that maximal concentration was reached at 30<sup>th</sup> min and then declined gradually. SQGD exhibited considerably lower levels of Asc $\cdot$  radical and significantly inhibited ( $p < 0.05$ ) lipid peroxidation in liver homogenate and serum (\* $p < 0.05$ ) against CCNU- induced toxicity. Higher DPPH scavenging activity of SQGD was observed in  $\gamma$ - irradiated (20 Gy) samples ( $81.11 \pm 2.94\%$ ).

In conclusion, SQGD demonstrates well –expressed antioxidant and radical-scavenging activity towards DPPH radicals and can be developed as good protector against radiation-induced oxidative changes.

**KEYWORDS:** SQGD; UV and  $\gamma$ -irradiation; DPPH capacity; **antioxidant activities**

## ANTIOXIDANT/ ANTICANCER POTENTIAL AND RADIATION-PROTECTION ABILITIES OF NATURAL ANTIOXIDANT *PSORALEA CORYLIFOLIA* LINN

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*Psoralea corylifolia* Linn (*P.corylifolia*) was collected from plants growing in India (fresh seeds/ coarse powder dissolved in 2l of distilled water and subjected to cold maceration process for 24h) and its antioxidant, anticancer and radiation-protection abilities were evaluated by using *in vitro/ ex vivo* assays. To observe antioxidant activities of *P.corylifolia* on body-stimulation, blood-kinetic experiments, organ biodistribution (10, 30, 60, 90 min, 4h, 24h), ascorbate levels and ROS production was analyzed in liver and serum (4h after i.p.). Natural stable free radical properties of *P.corylifolia* in powder/ solution form were estimated using Electron Paramagnetic Resonance (EPR) spectrometry. EPR analysis *in vitro* demonstrated high DPPH radical-scavenging capacity at 1.6% concentration ( $66.52 \pm 0.089 \%$ , alone; and  $93.35 \pm 1.67\%$ , after 10 Gy) before and after radiation. EPR distribution in organ homogenates (lungs, liver, spleen, pancreas, brain, kidneys) at different time intervals showed almost complete absence within 4h in all studied tissues, and stably localized in pancreas and spleen. Maximum concentration in blood of *P. corylifolia* was reached at 90 th min, p.i. and then declined gradually. Similarly, significant twice reduced levels of ascorbate radicals in liver ( $0.208 \pm 0.01$ , *P.corylifolia*, \*p<0.00001) and in serum (average  $0.0664 \pm 0.018$  *P.corylifolia* vs  $0.145 \pm 0.003$  controls, au, \*p <0.001) after administration with *P.corylifolia* were recorded, compared to controls and to CCNU- injected group. After treatment with *P.corylifolia*, in liver and blood was found statistically significant decrease in ROS production comparing to controls (\*p <0.002) and to CCNU- treated group (\*p <0.05).

Therefore, it can be concluded that *P.corylifolia* is a natural agent, possessing strong antioxidant/ anticancer activities, could effectively neutralize radiation and reduce free radicals formation in biological systems.

**KEYWORDS:** *P.corylifolia* ; UV and  $\gamma$ -irradiation; DPPH capacity; antioxidant activities



# Phenolics content and antioxidant activity of beverages on the Bulgarian market – wines, juices and compotes

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**Background:** Polyphenolics (PP) constitute a broad range of chemical compounds widely distributed in plants. They are classified into three important groups: phenolic acids, flavonoids, and tannins. Flavonoids include anthocyanins (AC), a water-soluble pigments, responsible for the red, purple, and blue colors in fruits. In wine, natural juices and compotes the taste is heavily influenced by the presence of phenolics. They contribute to the sensory quality (flavor taste, and color) as well as the antioxidant activity of fruits and processed foods derived from them. Moreover, these compounds have other beneficial effects such as antidiabetic, antibacterial, anti-inflammatory, and anticarcinogenic activities. These parameters are not included in the standard documentation related to food labeling and control.

**Aim:** To perform a comparative characteristic in terms of total phenolic content, anthocyanins and antioxidant potential of fruit alcoholic and non-alcoholic drinks on Bulgarian market.

**Material and methods:** Commercial fruit natural juices, compotes of stewed fruits, red wines, rose and white wines on Bulgarian market were used for testing total phenolics, anthocyanins and antioxidant activity. Total phenolics were determined spectrophotometrically by Folin-Ciocalteu method and pH-differential method was used for quantitation of anthocyanins. The antioxidant activity (AOA) was assessed by the ABTS radical-cation method.

**Results:** Among the tested alcoholic drinks the red wines revealed significantly higher PP ( $567.3 \pm 32.66$  mg/L) and AC ( $92.45 \pm 42.46$  mg/L) content vs rose ( $322.8 \pm 83.60$  mg/L,  $p < 0.0001$ ;  $90.32 \pm 8.82$  mg/L,  $p < 0.01$ ) and white ( $280.7 \pm 41.90$  mg/L,  $p < 0.0001$ ;  $0.15 \pm 0.21$  mg/L,  $p < 0.001$ ) wines. In the group of the red wines, highest PP content ( $625.3 \pm 13.32$  mg/L) was detected in Merlo wine, and the lowest in Syrah ( $534.5 \pm 20.18$  mg/L). The wine Malbek was found to be the richest in AC ( $157.3 \pm 2.65$  mg/L), while in Mavrud the AC content was the lowest ( $45.20 \pm 1.06$  mg/L). The tested red wines showed high AOA, especially strong in Aronia wine ( $45.55 \pm 0.35$  mM UAE). Strong positive correlation between AC content and AOA was found for rose wines ( $r = 0.965$ ,  $p < 0.01$ ). In the tested non-alcoholic drinks, highest PP content was found in Aronia natural juice ( $591.8 \pm 8.719$  mg/L) and compote ( $556.0 \pm 62.53$  mg/L). A borderline positive correlation between PP content and AOA in natural juices was detected ( $r = 0.83$ ,  $p = 0.058$ ).

**Conclusion:** The red wines and compotes have been an element of traditional nutrition in Bulgaria and nowadays their input in the healthy diet is reassessed, because of their high phenolics content and strong antioxidant potential.

## **Mulberry heartwood ethanol infusion modulates the expression of glutathione related enzymes in a model of oxidative stress**

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*Morus nigra* L. (mulberry tree) is well known in the ethno medicine for the healing effects of its leaves, fruits and roots bark preparations. In Bulgaria the heartwood of mulberry is traditionally used also for coloring of high alcoholic beverages and it is believed that colored drinks in small quantities might possess certain health beneficial effects.

In the present study a preliminary phytochemical analyses were made to evaluate the total flavonoid and tanin contents in the mulberry heartwood ethanol infusion. Furthermore, different concentrations of the infusion were applied in preadipocyte cell culture in the presence and in the absence of the oxidizing agent tertiary-butylhydroperoxide (t-ButOOH). With the aim to evaluate the effect of the infusion on the oxidative status in the cells, the transcriptional levels of glutamate-cysteine ligase and glutathione peroxidase 4 were measured.

The results showed that the total amount of flavonoids and tanins in 40% ethanol infusion from mulberry heartwood was respectively 14.39 µg QE/ml and 20.06 µg GAE/ml. The analyses of gene expression revealed that the pretreatment with the higher concentration of the infusion modulated the transcriptional levels of both antioxidant enzymes under t-ButOOH – induced oxidative stress.

In conclusion, the mulberry heartwood ethanol infusion could have a beneficial effect in the prevention and control of oxidative stress-based metabolic disorders. The contribution of the extracted active compounds in the infusion could be assumed.

## Flavonoid content and antioxidant potential of *Betonica bulgarica* Degen et Neic

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The Bulgarian endemic *Betonica bulgarica* Degen et Neič (syn. *Stachys bulgarica* Hayek) is a protected plant by the Biological Diversity Act and it is included in Red Data Book, of Bulgaria under the category “endangered”. Most of the species populations are found in protected territories – the Central Balkan National Park, Sinite Kamani Natural Park and in the protected areas of Natura 2000. The species is morphologically similar to medicinal plant *Betonica officinalis* L. (syn. *Stachys officinalis* L. Trevis). The aim of this study was to determinate the flavonoid content and antioxidant potential activity of different plant parts of this species (leaf, flower, root, stalk and seed), from four populations. Three flavonoids were found in significant amounts: rutin, quercetin and hispidulin. In largest quantity was rutin, followed by quercetin and hispidulin. The largest total flavonoid content was measured in leaves, followed by roots and flowers. The antioxidant potential activity of methanol extracts was tested by DPPH-method and total polyphenol assay. The correlation between flavonoid content and antioxidant potential activity of the studied plant parts and populations were established. The obtained results confirm the therapeutic potency of *Betonica bulgarica* Degen et Neič in traditional medicine and are good basis for future pharmacological investigations.

# Interfacial concentrations of phenolic antioxidants in model food emulsions: effects of acidity and surfactant concentration

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We have determined the effects of pH and emulsifier concentration ( $\Phi_I$ ) on the distribution of gallic (GA) and caffeic (CA) acids in stripped corn oil emulsions. GA and CA are essentially oil insoluble AOs and only one partition constant, that between the aqueous and interfacial region  $P_W^I$  is needed to describe their distribution in emulsions.<sup>[1]</sup>  $P_W^I$  is obtained in the intact emulsions from the kinetic analyses of the variation in the observed rate constant,  $k_{obs}$ , for the reaction between a hydrophobic arenediazonium ion, 16-ArN<sub>2</sub><sup>+</sup> and AOs and the results are interpreted on the basis of the pseudophase kinetic model. Results show that, the partition constant values  $P_W^I$  values are independent of emulsifier concentration, but change substantially with acidity following sigmoidal curves with upper limits of  $P_W^I \approx 280$  (GA) and  $P_W^I \approx 590$  (CA) at high acidity. The distributions of all phenolic acids between the aqueous and interfacial regions depend strongly on surfactant volume fraction,  $\Phi_I$ . Increasing the acidity produces substantial changes in the concentrations of antioxidants (AOs) in the interfacial region.<sup>[1]</sup> Results contribute to understand how different parameters control AO distributions in emulsified systems and how AO partitioning affects their AO efficiency.

[1] Losada-Barreiro *et al.* Eur. J. Lipid Sci. Technol., **2015**, 117, 1801-1813.

## Distribution of catechol in corn oil emulsions

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We have evaluated the effects of emulsifier concentration on the distribution of catechol, CAT, in intact food-grade emulsions composed of stripped corn oil, acidic water and Tween 20.<sup>[1]</sup> The distribution of CAT is described by two partition constants, that between the oil-interfacial region,  $P_O^I$ , and that between the aqueous and interfacial region,  $P_W^I$ .  $P_O^I$  and  $P_W^I$  are determined from the kinetic analyses of the variation in the observed rate constant,  $k_{obs}$ , for the reaction between the hydrophobic 4-hexadecylbencenediazonium ion ( $16\text{-ArN}_2^+$ ) and CAT with the emulsifier volume fraction ( $\Phi_I$ ). The results are analyzed according to the pseudophase kinetic model. Results indicate that CAT distributes between three regions and an increase in  $\Phi_I$  promotes the incorporation of CAT to the interfacial region of the emulsions, so that at  $\Phi_I = 0.037$ , more than ~70% is located in that region. Comparison of results with those obtained for different catechol derivatives indicate that partition constants do not parallel their solubility in water, and thus, their partition constants do not correlate with their polarity. These findings are basic to understand how the polarity of the antioxidants affects their distribution in emulsified systems and, therefore, their efficiency in inhibiting lipid oxidation.

[1] Losada-Barreiro *et al.*, J. Phys. Org. Chem. **2014**, 27, 290-296.

## Kinetic parameters of the copigmentation effect of caffeic acid and strawberry anthocyanins

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### Abstract

The effect of temperature on the stability of the copigmentation complex of strawberry anthocyanin extract as pigment and caffeic acid as copigment was investigated. The system was studied with high concentration of caffeic acid 1:20 to 1:100 molar ratio. Different temperatures and copigment concentrations were used for the investigated pigment:copigment interaction and kinetic parameters such as activation energy ( $E_a$ ),  $z$  – factor and degradation rates ( $k$ ) were calculated. According to the calculated results, at high temperatures (50°C) destruction of complex was observed. Decreasing the temperature in the range 20–30°C did not lead to restoration of the complex, indicating an irreversibility of the copigmentation process.

**Key words:** copigmentation, caffeic acid, anthocyanins, kinetic parameters

**Acknowledgements:** This work was funded by project DN 09/20 “Integrated approach for improving the quality, organoleptic properties and biological activity of new functional foods from chokeberry (*Aronia melanocarpa*) by copigmentation and synergy in antioxidant activity.” of the Bulgarian National Science Fund.

## **Valorization of bio-wastes: Extraction optimization of avocado seeds aimed at maximization of extract's antioxidant activity**

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### **SUMMARY**

Food supplements of vegetable origin are widespread for dietary use. Most often they are in the form of extracts rich in phytochemicals with high radical-scavenging action (antioxidants). Avocado (*Persea americana*) is a fruit widely cultivated throughout the world. There are many studies that attest to its health benefits. Avocado peels and seeds are considered as waste. However, there are indications that avocado seeds, which represent about 23 % of fruit weight, have even higher antioxidant activity than that of its flesh. So, the aim of this work is to study the influence of process parameters on the extraction of avocado seeds in order to determine the optimal conditions for obtaining extracts containing maximum amount of bioactive substances and having high antioxidant activity. An experimental approach is developed, which allows the optimal process parameters to be found by a reduced number of experiments. As a result, the following important process parameters are determined: selection of appropriate "green" solvent, which dissolves more antioxidant compounds; minimum solvent-to-solid ratio and minimum process duration necessary for complete extraction, which minimizes process costs; process temperature, which ensures an acceptable compromise between higher solubility and thermal stability of the antioxidant compounds. The results obtained are useful for development of technological schemes for production of antioxidant extracts for use as functional supplements obtained from bio-wastes.

## **Evaluation of polyphenolic content and antioxidant capacity of spent coffee grounds**

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### **SUMMARY**

Coffee is one of the most popular everyday drinks. In 2014 the world production is more than 8.5 million tons. Spent coffee grounds represent the main waste, generated in preparation of coffee drinks. Due to fast and short treatment of milled coffee with hot water, non-extracted bioactive substances remain in the spent coffee. Thus, the latter might be considered as a potential source of useful antioxidant compounds obtained from largely available wastes.

This study investigates the potentials of spent coffee grounds as a source of natural antioxidants. The polyphenolic content and antioxidant capacity of extracts from fresh roasted coffee is determined and compared to these of spent coffee grounds (after a common espresso preparation). The extracts are analyzed by Folin - Chicalteau reagent (for polyphenols) and DPPH method (for antioxidant capacity). It is found that the coffee remains still contain significant amount of polyphenols, and show high antioxidant capacity. In our case study, spent coffee grounds contain 15,68 mg of polyphenols (PPH) per gram of dry matter [mg PPH / g dm], and its antioxidant capacity is 68,49 mg DPPH per gram of dry matter [mg DPPH / g dm]. The remaining polyphenolic content and antioxidant capacity of spent coffee grounds represent 60,15% and 38,08% respectively as compared to fresh coffee. The conclusion is that spent coffee is far from being exhausted by a simple hot water extraction, and it can be considered as a rich and valuable source of useful bioactive substances with antioxidant activity.

This study is carried out in fulfilment of the research project No DN 07/12 – 2016, financed by the Bulgarian Fund for Scientific Research.



# Quality enhancement of chilled hake (*Merluccius merluccius*) by employing ice including ethanolic and aqueous extracts of alga *Fucus spiralis*

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## ABSTRACT

Marine foods deteriorate rapidly post-mortem as a consequence of a variety of biochemical and microbial breakdown mechanisms. According to the increasing demand for high-quality fresh products, different strategies have been tested to retard fish spoilage as long as possible. For centuries, edible marine algae have represented an important component in human diet in many Asian countries. Marine algae are reported to contain a wide range of constituents with important health benefits and preserving characteristics (i.e., antioxidants and antimicrobials), and can provide a profitable tool for the enhancement of food quality.

The basic objective of the present research was to investigate the effect that the inclusion of alga extracts in the icing medium may have on the preservation of fish. In it, a comparative study of different kinds of extracts of the alga *Fucus spiralis* was carried out. Thus, the effect of ice including: aqueous (AQ batch), ethanolic (ET batch) or ethanolic and aqueous (ET-AQ) alga extracts on lipid oxidation and microbial activity was monitored in chilled hake (*Merluccius merluccius*) throughout a 13-day storage and compared with fish kept under traditional ice (Control batch).

An inhibitory effect of both ethanol extracts (ET and ET-AQ batches) on the formation of thiobarbituric acid substances and fluorescent interaction compounds (i.e., secondary and tertiary lipid oxidation compounds) was observed, this effect being more intense in the case of fish belonging to the ET-AQ batch. Further, a low formation of primary oxidation compounds (i.e., peroxides) was obtained in all kinds of samples. Related to lipid hydrolysis development, lower mean free fatty acid values were observed in hake samples corresponding to both ethanolic conditions (ET and ET-AQ batches). Additionally, the study proved an antimicrobial effect against aerobes, psychrotrophs, proteolytic and lipolytic bacteria, derived of the presence of *F. spiralis* ethanolic extracts in the icing medium (ET and ET-AQ batches); again, this effect was found higher in the case of the ET-AQ batch.

A preservative effect derived of the use of an icing medium including both ethanolic and water alga extracts has been concluded, this result being linked to the presence of a wider range of preserving molecules (namely, lipophilic and water-soluble). The icing medium proposed in this study (combination of ethanolic and aqueous alga extracts) may open the way to the development of a natural biopreservation strategy for chilled seafood.

## ANTIOXIDANT MECHANISMS IN NEUROPROTECTIVE ACTION OF LIPOIC ACID ON LEARNING AND MEMORY OF RATS WITH EXPERIMENTAL DEMENTIA

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The aim of this study was to evaluate the antioxidant mechanism in neuroprotective effect of lipoic acid on cognitive functions in experimental dementia. Alzheimer's disease (AD) type dementia was produced via Scopolamine treatment (SC, 1 mg/kg i.p., 11 days) on male Wistar rats. Lipoic Acid (LA, 30 mg/kg i.p.) was applied for the same period. Hole-board test and Step through test were performed. Brain frontal cortex, hippocampus and striatum homogenates were used for determination of malondialdehyde (MDA), glutathione (GSH), and the activities of superoxide dismutase (SOD), glutathione peroxidase and catalase (CAT). The dementia model was verified by the cognitive tests used. In brain structures of SC-group increased MDA, decreased GSH and activated antioxidant enzymes were observed. LA significantly improved damaged by SC cognitive functions and oxidative status by increased GSH level, restored CAT and SOD activities. Thus LA significantly protects memory impairments of dement animals due to its antioxidant capacity and could be used in prevention and therapy of AD.

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# Estimation of DPPH radical-scavenging activity of Galantamine hydrobromide and Pymadine on the way to effective prevention and therapy of Alzheimer's disease

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The overproduction of reactive oxygen species (ROS) and the weakening of the antioxidant defense mechanisms in human body are the main reason for the oxidative stress, which underlies the development of several socially important neurodegenerative disorders, such as the Alzheimer's disease. The widely used in clinical practice alkaloid Galantamine acts as a nonselective acetylcholinesterase inhibitor, but it was recently found that also indicates a considerable antioxidant activity. On the other hand, Pymadine, as a non-depolarizing muscle-relaxant antagonist (potassium channel blocker), was discovered to also have a synergistic effect with the Galantamine on the symptomatic treatment of this widespread disease. As long as the pharmacological behavior of this drug combination is relatively well studied, there is almost no knowledge on its radical-scavenging and antioxidant potential.

The aim of this study was the evaluation of the radical-scavenging activity (RSA) of Galantamine hydrobromide, Pymadine and combination Galantamine hydrobromide/Pymadine towards 2,2-diphenyl-1-picrylhydrazyl (DPPH).

The decrease in the absorbance of 0.05 mM methanol solution of DPPH at  $\lambda = 516$  nm in presence of standard 1 mM methanol solution of Butylhydroxytoluene (BHT), resp. 1 mM ÷ 5 mM methanol solution of Galantamine hydrobromide, resp. 1 mM ÷ 5 mM methanol solution of Pymadine, resp. 5 mM methanol solution of Galantamine hydrobromide/Pymadine was monitored by spectrophotometry in equal time intervals of 5 s for a total period of 30 min. This method is particularly useful for studying the RSA of the investigated compounds, because they do not possess any measurable absorbance at this wavelength.

The regression equations for the plots RSA vs concentration were used to calculate the  $RSC_{50}$  values – the concentration, which achieves 50 % scavenging of DPPH:  $RSA_{(Galantamine\ hydrobromide)} = 3.419 \cdot e^{0.293 \cdot c}$ ,  $RSC_{50(Galantamine\ hydrobromide)} = 9.16$  mM;  $RSA_{(Pymadine)} = 0.460 \cdot e^{0.411 \cdot c}$ ,  $RSC_{50(Pymadine)} = 11.41$  mM. RSA of the investigated compounds was compared with the effect of standard – BHT and the relative radical-scavenging activity (RRSA) and relative decrease of radical-scavenging activity (RDRSA) were calculated. The experimental results showed that the combination 5 mM Galantamine hydrobromide/5 mM Pymadine has a slightly higher RSA (20.19 %), compared to 5 mM Galantamine hydrobromide (15.44 %) and 5 mM Pymadine (2.48 %) itself.

## Some structural effects on the radical-scavenging activity of phenolic acids

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The radical-scavenging activity allows to the highest extent the application of chemistry tools to forecasting the potential of a compound as an antioxidant working on scavenging principle. Phenolics are traditional scavengers and in this study quantum-chemistry models of a series of phenolic acids, featuring different number of OH-groups and length of side chain, were created to test their scavenging capacity. The effect of the structural factors was estimated based on the DFT assessment of the thermodynamic parameters characterizing the possible scavenging pathways.

**YSSBA 2017**

**Keynote Lectures**

## **Quantitative structure – activity relationships and molecular modelling of bioactive compounds**

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Quantitative structure – activity relationships (QSAR) and molecular modelling are well-established approaches to study bioactive compounds and they are constantly present in all modern drug design projects. The basics of these approaches will be discussed and their place in the frame of the *in silico* drug design concept will be outlined. The focus will be given to QSAR as the mostly used method to correlate activity of bioantioxidants to their chemical structures.

## Mass spectrometric analyses of bio-antioxidants: peptides and glycoproteins

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Several peptides and glycoproteins with antioxidante activity have excellent potential as pharmaceutical products. Inhibitory effect of the fraction, containing, on the reduction of NBT by photochemically generated superoxide anion radicals .

Tandem mass spectrometry, combines MALDI–MS/MS and Q-Trap MS/MS, was applied to analyse the structure of ten biologically active peptides isolated from *Cornu aspersum* with antioxidant activity. The molecular masses of peptides were measured by their MS spectra and the primary structures were determined by their MS/MS spectra.

Tandem mass spectrometry as a very useful method was also apply to analyse the primary structure of glycoproteins, superoxide dismutase from *K. marxianus* NBIMCC 1984 yeast (Cu/Zn-KmSOD) and from fungal strain *Humicola lutea* 103 (Cu/Zn-HISOD). The obtained fractions after treatment of the enzyme with trypsin were separated by HPLC and their amino acid sequences were determined by MALDI-TOF-TOF.

Both SODs are glycoproteins and their carbohydrate structures were also identified by MALDI–MS/MS and Q-Trap MS/MS. After treatment of the Cu/Zn-KmSOD with PNGase F a single peak at 1257.3 [M+Na]<sup>+</sup> was detected which suggests a uniform oligosaccharide chain. The structure of this carbohydrate chain was determined by Q-Trap MS/MS .

One putative linkage site was observed in the sequence of both glycoproteins. This linkage site (-Asn-Leu/Ile-Thr-) at position 33-35 is the glycosylated and is conserved in several SODs.

**Keywords:** antimicrobial peptides, glycopeptides, glycoproteins, *Cornu aspersum*, mass spectrometry

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## **Application of EPR spectroscopy for study free-radical structures at *in vitro* and *ex vivo* systems**

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Electron paramagnetic resonance (EPR) spectroscopy, known also as electron spin resonance (ESR) spectroscopy, is the only analytical technique for study and prove stable and unstable radical structures both *in vitro* and *in vivo* systems. EPR spectroscopy starts from 1944, and Academician E.K. Zavoyskiy from Kazan University is considered as its founder.

EPR spectroscopy is a technique used to investigate chemical structures (paramagnetic systems) with one or more unpaired or odd number of electrons. Paramagnetic structures are reactive, short - lived (transient), and under special conditions can be stabilized. At the base of EPR spectroscopy have laid the magnetic properties of unpaired electrons and their environment within the molecule.

What is EPR spectroscopy? It is resonance absorption of microwave radiation from a paramagnetic system placed in an external magnetic field. As a result an EPR spectrum is registered. The resulting EPR spectrum delivers information both for the structure of the investigated radical and for its conformation.

EPR spectroscopy is a method possessing a number of advantages: 1) identify only free radicals, which is an exclusive important when studying structures with unpaired electrons, and is also used for quantitative determinations; 2) can be proven nanomolar concentrations; 3) there is no background; 4) study time is quite short - it usually takes from 15 to 20 minutes, delivering very accurate information about the free radical properties of the tested sample; 5) results obtained are relatively easy to read.

In 2008 at the Department of Chemistry and Biochemistry Medical Faculty, Trakia University was created an EPR Center. At present EPR studies in our laboratory are performed in both directions:

- 1) *In vitro* direct and spin trapping EPR spectroscopy for study antioxidant activity of natural extracts, fractions and compounds.
- 2) *Ex vivo* direct and spin trapping EPR spectroscopy for evaluation of oxidative stress in biological systems.



## Computational approaches to the assessment of radical-scavenging activity

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The radical-scavenging activity is deemed to be an indication of the antioxidant potential of a substance and is therefore studied intensely both experimentally and theoretically. The computational approaches employ methods of both classical and quantum mechanics to investigate the scavenging mechanisms by means of assessment of static and dynamic properties of the prospective scavengers as well as of the intermediates and products of the reactions they are involved in. Relationship between the radical-scavenging activity and various descriptors of the molecular spatial structure and electron distribution is sought and in silico models are built. The knowledge acquired allows the decoding of mechanisms and control of processes, diversification of the existing and design of new active compounds.

**YSSBA 2017**

**Oral Presentations**

# INTERACTION OF NATURAL TIOLS AND CATECHOLAMINES WITH REACTIVE OXYGEN SPECIES

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Oxidative stress is characterized by a high content of reactive oxygen species (ROS), and reflects imbalance between the rates of ROS generation and their utilization. There are biologically active compounds in all living organisms, which produce ROS (generating of free radicals) and bioantioxidants (BAO) utilizing ROS by reduction of peroxides and radical scavenging. Strong influence of the media on bioantioxidant behavior has been established. BAO can be divided into fat-soluble (tocopherols, carotenoids, polyphenols) and water-soluble (natural thiols, catecholamines and catechins) antioxidants.

It is well known, that natural thiols (RSH) perform a variety of antioxidant function such as interaction with hydroxyl radicals, reduction of hydroperoxides, prevention of protein oxidation. Low molecular natural thiols such as glutathione (GSH) and cysteine (CSH) are used as drugs. Contrary to them, a high level in blood of homocysteine (HSH), which is an amino acid that differs from CSH by one methylene group, indicates a lack of folic acid and vitamin B12 and may lead to coronary heart disease and neurodegeneration [1].

Epicatechin (EP) and catecholamines (CA), namely dopamine (DA), epinephrine (EN), norepinephrine (NE) are derivatives of catechin (dihydroxybenzene). They easily split off hydrogen in reactions with radicals. Catecholamines are neurotransmitters and neurohormones in animals and humans, and they also function as endogenous antioxidants in the nervous system.

In this work the kinetic of reactions of natural thiols GSH, CSH, HSH and catecholamines DA, EN, NE, EP with ROS was studied. ROS are hydrogen peroxide and peroxy radicals ( $\text{RO}_2\bullet$ ), which are generated from AAPH in aqueous media.

A new water-soluble free radical scavenger – the polymethine dye A (pyridinium salt of 3,3' di-Y-sulfopropyl-9- methylthiacarbocyanine betaine) were used. Spectral and kinetic characteristics of A are determined in [2]. The rate constants for the reactions of GSH, HSH and CSH with  $\text{RO}_2\bullet$  ( $k_{\text{RO}_2\bullet}$ ) were determined by the method of competing reactions using dye A as a basic radical acceptor with known kinetic characteristics:  $k_{\text{RO}_2\bullet} = 5.4 \times 10^4 (\text{Ms})^{-1}$ , stoichiometric coefficient  $f=1$  (in water solutions at 37°C). The values of  $k_{\text{RO}_2\bullet}$  for GSH, HSH, and CSH were determined to be equal to:  $10^5 (\text{M}\cdot\text{c})^{-1}$  0.84, 2.16 and 4.4, respectively. It was found that  $k_{\text{RO}_2\bullet} \geq 10^6 (\text{Ms})^{-1}$  for DA, EN, NE и EP and corresponding stoichiometric coefficients for DA and EP are equal to 2; for EN and NE  $f < 1$ .

It was established for the first time that the reduction of  $\text{H}_2\text{O}_2$  by natural thiols is accompanied with free radical formation. The rates of radical formation were measured by inhibitors method with use of A as a radical acceptor. Specific radical formation rates  $\varpi = W_A / \{[\text{H}_2\text{O}_2] \times [\text{RSH}]\}$  for this reaction were determined.

Values of  $\varpi$  characterize the activity of thiols to form free radicals in a reaction with  $\text{H}_2\text{O}_2$ . GSH and CSH almost equally generate radicals ( $\varpi = 7.0 \cdot 10^{-5} (\text{M}\cdot\text{c})^{-1}$ ), CSH activity in this reaction is in 30 times higher than activity of GSH and HSH ( $\varpi = 2.1 \cdot 10^{-3} (\text{M}\cdot\text{c})^{-1}$ ).

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## References

1. McCully, Kilmer S. // *Comprehensive Physiology*. 2016, 6 (1): 471.
2. Zinatullina K.M., Kasaikina O.T. et al. *Russ.Chem. Bull.* 2016, 12: 2825.

## Effect some membrane lipids on radical generation in the system Ach-LOOH.

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Hydroperoxides (LOOH), the primary oxidation products of most organic compounds, which are amphiphilic compounds and have surface activity at interphase boundaries. With micelle-forming surfactants hydroperoxides form mixed micelles. In the case of cationic surfactants, in particular quaternary ammonium salts (QUATS), it has been found that they catalytically accelerate the decomposition of hydroperoxides into radicals [1, 2]. In micelles with QUATS peroxide bond-O-O-, it evidently falls into a strong electric field of a double electric current with a voltage of  $\sim 105\text{-}107\text{ V / m}$ , which attenuates this bond and stimulates homolytic decay. The radicals formed in the micelle react with LOOH and in the scope of supply of the peroxy radicals  $\text{LO}_2^\bullet$ . The known neurotransmitter acetylcholine (ACh) of simple QUATS contains a tetraalkylammonium group and, as established in [3], catalyses the decomposition of hydroperoxides into radicals in a hydrocarbon and lipid medium:



In this paper, the influence of sphingomyelin (0.001 - 0.1 mM) lipid components of cholesterol and phosphatidylcholine on the rate of formation of radicals in acetylcholine-hydroperoxyhydrobutyl (TBHP) microaggregates in n-decane (37 ° C) was studied. Cholesterol - a natural polycyclic lipophilic alcohol, which is contained in the cell membranes of virtually all living organisms. Phosphatidylcholines (PC) (1, 2-diacyl-sn-glycero-3-phosphocholine, egg) are the main lipid component cell membranes, natural surfactants, their polar head have the structure of zwitter ions over a wide pH range. Sphingomyelin is part of the myelin sheath of axons of nerve cells. The rate of radical formation in the decay of TBHP was determined by the inhibitor method using  $\beta$ -carotene as a free radical scavenger. The rate of consumption of hydrophobic  $\beta$ -carotene, which is localized in the organic phase and reacts actively with radicals of different types, was determined by kinetic spectrophotometry. The dimensions of the microaggregates were determined by dynamic light scattering.

The results obtained show that the lipid additives affect the size and structure of the resulting microaggregates  $\{m\text{ACh} \dots n\text{THPB}\}$ , which in turn affects the yield of radicals and the rate of initiation. It has been established that cholesterol supplements several times increase the initiation rate of radicals in the acetylcholine system -hydroperoxide, and phosphatidylcholine, forming mixed aggregates with blocking the yield of radicals into the volume.

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### Literature

1. Kasaykina OT, Golyavin AA, Krugov DA and others. // Vest. Moscow State University. Ser.khim. 2010. (3). 246.
2. Trunova NA, Krugovov DA, Bogdanova Yu.G. and others // Vest. MSU Ser. Chem. 2008. (4). 260.
3. Krugovov DA, Mengele E.A. Kaseikina O.T. // Izv. AN, Ser. Chem. (8). 1837.

## Antioxidant properties of some C<sub>60</sub> fullerene with $\alpha$ -tocopherol analogue – a hybrid of natural and synthetic antioxidant

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Fullerenes can find many potential applications in science, biology and industry.<sup>1,2</sup> Since fullerenes (with their main representative C<sub>60</sub> molecule) act like radical sponge able to trap several radicals per molecule, they can be regarded as an interesting initial structure in the development of novel radical-scavenging compounds.<sup>3,4</sup> Together with the attached phenolic antioxidant (e.g. phenol or  $\alpha$ -tocopherol), the C<sub>60</sub> sphere can form a new hybrid molecule with combined properties of both parts: phenolic antioxidants which can effectively neutralize peroxy radicals, and C<sub>60</sub> molecules that are more active with respect to alkoxy and alkyl radicals. In order to study the structure – activity relationship we synthesized an adduct of C<sub>60</sub> molecule with Trolox<sup>TM</sup> (a water-soluble derivative of vitamin E) and also the adducts of fullerene C<sub>60</sub>(X)<sub>n=1-6</sub> (where X = -phenol; - p-aminophenol; -OH) or obtained by Prato reaction C<sub>60</sub>(C<sub>3</sub>H<sub>6</sub>NX)<sub>1</sub> (where X = -vanillin; -ferulic acid). By means of Differential Scanning Calorimetry, Clark Electrode and Oxygen Uptake Method we studied the antioxidant properties during the oxidation of cumene, methyl linoleate or stearic and linolenic acids as models of oxidizable organic materials. During oxidation of cumene or methyl linoleate (monitored by means of Clark Electrode and Oxygen Uptake Method at 40°C) the tested compounds show similar antioxidant properties compared to natural antioxidants such as synthetic analogue of tocopherol 2,2,5,7,8-pentamethyl-6-chromanol (PMHC) or 2,6-di-tert-butyl-4-methylphenol (BHT). But during non-isothermal oxidation of stearic or linolenic acids in the bulk phase at temperatures above 100°C the C<sub>60</sub> derivatives are stable and can break the propagation chain of hydrocarbon autoxidation. This feature is useful because new derivatives can be applied as antioxidants working in heavy conditions (increased temperature).

<sup>1</sup> Bakry, R.; Vallant, R. M.; Najam-ul-Haq, M.; Rainer, M.; Szabo, Z.; Huck, C. W.; Bonn, G. K. *Int. J. Nanomedicine*, **2007**, 2, 639-649.

<sup>2</sup> Huang, S. S.; Tsai, S. K.; Chih, C. L.; Chiang, L.-Y.; Hsieh, H. M.; Teng, C. M.; Tsai, M. C., *Free Radic. Biol. Med.*, **2001**, 30, 643-649.

<sup>3</sup> McEwen, C. N.; McKay, R. G.; Larsen, B. S., *J. Am. Chem. Soc.*, **1992**, 114, 4412-4414.

<sup>4</sup> Morton, J. R.; Negri, F.; Preston, K. F., *Acc. Chem. Res.*, **1998**, 31, 63-69.

## Rapid determination of antioxidant compounds in *Portulaca oleracea* L. (purslane) by the optimal ultrasound extraction combined with liquid chromatography - high resolution mass spectrometry (LC-HRMS)

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*Portulaca oleracea* L. (Portulacaceae) is well known edible and ethno-medicinal plant. Purslane has been called “vegetable for long life” in Chinese herbal medicine. A rapid and reliable ultrasound extraction and liquid chromatography (LC) coupled with quadrupole-orbitrap mass spectrometer method was developed for the determination of 12 phenolic acids together with 2 flavonol glycosides in twelve purslane batches. The ultrasound-assisted extraction, optimized by orthogonal design, was performed at 50°C 2 × 15 min with methanol-water (50:50, v/v) as the extracting solvent, and the solvent to solid ratio was 40:1. The total polyphenol and saponin contents as well as antioxidant activity (DPPH) were used as the criteria for selecting the optimal extraction condition. The accessed phenolic acids and flavonoids were eluted in 10 min on a Kromasil EternityXT C18 (1.8 µm, 2.1 × 100 mm) column using acetonitrile and formic acid-water solution as a mobile with a flow rate at 0.3 ml/min. All calibration curves showed good linearity ( $r > 0.996$ ) within the tested ranges. Ferulic acid was the major compound being presented with up to  $315.39 \pm 2.349$  ng/g dry weigh. The highest content of gentisic acid was found in Greek purslane batch ( $35.590 \pm 0.419$  ng/g dw). With respect to flavonol glycosides, rutin was present in the highest amount, up to  $40.135 \pm 0.264$  ng/g dw in a Bulgarian sample. Data were analyzed by principal component analysis (PCA). The PCA scoring plot grouped the majority of the Bulgarian batches into one well defined cluster. Two Bulgarian samples displayed the highest combined levels of studied compounds. The PCA loading plot pointed gentisic and caffeic acids as the compounds with the highest contribution in PC1, while chlorogenic and neochlorogenic acids dominate in PC2. In conclusion, the improved LC-HRMS method enables the simultaneous determination of common antioxidants in relatively low concentration levels as well as fingerprint analysis in *P. oleracea* herb.

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## Can sulforaphane and its analogues exhibit direct antioxidant activity?

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1-isothiocynato-4-methylsulfinylbutane (sulforaphane, SFN) belongs to the group of isothiocyanates (ITCNs) and could be found in *Cruciferous* vegetables. SFN has attracted much attention due to valuable nutritional and pharmacological properties. Its biological properties are considered to be due to “indirect” antioxidant properties – SFN is a potent inducer of phase II enzymes and increases the cellular antioxidant capacity.<sup>1</sup>

Some papers suggest that ITCNs cannot act as direct preventive or chain-breaking antioxidants but on the other hand, ITCNs are able to react with radicals, *i.e.* 2,2-diphenyl-1-picrylhydrazyl (dpph<sup>•</sup>), hydroxyl (OH<sup>•</sup>), superoxide anion (O<sub>2</sub><sup>•-</sup>) and peroxy radicals (ROO<sup>•</sup>) as well.<sup>2,3</sup> The latter kind of action of ITCNs have not been systematically recognized and explained yet.

Trying to answer for the title question, we employed advanced kinetic techniques (Stopped Flow, Differential Scanning Calorimetry, Oxygen Uptake Method) and *ab initio* calculations (DFT M062-2X/6-31+G\* level of theory) to study the reactions of ITCNs with series of natural and artificial radicals in model systems. Obtained kinetic and thermodynamic parameters indicate that direct antioxidant activity of ITCNs depends on microenvironment – polarity of the solvent, presence/absence of acids and bases, nature of the system (homo- or heterogenic). In this talk, the possible mechanism of action of SFN toward dpph<sup>•</sup> radical will be discussed. We propose that this reaction proceeds *via* the electron transfer (ET) rather than *via* a one-step hydrogen atom transfer (HAT).

<sup>1</sup> Y.S. Zhang, P. Talalay, *Cancer. Res. (Suppl.)*, **1994**, 54, 1976.

<sup>2</sup> J. Barillari *et al.*, *J. Agric. Food Chem.*, **2005**, 53, 2475.

<sup>3</sup> H. Yuan *et al.*, *Chin. J. Chem. Eng.*, **2010**, 18, 312.

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## Evaluation of the antioxidant capacity of wild berries extracts

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Wild berries are small forest shrubs with the most widely distributed genus *Vaccinium*. Blueberries (*Vaccinium myrtillus* L.) and redberries (*Vaccinium vitis-idaea* L.) are rich in flavonoids, phenolic acids, anthocyanins, stilbenes and tannins, as well as nutritive compounds such as sugars, essential oils, carotenoids, vitamins and minerals. Bioactive compounds from berries have potent antioxidant, anticancer, antimicrobial, anti-inflammatory properties. Traditionally, berries fruits are used to treat disorders of the gastrointestinal tract and diabetes. The objective of this study was to investigate the antioxidant activity of selected berries collected from Polish forest. Ethyl acetate, water, ethanol and its mixture were used for the extraction. Antioxidant capacity of the prepared extracts was screened by several spectrophotometric methods. The total reducing capacity was evaluated by Folin-Ciocalteu (FC) method (so-called total phenolic content) and cupric ion reducing antioxidant capacity (CUPRAC). The content of flavonoids was determined by spectrophotometric methods based on the formation of Al(III)-flavonoid complexes. The total content of anthocyanins was estimated according to pH differential method. Scavenging ability on 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH method) was also studied.



## The influence of different fermentation types on dopamine precursor content in fava beans

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Fava bean (*Vicia faba* L.) is worldwide known nutrient rich plant. Additionally fava bean may be also considered as an alternative source of dopamine precursor – L-3,4-dihydroxyphenylalanine (levodopa, L-dopa). Synthetic form is used as a drug for people suffering from Parkinson's disease (PD). PD is a progressive neurodegenerative disease of the central nervous system comprising combinations of motor problems caused by an imbalance of dopamine and acetylcholine in the brain. It has been reported that administration of synthetic drugs containing L-dopa may cause serious hallucinations and dyskinesias in addition to gastrointestinal disturbances like nausea and vomiting. Since synthetic L -dopa has side effects, the necessity of searching for the natural source is becoming more important. Patients with PD who were taken fava bean as natural therapy did not show side effects. This may suggest that fava beans may contain compounds which can complement anti-Parkinsonian effect. In the research carried out by Randhir et. al (2007) it was shown that there is a possibility of enriching fava bean in L-dopa via solid-state bioconversion system using the food-grade fungus *Rhizopus oligosporus*.

This study was conducted to examine the influence of other types of fermentations on L-dopa content in fava beans. Four types of fermentation were carried out and each lasted 6 days. The microorganisms used for fermentations were: *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Lactobacillus rhamnosus* and *Rhizopus oligosporus*. It was proved that *Saccharomyces cerevisiae* increased L-dopa content 3.5 times in 2<sup>nd</sup>, *Lactobacillus rhamnosus* 3.3 times in 2<sup>nd</sup>, *Bacillus subtilis* 2.6 times in 1<sup>st</sup> day of fermentation.

Randhir R., Vattem D., Shetty K. 2004. Solid-state bioconversion of fava bean by *Rhizopus oligosporus* for enrichment of phenolic antioxidants and L -DOPA. Innovative Food Science and Emerging Technologies, 5: 235–244.

# Green extraction of grape seed oil as a potential source of antioxidants

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## ABSTRACT

The predisposition of fatty acids to oxidation is believed to be directly dependent on their degree of unsaturation. However, some *in vitro* and *in vivo* studies suggest that the relation between chemical structure and susceptibility to oxidation is not as direct as hypothesized from theoretical viewpoints. Actually, long chain polyunsaturated fatty acids (PUFAs) might be less oxidizable than others under specific experimental conditions.

Seed biomass from *Vitisvinifera* L. contains typically (8–15) % (w/w) of oil which is rich in unsaturated fatty acids and antioxidants. Yet, in many countries the seeds, which represent about (20-25) % of the biomass generated by the wine industry, are considered a disposable material. Conventional extraction with *n*-hexane of the pressed seeds is the typical technique applied at present in an attempt to reuse the seeds biomass. However, the solvent is highly flammable and with hazardous effects on human health. A viable green alternative is extraction with supercritical CO<sub>2</sub> (scCO<sub>2</sub>) as it alleviates the addition of environmental footprint.

The aims of our work are to present novel data on the supercritical extraction (SCE) of grape seeds and to examine and compare the influence of the extraction method on the extract compositions.

The SCE of oil from grape seeds samples obtained from a Portuguese industry was carried out in a flow apparatus (Applied Separations, Spe-edTM SFE) at temperatures from (313 to 333) K, pressures up to 40.0 MPa and flow rate of 0.10 kg/h of CO<sub>2</sub>. The qualitative analysis of the crude oils was carried out by Nuclear Magnetic Resonance, 400 MHz NMR spectrometer (Bruker Advance), operating at 400.13 MHz for 1H-NMR and at 100.61 MHz GC-Fid analyses, to the fatty acids, were carried out with reference to the parameters in Annex I to Commission Regulation (EEC) No 2568/91(1), published 04.12.2016.

The results show that the content of triacylglycerols (TAGs) and diacylglycerols (DAGs) both in the *n*-hexane and SCE extractions were similar. However, higher PUFA content, namely C18:2 (ω-6) - diunsaturated fatty acids (DUFAs), and (C16:0, C18:0 and C20:0) - low saturated fatty acids (SFA), was obtained in the SCE oil. It is well-known that the consumption of lipids rich in saturated fatty acids and cholesterol increases atherogenesis, while lipids rich in MUFA and PUFA reduce atherogenesis and thrombogenesis. Our work, thus, demonstrates that the eco-friendly SCE is better than *n*-hexane extraction - with comparable yields obtained but with higher extraction efficiency in favour of the PUFAs.

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## **Antioxidant activity of red fruits and application of berry extracts and films in food conservation**

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Red fruits are rich in antioxidants, and have gained, over the last years, capital importance for consumers and manufacturers due to their characteristics. Phenolic compounds and anthocyanins are key for their antioxidant properties and appealing color. Red fruits are being included nowadays in a variety of foods, mainly as whole berries, whether fresh or dried; and in cosmetics and nutraceuticals as extracts or juices. However, it is still difficult to substitute synthetic preservatives completely from the ingredient list using natural antioxidants, especially in food applications involving risk of microbiological colonization.

The main goal of this project is to determine the application of red fruit antioxidants in several food models, including emulsions and meat burgers. Gelatin and PLA films were used as matrices for the application of several berry extracts on food models. The aim of film utilization as carriers for natural antioxidants is the future commercial application of these solutions to effectively replace substances such as BHA. *Rosa Canina*, *Vaccinium myrtillus*, *Rubus idaeus* and *Sambucus nigra* were the four selected species to perform the experiments. 50% Ethanol was used to obtain extracts from the different berries, and ultrasound assistance and acidification was used to enhance Total Polyphenol and Anthocyanin Content (TPC: 99.55 mmol GAE/g DW, TAC: 71.10 malvidin-3-glucoside eq./g DW). Non ultrasound, lightly acidified, 50% aqueous ethanol was found to be the best extraction medium to obtain higher antioxidant capacity, evaluated by radical scavenging assays (DPPH: 3.33 Trolox eq./g DW, ORAC: 22.14 mmol Trolox/g DW and ABTS-TEAC: 3.19 mmol Trolox/g DW). HPLC analyses were performed to find the chemical profile of fruits. Peroxide value and TBARS assays were performed in food models with satisfactory results. Red fruits were able to inhibit oxidation of emulsions up to more than 50%, both using direct application of extracts and loaded PLA films, respectively. Gelatin films containing rose hip extracts were especially protective against oxidation of meat burgers. Sensory analysis carried out with 30 tasters eating cooked meat burgers containing red fruits exhibited surprising results. In conclusion, this research proves red fruits can act as natural alternatives to some synthetic preservatives in food conservation.

## Antioxidant activity of walnuts (shell and seed) of different origins

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The oxidation of lipids in food decreases the nutritional value and affects the organoleptic characteristics. In this study, industrial by-product like walnuts shell and, also walnut seed of different origins (Spanish, French, Californian, Indian and Mexican) were selected for antioxidant activity research.

The Total Polyphenol Content (TPC) by Folin Ciocalteu method and the radical scavenging activity by Oxygen Radical Absorbance Capacity (ORAC) were determined. The antioxidant activity was measured in oil in water (O/W) model food emulsions by Peroxide Value (PV) as primary oxidation product. The Minitab statistical software was used to define the Response Surface Methodology (with time, Temperature, % EtOH, mass walnut –shell and seed-/volume as variables) and to treat all the results.

The Spanish Chandler walnut shell had the highest TPC (24,83 milimols Gallic Acid Equivalent –GAE-/g dry sample) and the minimum value was obtained for the French (11,42 milimols GAE/g dry sample). For the radical scavenging the order was similar (Spanish > Mexican > Californian > Indian > French) and were in the range 17,30-8,92 milimols Trolox Equivalent/g dry sample. For the walnut seed the maximum TPC was obtained for the Californian seed. And the minimum was for French. All of them were in the range (21,72-11,69 milimols GAE/g dry sample). The ORAC value followed similar order (8,07-4,82 milimols Trolox Equivalent/g dry sample). Based on the results acquired, RSM was performed to optimize the extract conditions. For the Spanish Chandler walnut shell and the Californian walnut seed the best condition was the minimum concentration used (0,2g/12,5 mL) and 48-52% EtOH. The time and the Temperature had not influence.

For the extracts of walnut shell and seed were evaluated for their ability to inhibit lipid oxidation using (O/W) emulsions as the model food system. The emulsions samples (o/w) were stored for 32 days at 30°C to accelerate the lipid oxidation. Control (without any walnut or GA extract) < GA 0.21 g/L < walnut seed at 1.09 g/L < walnut shell at 1.09 g/L < GA at 0.39 g/L < walnut seed at 6.52 g/L < walnut seed at 4.35 g/L (between 7 and 32 days). The other samples, walnut shell at 4.35 g/L, walnut shell at 6.52 g/L and GA at 0.78 g/L were stable until the end of the experiment (after 32 days, PV was < 50 meq hydroperoxides/kg).

All these results indicate that the walnut (seed and shell) has a high antioxidant potential and could be used as a substitute for synthetic antioxidants.

# Antioxidant effectiveness of alga *Bifurcaria bifurcata* extracts when employed as filling medium during Atlantic mackerel (*Scomber scombrus*) canning

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## ABSTRACT

Canning is one of the most important means of fish preservation. This is done by two thermal steps (cooking and sterilisation), so that both enzymes and bacteria should be inactivated provided reinfection does not occur and no negative interaction with the container is produced. However, marine lipids are known to possess a high content of polyunsaturated fatty acids, which during heat treatment can be oxidised and lead to browning, flavour changes and loss of essential nutrients. Marine algae are reported to contain a wide range of constituents with important health benefits and preserving characteristics. Among them, *Bifurcaria bifurcata* is a widely extended brown macroalga, whose antioxidant properties have previously been described.

The present research focussed on the quality of canned Atlantic mackerel (*Scomber scombrus*). In it, an aqueous *B. bifurcata* extract was prepared and included in the filling medium during the canning process. In agreement with previous preliminary trials, different alga extract contents were tested and compared to a control without alga extract (i.e., water as filling medium). After a 3-month storage at room temperature (18-20°C), cans were opened and analyses related to lipid damage were carried out on the mackerel muscle.

An inhibitory effect on lipid oxidation development (thiobarbituric acid index and fluorescent compounds formation) and total volatile amines formation could be observed as a result of the alga extract presence in the filling medium; this effect was found more important by increasing the extract presence. However, no effect could be concluded on primary lipid oxidation (peroxides formation), lipid hydrolysis (free fatty acids content) and trimethylamine formation. Concerning physical colour assessment, *B. bifurcata* extract presence showed an inhibitory effect on L\* and b\* parameters increase, this effect being more intense by increasing the alga content in the filling medium.

A preservative effect derived of the use as filling medium of an aqueous *B. bifurcata* extract has been concluded, this result being mostly linked to the presence of hydrophilic antioxidant molecules. The filling medium proposed in this study may open the way to the development of a natural biopreservation strategy for canned seafood.

# Structure and antibacterial activity of glycopeptide, isolated from snail *Cornu aspersum*

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The world provides a rich source of peptides with antimicrobial, antiviral and antitumor activity. Glycopeptides are an expanding group of structurally complex anti Gram positive antibacterial agents, representatives of which have been used in human and veterinary medicine. Quite a series of proline-rich peptides, isolated from arthropods and molluscs, were considered to be promising candidates for the treatment of microbial infections and suppression of microbial resistance.

We now report on the primary structure and mass of a peptide isolated from the mucus of the molluscan garden snail *Cornu aspersum*, and compare its antimicrobial activities with those of other similar peptides isolated from the hemolymph of the marine snail *R. venosa* and garden snail *H. lucorum*.

The structure of a glycopeptide isolated by high performance reverse phase chromatography (HPLC) from the mucus of the garden snail *Cornu aspersum* was determined by mass spectrometric analyses. Its molecular mass of 4113 Da was measured by MALDI-TOF and the amino acid sequence of the glycopeptide KARYCGAEVTAN was determined by its MS/MS analysis.

In the test against Gram+ (*C. perfringens*, *P. acnes* и *S. aureus*) and Gram- (*E. coli*) bacteria the peptide shows an inhibition effects against *S. aureus* and *E. coli*.

**Keywords:** antimicrobial peptides, glycopeptides, *S.aureus*, *E. coli*, *Helix lucorum*, *Rapana venosa*, *Cornu aspersum*, mass spectrometry

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# The evolution of natural bio-antioxidants: types, mechanisms of action and new biochemical implications

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Complex life on Earth evolved around oxygen – a highly reactive molecule, able to cause significant damage to other biologically relevant molecules. Free radicals and reactive species (ROS and RNS) are generated by our body by various biological processes, or in response to different exogenous stimuli. Thus, the ongoing research of natural antioxidant synthesis and mechanisms of action has been receiving close review in recent years. This study aims to inform about some of the major antioxidant systems in aerobic cells and their role in preventing disease, with a focus on some of the more promising future implications of antioxidants found in nature.

Discussion on the different types of naturally synthesized antioxidants, both enzymatic and non-enzymatic (e.g. superoxide dismutases, catalases, glutathiones and tocopherols) and their levels of action in the organism serves as a base for understanding the clinical value of antioxidants and the benefits of supplementation. Furthermore, the study points out the importance of phytochemical antioxidants and antioxidant peptides from marine invertebrates for the development of future multifunctional therapeutics.

The presentation is supported by a brief history of antioxidants, from James Lind's clinical trial on citrus fruit's effect on scurvy, to the newest discoveries on redox regulation on gene expression and antioxidants' effect on malignancy. Studies on oxidative stress-induced DNA damage suggests careful evaluation on the use of antioxidants in melanoma, which will be broadly discussed. A short mention of synthetic antioxidants (e.g. BHT and BHA) will act as a comparative study between their impacts on human health and potential risk factors.

Finally, the presentation concludes with novel analysis of potential antioxidant agents for the treatment of neurodegenerative diseases and action on signal transduction cascades and gene regulation.

**Keywords:** antioxidant, oxidative stress, free radical, disease prevention

## 4-methoxy aroylhydrazones - promising agents protecting biologically relevant molecules from free radical damage

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The concomitant toxicity of iron observed in many disorders associated with excess of this essential trace element is mainly due to its ability to promote and participate in free radical generation reactions and the subsequent oxidative damage of the basic cellular macromolecules. The dietary recommendations for these patients and the restriction for supplementation with essential antioxidants have led to the necessity of developing novel high efficiency pharmacologically active substances with multipotent antioxidant properties.

The present investigation aimed to determine the protection effect of three structurally characterized derivatives of the active chelator salicylaldehyde benzoylhydrazone (SBH). The capability of the tested compounds (4-methoxysalicylaldehyde benzoylhydrazone (4mSBH), 4-methoxysalicylaldehyde-4-hydroxybenzoylhydrazone (4mShBH) and 4-methoxysalicylaldehyde isonicotinoylhydrazone (4mSIH)) to preserve the oxidative damage of biologically relevant molecules was evaluated in *in vitro* spectrophotometric model systems with diverse mechanism of free radical generation containing deoxyribose and egg yolk homogenate. Additional experiments have been performed in chemiluminescent systems containing different type of reactive oxygen species.

The obtained results indicate that the performed structural modification associated with incorporation of methoxy group at 4<sup>th</sup> position in the aldehyde part of the molecule ameliorates the evaluated properties. The extent of the observed improvement depends on the subsequent modifications in the hydrazide moiety. The hydroxyl bearing compound demonstrated better protection effect and higher activity against OH<sup>•</sup> and O<sub>2</sub><sup>•-</sup> in all model systems. Comparing the C-50 values from the different model systems we suggest that the observed protection effect is associated with antioxidant activity different from Fe-chelation.

### Acknowledgements

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**YSSBA 2017**

**Poster Presentations**

## Antioxidant activity of buckwheat seed extract and fractions thereof

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Buckwheat is recognized as a good source of nutritionally valuable protein, lipid, dietary fiber and minerals. This pseudocereal seeds, both groat and hull, and sprouts are an important source of rutin (quercetin-3-rutinoside) the content of which depends on the variety of seeds and growth conditions. The C-glucoglavones present mainly in buckwheat seeds are vitexin, isovitexin, orientin and homoorientin, which exert antioxidant activity *in vitro*

The objectives of this research were to investigate the antioxidant and antiradical activities of a crude acetone buckwheat seed extract and its isolated low-molecular-weight phenolics and tannin fractions.

Phenolic compounds were extracted from buckwheat seed using 80% (v/v) aqueous acetone. The crude extract was applied to a Sephadex LH-20 column. Fraction I, consisting of low- molecular-weight phenolics, was eluted from the column by ethanol. Fraction II, consisting of tannins, was obtained using acetone-water (1:1; v/v) as the mobile phase. Phenolic compounds present in the crude extract and its fractions showed antioxidant and radical scavenging properties as revealed following studies using a  $\beta$ -carotene-linoleate model system, the total antioxidant activity (TAA) method, the DPPH radical scavenging activity and a reducing power evaluation.

Results from the *in vitro* assays showed the highest values when tannins (fraction II) were tested. Specifically, the TAA of the tannins fraction was 2.44  $\mu\text{mol Trolox eq./mg}$  fraction; whereas, the crude extract and fraction I were 1.89 and 1.63  $\mu\text{mol Trolox eq./mg}$  extract or fraction, respectively. The content of total phenolics in fraction II was the utmost (477 mg/g); the tannins content, assayed by the vanillin method and expressed as absorbance units at 500 nm per 1 g, was 1396.

# Responsible structural features for cytotoxic and other kind activity of neo-clerodane diterpens from genus *Scutellaria*

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## Abstract

For years natural neo-clerodane diterpenoids have attracted the attention of the scientific community with their biological properties. They have been proven to act as potential insect antifeedant and antifungal agents. Jodrellin B was reported to be the most powerful antifeedent up to date. However, little or no research was found testing the neo-clerodane diterpenoids for cytotoxic activity. Therefore, this research covers the results from the bioassay of natural neo-clerodane diterpenoids, isolated from the acetone extracts of the aerial parts of *Scutellaria* species, for cytotoxic activity on H1299 and HUVEC cell lines.

For the purpose of the research twelve diterpenes\* from different *Scutellaria* genus were tested on the two cell lines, H1299 (human tumors of the lung) and HUVEC (normal cells from a navel string). The MTT (thiazolyl blue tetrazolium bromide) method was performed as a result of which the cytotoxic activity (IC<sub>50</sub>) was observed when a compound killed half of the cells in a cell line.

The testing showed that neoajugapyrin A is not sensitive to cytotoxicity. Scutalpins A, E and F exhibit moderate cytotoxic properties on both cell lines. The rest of the compounds show inactivity with in the studied concentration range.

The achieved results from the series bioassays of antifeedant, antioxidant, antimicrobial and cytotoxic activities of neo-clerodane diterpenoids, isolated from the plants belonging to genus *Scutellaria*, conclude that higher activity depends on the characteristic features of the whole molecular structure and not on the correlation between the separate biological properties.

\* 14,15-dihydrojodrellin T, neoajugapyrin A, scutegalerin A, scutecolumnin C and scutegalin D were received from *Scutellaria galericulata* L. Scutecyprol A, scupolin H, clerodin and scutaltisin G were isolated from *Scutellaria altissima* L. Diterpenoids scutalpin A, scutalpin F and scutalpin E were obtained from *Scutellaria alpina* L.

## ANTIOXIDANT ACTIVITY OF HEMOCYANINS

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Hemocyanins (Hcs) are complex oxygen-transport proteins found in the hemolymph of many arthropods and molluscs. In addition to their important biological function, molluscan Hcs have shown promising properties in the development of various medicinal products. In this study, we present for the first time an investigation on the antioxidant activity of two representatives of Hcs from molluscan species, namely those purified from marine snails *Rapana thomasiana* (RtH) and terrestrial snails *Helix pomatia* ( $\alpha$ -HpH), using various experimental models. The free radical scavenging activity was determined against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] free radical cation (ABTS). Cupric ion reducing antioxidant capacity (CUPRAC) and Ferric-reducing antioxidant power (FRAP) assay also were carried out. The results show that RtH exhibits good radical scavenging activity by 32.89 % reduction of the DPPH and 62.9 % inhibition of ABTS radicals. The antioxidant activity of  $\alpha$ -HpH according to these methods is weaker: 6.87 % and 52.67 % inhibition of DPPH and ABTS radicals, respectively. Furthermore, RtH demonstrated a strong chelating effect on copper ions measured through CUPRAC method. Here, the highest values for an antioxidant activity of RtH – 450.65 mM Trolox equivalents/g protein were detected. The result for  $\alpha$ -HpH from the same measurement was 110.32 mM Trolox equivalents/g protein.

In conclusion, the present study revealed that molluscan Hcs possess antioxidant properties. The antioxidative activity of these proteins involves quenching of reactive oxygen species and metal ion chelation, thereby reducing the potential of prooxidants to attack cellular components.

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## Partitioning of gallic acid and alkyl gallates in binary olive oil-water mixtures

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Estimating antioxidant (AO) distribution in food emulsions is important because its efficiency in inhibiting lipid peroxidation depends, among other aspects, on its distribution within the different regions of the system. In emulsified systems, the distribution is governed by two partition constants, that between the water and interfacial region,  $P_W^I$  and that between the oil and interfacial region,  $P_O^I$ .<sup>[1]</sup> The partition constant  $P_W^O$  in immiscible binary systems (e.g. oil and water) is numerically equal to the ratio  $P_W^I/P_O^I$ , and thus it is a very valuable parameter to determine the antioxidant distribution in an emulsified system.  $P_W^O$  is also a measure of the lipophilic/hydrophilic nature of the AOs. Here we determined the  $P_W^O$  values for gallic acid (GA) and some alkyl ester derivatives in binary stripped olive oil–water mixtures (in the absence of emulsifier) by employing a modified shake-flask method. Results show that  $P_W^O$  values increase with increasing hydrophobicity of the AO ranging from  $P_W^O = 0.09$  (GA) to 210 (lauryl ester), an increase of ca.  $2 \cdot 10^4$ . The value obtained for GA,  $P_W^O < 0.1$ , is consistent with the expected higher solubility of phenolic acids in water than in oil. Analyses of the effects of acidity on  $P_W^O$  values for GA show that they decrease upon decreasing the acidity.

[1] Bravo-Díaz *et al.* Langmuir, **2015**, 31 (33), 8961-8979.

## **PLA FILMS FOR PACKAGING TECHNOLOGIES**

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Poly-lactic acid (PLA) is a biopolymer obtained from renewable sources, as starch and sugar. Nowadays is used as a compostable, biodegradable thermoplastic alternative material to petrochemical-based polymers. PLA can present a more crystalline or a more amorphous structure with a visible difference in colour of the raw material. It has a good application in the packaging field, even for food because is totally safe. The addition of a compound could lead to an increase in PLA crystallinity structure avoiding to increase the amount of the high crystalline PLA form that is more expensive.

The aim of this work consists in to investigate the efficiency of some different PLA films in releasing natural antioxidants compounds in food simulants and to investigate the effect of the release of a specific amount of gallic acid (GA) (1,5% in weight) originally incorporated in three different PLA films (highly amorphous, semi crystalline and PLA Bio-Flex® F 2110 with some additives).

Simulants used were 95% EtOH (simulants for fats, oil and fatty foods), 50% EtOH (simulants for oil in H<sub>2</sub>O emulsions and alcoholic beverages) and H<sub>2</sub>O. The Folin-Ciocalteu method was used to measure the total phenolic content (TPC). The maximum values of GA released into the solvent are expressed as a percentage respect to the total initial amount. For almost all PLA films the diffusion in H<sub>2</sub>O reached the highest value after one day while for the ethanol food simulants the peak of release was in four hours. The more crystalline film diffuses GA in ethanol better than the amorphous one (19,7%,15,96% and 0,49% for the crystalline and 14,98%, 1,04% and 1,08% for the amorphous one respectively in EtOH 95%, EtOH 50% and H<sub>2</sub>O). The film of PLA Bio-Flex® F 2110 released a 19,46%, 17,08%, 15,38% of the original GA amount in EtOH 95%, EtOH 50% and H<sub>2</sub>O respectively. Differences between the pure PLA films behaviours (amorphous and crystalline) and that of the PLA Bio-Flex® F 2110 (that “works” very well also in H<sub>2</sub>O but with a slower GA content diffusion) were found. These films were used also for protecting the oxidation of oil/water emulsion and a life extended 50% (comparing to the control). The film has been characterized by Differential Scanning Calorimetry (DSC) and not substantial difference between the control film (without GA) and the film containing 1,5 % GA has been found. In conclusion, PLA shows useful properties for the antioxidant film packaging field and could be “green” production process in the near future.

## **Antioxidant mechanisms in preventive effect of Ellagic Acid on cognitive disorders on experimental model of Alzheimer's disease**

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Antioxidant mechanisms in protective effects of some natural compounds on progression of Alzheimer's disease (AD) were reported during last years. Our previous data revealed significant improving effect of a natural polyphenol Ellagic Acid (EA) on rodent cognitive functions. The goal of this study was to evaluate the effect of EA on cognition of mice with chemically induced dementia from AD type. This animal model was produced via Scopolamine treatment of male Albino mice and was verified by cognitive and biochemical methods. After 5-days treatment with EA both the changes in the cognitive functions of animals and biochemical correlates were evaluated. Significant preventive effect of EA on the processes of learning and memory (Passive avoidance test) and exploratory activity (Hole board test) of dement animals was established. The high percent (50%) of memory prevention by EA was accompanied by significant antioxidant effect (decreased lipid peroxidation) and inhibited activity of acetylcholine esterase in the brains of EA-treated animals. An increase of dopamine uptake in the brains of EA-treated dement animals was also found. Our results revealed some of the complex mechanisms underlying the EA preventive effect on the cognitive disorders in mouse model of dementia.

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## Neuroprotective mechanisms of combination Myrtenal and Lipoic Acid on experimental Alzheimer's disease model

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Many plant substances with complex neuroprotective activity are known as promising agents in Alzheimer's disease (AD) studies. Our previous data demonstrated improved memory effect of bicyclic monoterpene Myrtenal in rodents. We aimed to evaluate influence of Myrtenal (alone or in combination with Lipoic acid) in scopolamine mouse model of dementia from AD type, where Myrtenal (M, 20 mg/kg) and Lipoic acid (LA, 30 mg/kg) i.p. alone or in combination were applied. Cognitive functions were evaluated using Passive avoidance test. Brain acetylcholine esterase (AChE) activity and lipid peroxidation levels (TBARS) were measured. Data were analyzed using Student-Fisher *t*-test. Scopolamine treated animals demonstrated severe memory-loss, decreased brain mass, accompanied with increased AChE-activity (by 15%) and lipid peroxidation (by 83 %). M-treated animals showed significant cognitive function restoration. M and LA combination demonstrated better prevention on memory than their single administration and also preserved the brain tissue mass near to healthy control values. M alone has no effect on AChE brain activity but in combination with LA the AChE activity was inhibited by 25%. In healthy mice M has antioxidant activity (decreased brain lipid peroxidation levels). In dement mice M showed moderate pro-oxidant activity but combination with LA decreased pro-oxidant activity of M (by 30%). In conclusion, at least two different mechanisms (antioxidant and AChE inhibition) underlied the established effect of M+LA combination.

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## **Inhibition of lipid oxidation development in refrigerated salmon (*Salmo salar*) paste by addition of different stevia (*Stevia rebaudiana* Bert.) extracts**

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### ABSTRACT

Stevia (*Stevia rebaudiana* Bert.) is a relevant source of natural phenolic compounds with antioxidant and antimicrobial properties. The aim of this study was to evaluate the potential protective effect of crude stevia extracts on the quality and shelf-life of salmon (*Salmo salar*) paste.

For this purpose, polyphenol extracts obtained by stevia extraction with water, ethanol/water or ethanol-supercritical CO<sub>2</sub> were evaluated throughout the refrigerated storage (5 °C for 21 days) of salmon paste. Primary, secondary and total lipid oxidation compounds were monitored along storage by means of peroxide, p-anisidine and TOTOX indices, respectively. In addition, ω3/ω6 fatty acids ratio, polyene index and α-tocopherol content were monitored. Additionally, microbial development was analysed by means of the evolution of the aerobic mesophiles and psychrotrophes counts.

Salmon paste samples treated with ethanol/water and ethanol-supercritical CO<sub>2</sub> stevia extracts exhibited the highest (p<0.05) ω3/ω6 ratio and α-tocopherol content. Besides, partial inhibition of both primary and secondary lipid oxidation events as well as aerobes and psychrotroph growth was also observed in both kinds of paste samples. These results agreed with the fact that ethanol/water and ethanol-supercritical CO<sub>2</sub> extracts provided the highest DPPH and FRAP values.

It is concluded that, on the basis of employing the present stevia extracts, it is possible to obtain a preservative effect on salmon paste throughout its refrigerated storage, as a result of inhibiting the two most important damage pathways (i.e., development of lipid oxidation and microbial activity). The results obtained in this research show the possibility of using stevia and/or its derivatives of the sweetener industry as an alternative source of natural antioxidants to be applied for the refrigerated storage of fatty fish pastes.

## Antioxidant activity of selected o-methoxyphenols, benzenediols and biphenols. Theoretical and experimental studies

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Combination of theoretical and experimental approaches were applied to study and to explain the structure – antioxidant activity relationship for selected *ortho*-methoxyphenols, benzenediols and biphenols. They are natural and natural-like phenols and corresponding dimers with selected positions of the phenol-OH group in order to study the influence of the conformation and substituents in the aromatic ring on the antioxidant activity. Chain-breaking antioxidant activities of compounds under study were determined from the kinetic curves of bulk lipid autoxidation. Predicted pharmacokinetics and biological properties of all selected compounds is presented using molinspiration program (Lipinski Rule of Five). Full geometry optimization of neutral molecules and their corresponding phenoxyl radicals for all compounds under study were obtained by using DFT (B3LYP/6-31+G\*\*) calculations. Good correlation between experimental and predicted activity was achieved which is helpful for explanation the structure-activity relationship. All studied compounds are in agreement with Lipinski Rule of Five that means they have good predicted pharmacokinetics and may be used in pharmaceutical industry. Furthermore, the predicted biological activity by applying molinspiration program is presented for the first time.

## Comparative study of antioxidant potential of curcumin and its degradation products – vanillin, ferulic acid and dehydrozingerone.

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Curcumin is one of the most intensively studied biologically active antioxidants during the last decade. Its chemical instability and rapid degradation at physiological conditions ( $\text{pH} \approx 7$ ) was stated as the most important limitation for its potential applications. Numerous approaches have been undertaken to overcome the problem with the bioavailability of curcumin, including the use of: adjuvants such as piperine, liposomes, curcumin nanoparticles, phospholipid complexes and design of new structural analogs of curcumin [1]. On the other hand degradation of a compound does not necessarily lead to loss of its activity. Whether and how the degradation and oxidation pathways contribute to the biological and antioxidant activities of curcumin has also been discussed [2]. The aim of this study is to compare the antioxidant activity of curcumin with those of its degradation products (ferulic acid, vanillin and dehydrozingerone) identified by Wang *et al.* [3], when added in binary and ternary (triple) antioxidant mixtures. Lipid autoxidation has been used for assessing the chain-breaking antioxidant efficiency and reactivity of the phenols. The results obtained manifested much stronger activity of curcumin than that of all the individual compounds and their binary and triple mixtures.

[1] Pari, L and Eckel, J. *Arch. Phys. Biochem.* **114**, 127 (2008); [2] Schneider, C. *et al.*, *J. Agric. Food Chem.* **63**, 7606 (2015); [3] Wang, Y. J. *et al.*, *J. Pharm. Biomed. Anal.* **15**, 1867 (1997).

# QUERCETIN COMPLEXES FORMED ON INITIAL SILICA AND Zn-MODIFIED MESOPOROUS MATERIALS

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Quercetin is bioflavonoid with proven antioxidant activity and many studies are focused on its pharmaceutical properties and medical applications. Problems associated with low solubility and poor bioavailability of this compound can be solved with its incorporation in appropriate delivery systems. Mesoporous silica materials are promising carriers of different biologically active molecules because of their advantages such as high specific surface area, tunable pore and particle size and dual-functional surface (external and internal).

**In the present study** we developed delivery systems of quercetin on the base of initial and zinc modified mesoporous silicas (MCM-41 and SBA-16) and investigated the complexes' formation of the antioxidant molecules with surface of the obtained materials. *In-vitro* release profiles of quercetin were studied in respect to their possible application as dermal formulations.

MCM-41 and SBA-16 were synthesized and modified by post-synthesis method with different amounts of Zn (2 and 4 wt.%). Incipient wetness impregnation method was used for quercetin loading on the initial and Zn-modified mesoporous supports. The initial and drug loaded formulations were characterized by powder XRD, N<sub>2</sub> physisorption, TEM, TG analysis, UV Vis and FT-IR spectroscopy.

Results from analysis show high loading capacity of quercetin in the pores of initial and Zn-modified mesoporous materials. For the first time the formation of complex by quercetin and Zn incorporated in mesoporous silica materials was determined. *In-vitro* experiments show slower quercetin release from Zn-modified materials in comparison to initial ones. Additionally the comparative cytotoxic experiments show that quercetin encapsulated in Zn-modified silica carriers proved to exert superior antineoplastic potential against HUT-29 cells compared to free antioxidant. Thus, it can be concluded that Zn-modified silica particles are promising carriers for dermal delivery of quercetin.

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# Hepatoprotective and antioxidant properties of novel hydrazone derivatives of the *N,N'*-disubstituted benzimidazole-2-thione synthesized as melatonin analogues and study of their mechanism of action

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Based on the structural resemblance of melatonin, series of novel hydrazone derivatives of the *N,N'*-disubstituted benzimidazole-2-thione have been synthesized as potential hepatoprotective and antioxidant drugs for the treatment of liver disorders. The compounds were tested on isolated rat hepatocytes and the antioxidant properties of the ones that exhibited lowest hepatotoxicity were evaluated by inhibition of the *tert*-butyl hydroperoxide (*tert*-BOOH)-dependent lipid peroxidation in induced oxidative stress. Three 3,3'-(2-thioxo-1*H*-benzo[d]imidazole-1,3(2*H*)-diyl)bis(*N'*-substituted-methoxybenzylidene)propanehydrazides and three 3,3'-(5-benzoyl-2-thioxo-1*H*-benzo[d]imidazole-1,3(2*H*)-diyl)bis(*N'*-substituted-methoxybenzylidene)propanehydrazides showed significant cytoprotective and antioxidant effects similar to those of the referent quercetin. For further investigation of the antioxidant properties, the ability to inhibit the xanthine oxidase was evaluated *in vitro*. Since hepatoprotective effect may be exerted also by reduction of endonuclease catalyzed DNA fragmentation (decreased DNase activity), the DNase I inhibition has also been studied. In order to estimate the structure-activity relationship (SAR), DFT analysis was performed. On the base of the calculated reaction enthalpies of hydrogen atom abstraction (HAT) and single-electron transfer (SET) it could be concluded that the preferred mechanism of antioxidant action for the hydrazone derivatives of the *N,N'*-disubstituted benzimidazole-2-thione is SET.

## **Effect of Pr(III) nitrate and Pr(III) complex with 5-aminoorotic acid on the accumulation of hydroxyl radicals in rat blood serum.**

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The free radicals formation near cancerous cells facilitates the tumor deterioration. The success of the cancer treatment depends on the good control over the oxidative stress level, to avoid damaging healthy tissues in the body. Lanthanides and their compounds are promising anticancer agents due to the prooxidant activity of the Ln cations. The 5-aminoorotic acid (HAOA) proved to be antioxidant at homeostatic pH. A complex formed by coordinatively bonding lanthanide ion with antioxidant ligand is promising strategy in the search of efficient anticancer agents. In this investigation, the effect of Pr(III) complex with 5-aminoorotic acid (PrAOA) on the accumulation of free radicals in blood serum was estimated. The free radicals were created in the presence of Fe(II)/H<sub>2</sub>O<sub>2</sub>/EDTA/Ascorbate model system. K, Na-PBS (50 mM) was used as reaction medium.

The Fe(II)/EDTA model system was used as a source of hydroxyl radicals, to initiate the free radicals chain reactions. The effect of the NO<sub>3</sub><sup>-</sup> was taken into account by investigating the free radicals formation in the presence of Na nitrate (no free radicals are formed in the presence of Na ions). The effects of HAOA and PrAOA were monitored and compared with this of the Pr(II) cation, at concentration range 10<sup>-4</sup> - 10<sup>-7</sup> M. MTT formazan (characteristic  $\lambda$  at 576 nm) formed due to interaction of Nitroble tetrazolium (MTT) with free radicals, was used as a marker for the extent of free radicals accumulation in the blood serum.

The stronger prooxidant effect of Pr(NO<sub>3</sub>)<sub>3</sub> than this of the NaNO<sub>3</sub> was associated with Pr(III). At concentrations below 10<sup>-6</sup> M both HAOA and PrAOA did not effect the Fe(II)/H<sub>2</sub>O<sub>2</sub> - provoked free radicals accumulation in rat blood serum. Above this concentration, both molecules were antioxidants, the complex being weaker than the ligand alone. It was proposed that the antioxidant effect of PrAOA resulted from both antioxidant properties of the ligands and prooxidant properties of the Pr<sup>3+</sup>.

## Fluorescence spectroscopy and antioxidant activity from Lingonberry (*Vaccinium vitis-idaea*) fruit juice

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*Vaccinium vitis-idaea* is a woody shrub from Pirenovi family. In the present investigation lingonberry (*Vaccinium vitis-idaea*) from region of Velinograd have been used for obtaining the juice. The aim of the present study was to show the opportunity of fluorescence spectroscopy for quality detection of some biologically active substances and the antioxidant activity of fruit juice from lingonberry. The samples are produced by crushing and squeezing of fruits after which the juice was filtered and poured in glass bottle with the metal cap. The probes have been enriched with lactulose whose concentration are 0.5%, 1 % and 1.5% respectively.

The contents of biologically active substances such as anthocyanin and phenolic components in the juice samples were measured spectrophotometrically. Ferric reducing antioxidant power (FRAP) assay was used for determining the antioxidant activity. The reaction was started by mixing 3.0 ml FRAP reagent with 0.1 ml of investigated juice. The reaction time was 10 min at 37 °C in darkness and the absorbance was measured at 593 nm. The fluorescence spectra have been obtained by exciting the samples with 245 nm, 265 nm, 275 nm and 295 nm. A fiber optic spectrometer (AvaSpec-2038, Avantes) with sensitivity in the (200-1100) nm range and a resolution of about 8 nm was used to measure the fluorescence spectra. The juice samples were placed in a cuvette 10 mm x 10 mm and irradiated by LEDs.

The excitation/emission ranges in the fluorescence spectra can be connected with the following phenolic components and flavanoids

-Chlorogenic acid – (245-250 nm)/ (430-440 nm); Caffeic acid – (230-350 nm)/ (405-470 nm); Vanilic acid – (295-300 nm)/ (305 -355 nm); Tannins – (230- 315 nm)/ (345-405 nm); Catechin and epicatehin – (275-280 nm)/ (320 – 335 nm)

The high value for ratio between the intensity of emission and intensity of excitation is obtained for wavelengths 265 nm and 275 nm. The emitted fluorescence signal is the lowest for exciting wavelength 245 nm. Linear dependences exist between:

Total phenolic content (TPC) and anthocyanins (A) –  $TPC = -132.66 + 16.83 \cdot A$ ; with correlation coefficient  $R^2 = 0.96$ .

-Antioxidant activity (AA) and total phenolic content (TPC) –  $AA = 8.88 \cdot TPC - 1609.2$  with correlation coefficient  $R^2 = 1$

Total phenolic content and the ratio  $I_{\text{emission}}/I_{\text{excitation}}$  for exciting wavelength 275 nm.

$TPC = 1750 + 262.20 \cdot I_{\text{emission}}/I_{\text{excitation}}$  with correlation coefficient  $R^2 = 0.99$ .

Lightness of the samples and the ratio  $I_{\text{emission}}/I_{\text{excitation}}$  for exciting wavelength 275 nm

$I_{\text{emission}}/I_{\text{excitation}} = -20.31 + 0.99 \cdot L$  with correlation coefficient  $R^2 = 0.937$ .

Front –face fluorescence spectroscopy give the possibility for qualitative detection of phenolic components in juice from lingonberry, which are important for hygiene of eating. The proposed technique represents fast, cheap and not time consuming methods, without using chemical reagents.

## Changes of lipid composition of stabilized pumpkin seed oil with different antioxidants during long-term storage

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**Abstract.** Oxidative stability of pumpkin seed oil during long-term storage at different conditions was investigated. The examinations were carried out with cold pressed pumpkin seed oil (as a control sample) and stabilized oil with different natural antioxidants such as caffeic acid, ethyl gallate and combination of both of them. Long-term storage for all samples was leaded for 6 months at atmospheric conditions and at 10°C. Indicators that defined oxidative stability of the oils (acid and peroxide value, fatty acid and tocopherol composition) were monitored during the long-term storage. Significant changes were detected in peroxide value in all samples during the whole period, especially in those that were kept at atmospheric conditions, while in acid value and fatty acid composition deterioration was minor. Same tendency was observed in total tocopherol content where the later decreased insignificantly in samples, stored at 10°C in dark place. Generally, could be considered that pumpkin seed oil was more stable when was stabilized with ethyl gallate and stored at 10°C.



# Phytochemical characteristics and *in vitro* antioxidant activity of fresh, dried and processed fruits of Cornelian cherries (*Cornus mas* L.)

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## Abstract

In this study, chemical and technological properties and antioxidant activity of fresh, dried and processed cornelian cherry fruits were evaluated. Several fruit characteristics, total titratable acidity (TA), pH, total phenolic compounds, total monomeric anthocyanin (TMA), total antioxidant activity and sugar composition were studied. Average fruit mass and flesh ratio were as follows: for fresh fruits (1.53 g and 77.16%), for dry fruits (0.86 g and 67.61%) and for compôte (1.68 g and 63.15%), respectively. In fruits of Cornelian cherry dry matter varies from 18.7 to 81.4%, ash content was 0.5 to 2 % and TA from 1.5 to 3.4%. The highest content of TMA was found in fresh fruits – 32.1 mg cyd-3-glu/100 g fresh weight (fw), as well as total phenolic compounds 4.56 mg GAE/g fw. Fresh Cornelian cherry demonstrated the highest antioxidant potential - 36.5 mM TE/g fw (DPPH assay) and 29.6 mM TE/g fw (FRAP assay). The sugar composition in all Cornelian cherry products was presented mainly from sucrose, glucose and fructose, as sucrose was in low levels from 0.15 to 0.30 % fw. The polyuronic content was in the range from 1.1 to 1.3 %. The current study demonstrated nutritional characteristics of Cornelian cherry fruits suitable for direct consumption and various forms as infusions or compôte. The analyzed products were evaluated as natural foods with high antioxidant activity and potential health benefits.

# Structure and stability of peptides and hemocyanin from garden snail *Cornu aspersum*

Radostina Velikova<sup>1</sup>, Lyudmila Velkova<sup>1</sup>, Aleksandar Dolashki<sup>1</sup> and Pavlina Dolashka<sup>1</sup>

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The hemolymph of molluscan snails is a complex mixture of biochemically and pharmacologically-active components such as peptides and proteins with antioxidante activity.

The peptides with antioxidant activity were isolated from the extract of garden snail *C. aspersa* and the temperature and pH-dependent denaturations of one isolated peptide were determined by circular dichroism (CD). Loss of  $\alpha$ -helical structure occurred in the range of 50-85°C depending on pH. The peptide was not stable towards thermally-induced unfolding.

A copper-containing respiratory glycoprotein (hemocyanin) was also isolated from the hemolymph of garden snail *C. aspersa* (CaH). The structural and conformational stability of CaH,  $\beta$ c-CaH subunit and functional unit  $\beta$ c-CaH-g were analyzed by CD in wide pH-T range (T°C 20÷85°C and pH 2÷12).

The obtained results show that the native CaH is more stable compared to the  $\beta$ c-CaH subunit and  $\beta$ c-CaH-g functional unit, which was explained by the formation of quaternary structures, and non-ionic forces (intra-subunit, hydrophobic and hydrogen-bonded networks of carbohydrate moiety interactions). The difference in the stability of the native CaH and  $\beta$ c-CaH indicates the influence of the quaternary structure and the difference in the stability of  $\beta$ c-CaH and  $\beta$ c-CaH-g shows the influence of the tertiary structure.

The carbohydrate structure influences the stability of the native molecule CaH and  $\beta$ c-CaH subunit, but does not affect the stability of one isolated functional unit.

**Keywords:**  $\beta$ c-CaH subunit (SU),  $\beta$ c-CaH-g functional unit (Fu), circular dichroism (CD), stability, temperature and pH-dependent denaturation.

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# NMR spectroscopy of Bulgarian wine – an alcoholic beverage rich in bio-antioxidants

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Wine is the first alcoholic beverage and the first medicine, produced by humans. It is well known by its antioxidant and antimicrobial properties. It is believed it can protect against heart disease, atherosclerosis, certain types of cancer, type two diabetes and neurological disorders. Bulgarian wine is worldwide famous for its high quality, aroma, taste, vinicultural traditions and antioxidant activity. Red wines Cabernet Sauvignon, Thracian valley possess highest antioxidant capacity compared to all known natural wines in the world (1). Health effects of wine are mainly determined by the chemical composition. Wine antioxidant activity depends on the content of polyphenols, sugars, acids and alcohols. Their quantities are affected by many factors, including soil, climatic conditions, yeast, method of production, wine regions and grape varieties.

1D (<sup>1</sup>H, <sup>13</sup>C) and 2D (HSQC, TOCSY) NMR spectra were used to identify and quantify the chemical profile of wine. The composition from several varieties with high antioxidant activity – Cabernet Sauvignon, Merlot, Syrah, Sauvignon Blanc and Chardonnay – was explored. Using chemometric analysis (ANOVA, PLS-DA) on semi-quantitative NMR data allows differentiation of the botanical and geographical origin of the studied wines based on the quantities of the identified components.

## Reference:

1. Burns, J., Gardner, P., O’Neil, J., Crawford, S., Morecroft, I., McPhail, D., Lister, C., Matthews, D., MacLean M., Lean, M., Duthie, G., and Crozier, A. Relationship among antioxidant activity, vasodilation capacity, and phenolic content of red wines. *J. Agric. Food Chem.* **2000**, 48, 220–230.

# Synthesis and antioxidant properties of metallic nanoparticles functionalized with $\alpha$ -tocopherol derivatives

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Tocopherol is the most active antioxidant present in mammalian tissues. One molecule of  $\alpha$ -TOH reacts with two peroxy radicals: the inhibition rate constant ( $k_{inh}$ ) for  $\alpha$ -TOH + peroxy radical in a model system AIBN (azobisisobutyronitrile)-styrene, PhCl is  $3.2 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$  at 30°C.<sup>1</sup> In this work we use derivative of tocopherol to functionalize gold nanoparticles to obtain nanogold antioxidants.

Derivatives of  $\alpha$ -tocopherol are promising building blocks of novel antioxidants<sup>2</sup> and antioxidative stress markers. One of these derivatives is 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid known as Trolox<sup>TM</sup>. It is characterized by a lower inhibition rate constant than tocopherol ( $1.1 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ ) but is soluble in water and thanks to reactive carboxylic group Trolox has wider application in organic chemistry.

To synthesize gold nanoparticles modified Brust-Schiffrin method<sup>3</sup> was used, reducing  $\text{HAuCl}_4$  in two-phase system of water-toluene with TOAB (tetraoctylammonium bromide) as the phase transfer ligand and functionalize these nanoparticles with derivative of Trolox (TroloxSH, where SH means:  $(\text{C}=\text{O})\text{NH}(\text{CH}_2)\text{SH}$  instead of  $\text{COOH}$ ), which was synthesized and its structure was confirmed by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy. Obtained AuNPs@STrolox particles were characterized by UV-Vis and FT-IR spectroscopy and size of functionalized nanoparticles was determined by TEM measurements.

In order to determine potential antioxidant properties of obtained AuNP@STrolox, the oxygen consumption measurements were monitored for autoxidation of styrene in PhCl at 30°C.

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<sup>1</sup> G.W Burton, K.U. Ingold, *Ann. N. Y. Acad. Sci.*, **1989**, 570, 7-22.

<sup>2</sup> G.W Burton, K.U. Ingold, *Acc. Chem. Res.*, **1986**, 19, 194-201

<sup>3</sup> M. Brust, M. Walker, D. Bethell, D. J. Schiffrin, *Chem. Soc., Chem. Commun.*, **1994**, 7, 801-802

# NEAR INFRA-RED SPECTROSCOPY AS A TOOL FOR RAPID ESTIMATION OF THE ANTIOXIDANT CAPACITY OF RED WINES

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The present investigation evaluates the feasibility of using near-infrared spectroscopy as an accurate, fast and non-destructive analytical tool for estimation of the antioxidant properties of red wines. The evaluation of the antioxidant properties was conducted using stable free radicals (ABTS and DPPH) in spectrophotometric model systems for total antioxidant activity determination. The obtained AOA data have been used to calculate the volume bottled wine product reducing the quantity of free radicals in the system by 50% -  $V_{50}$ . NIR measurements were performed by NIRQuest 512 spectrometer in the region 900-1700 nm using transmittance mode and 10 mm cuvette. Partial least square regression with internal cross-validation was used for calibration models development for determination of tested parameters and SIMCA for creation of model for classification on the base of spectral data.

All the investigated wines have demonstrated better antioxidant properties in the ABTS model system compared to the DPPH one. Although the observed activity vary highly the wines containing the varieties Syrah and Malbec presented better antioxidant effect in both model systems. The comparison of the data for the Merlot wines produced from the vineyard situated on the southern slopes of Sakar Mountain in the time interval 2012-2016 denote tendency of decrease of  $V_{50}$  values from year to year. In both model systems Merlot 2016 demonstrated best antioxidant effect correlating to lowest  $V_{50}$  values.

The evaluation of the antioxidant activity of the tested wines on the basis of their spectra in the near infrared region revealed high degree of accuracy of estimation. This indicates that NIR spectroscopy could be a promising technic in quantitative determination of antioxidant activity and building classification model for discrimination of wine according to their antioxidant properties.

## POSTERS PRESENTATION

**Monday 26.06.2017 and Tuesday, 27.06.2017**

**Bulgarian Academy of Sciences - Presidium**

Moderators: Prof. P. Dolashka, Prof. P. Denev, Prof. I. Kostova

- |      |  |  |
|------|--|--|
| PP1  | <u>Gaia Rocchitta</u> , P. Carta, L. Koleva, M. A.Dettori,<br>A. Slavova-Kazakova, D. Fabbri, V. Kancheva, P.<br>A. Serra, G. Delogu,<br>Italy, Bulgaria | Antioxidant activity of naturally occurring phenols and their corresponding biphenols as individual compounds and in mixtures with anticancer drug 5-fluorouracil  |
| PP2  | <u>Adrana K. Slavova-Kazakova</u> , V. D. Kancheva, S.<br>E. Angelova, D. Fabbri,<br>G. Delogu,<br>Bulgaria, Italy                                       | Chain breaking antioxidant activity of new synthetic analogues of curcumin as individuals and in mixtures with conventional antioxidants                           |
| PP3  | <u>Davide Fabbri</u> , M. A Dettori,<br>G. Delogu ,<br>Italy   | Sustainable oxidative coupling of natural bioactive hydroxylated phenols   |
| PP4  | <u>Maria Antonietta Dettori</u> , D. Fabbri ,<br>G. Delogu,<br>Italy   | Sustainable synthesis of natural-like hydroxylated biphenyls as potential bioactive compounds  |
| PP5  | <u>Antoaneta Trendafilova</u> , T. Lefterova,<br>A. Konakchiev, S. Bozhanov,<br>Bulgaria   | Phytochemical screening and antioxidant capacity of <i>R. damascena</i> Mill. flowers dry extract  |
| PP6  | <u>Diana Ivanova</u> , S. Boyadzhieva, G. Angelov, P.<br>Nedialkov, F. Tsvetanova,<br>Bulgaria   | Activity-guided extraction optimisation of highly efficient antioxidant plant species: Study of <i>Rhodiola rosea</i> L. (Golden root)                             |
| PP7  | <u>Diana Ivanova</u> , I. Iliev, P. Nedialkov, Y. Ilieva, T.<br>Atanassova, G. Angelov,<br>F. Tsvetanova, G. Yotov,<br>Bulgaria                          | Antioxidant and antiproliferative activities of <i>Juniperus virginiana</i> "Grey Owl" cultivated in Bulgaria  |
| PP8  | Mihail Chervenkov, T. Ivanova, E. Stoyanova, A.<br>Alexandrova, E. Tzveta-nova, <u>L. Tancheva</u> , A.<br>Georgieva, E. Kistanova,<br>Bulgaria          | Effect of <i>m. Officinalis</i> l. On the level of induced lipid peroxidation in mouse liver   |
| PP9  | <u>Yanka Karamalakova</u> , G. Nikolova,<br>R. Kumar, A. Zheleva, V. Gadjeva,<br>Bulgaria  | Semiquinone glucoside derivative (SQGD) isolated from <i>Bacillus</i> sp. INM-1: antioxidant activities and protection against radiation-induced oxidative changes |
| PP10 | <u>Galina Nikolova</u> , Y. Karamalakova, R. Arora, A.<br>Zheleva, V. Gadjeva,<br>Bulgaria   | Antioxidant/ anticancer potential and radiation-protection abilities of natural antioxidant <i>psoralea corylifolia</i> linn                                       |
| PP11 | <u>Miglena Todorova</u> , M. Pasheva, Y. Kiselova,<br>D. Ivanova, B. Galunska,   | Phenolics content and antioxidant activity of beverages on the Bulgarian market – wines,   |

	Bulgaria	juices and compotes
PP1 2	<u>Milena Pasheva</u> , M. Todorova, O. Tasinov, M. Nashar, D. Ivanova, Bulgaria	Mulberry heartwood ethanol infusion modulates the expression of glutathione related enzymes in a model of oxidative stress
PP1 3	Milena Tzanova, <u>Neli Grozeva</u> , M. Gerdzhikova, M. Argirova, D. Pavlov, S. Gospodinova, Bulgaria	Flavonoid content and antioxidant potential of <i>Betonica bulgarica</i> Degen et Neic
PP1 4	<u>Sonia Losada-Barreiro</u> , C. Bravo-Díaz, L. S. Romsted, F. Paiva-Martins, Spain	Interfacial concentrations of phenolic antioxidants in model food emulsions: effects of acidity and surfactant concentration
PP1 5	<u>Sonia Losada-Barreiro</u> , C. Bravo-Díaz, F. Paiva-Martins, L.S. Romsted, Spain	Distribution of catechol in corn oil emulsions
PP1 6	<u>Ivalina Petrova</u> , V. Gandova, P. Denev, V. Shikov, K. Mihalev, Bulgaria	Kinetic parameters of copigmentation effect of caffeic acid and strawberry antocyanins
PP1 7	<u>Vessela D. Kancheva</u> , A. K. Slavova-Kazakova, S. E. Angelova, S .K. Singh, Sh.Malhotra, B. K. Singh, L. Saso, A. K. Prasad, V. S. Parmar, Bulgaria, India, Italy	Synergistic effects of new antioxidant compositions. Assessing the role of each component of equimolar triple mixtures
PP1 8	<u>Stanislava Boyadzhieva</u> , S. Georgieva, G. Angelov Bulgaria	Valorization of bio-wastes: Extraction optimization of avocado seeds aimed at maximization of extract's antioxidant activity
PP1 9	<u>Stanislava Boyadzhieva</u> , G. Angelov, S. Georgieva, D. Yankov, Bulgaria	Evaluation of polyphenolic content and antioxidant capacity of spent coffee grounds
PP2 0	Jose M. Miranda, M. Trigo, M. López, J. Barros-Velázquez, and <u>Santiago P. Aubourg</u> , Spain	Quality enhancement of chilled hake ( <i>Merluccius merluccius</i> ) by employing ice including ethanolic and aqueous extracts of alga <i>Fucus spiralis</i>
PP2 1	Elina Tzvetanova, A. Alexandrova, <u>Lyubka Tancheva</u> , M. Lazarova, S. Dragomanova, L. Alova, M. Stefanova, R.Kalfin, Bulgaria	Antioxidant mechanism in neuroprotective action of lipoic acid learning and memory of rats with experimental dementia
PP2 2	<u>Dobrina Tsvetkova</u> , St.Ivanova, Bulgaria	Estimation of DPPH radical-scavenging activity of Galantamine hydrobromide and Pymadine on the way to effective prevention and therapy of Alzheimer's disease
PP2 3	<u>Zhivko Velkov</u> , Lili Muteva, Alia Tadjer, Bulgaria	Some structural effects on the radical-scavenging activity of phenolic acids

**Wednesday, 28.06.2017, 16.00h -17.00h**  
**Bulgarian Academy of Sciences - Presidium**

Awards commission: Prof. O. Kasaikina, Prof. L. Antonov, Prof. R. Amarowicz

YPP1	Kamila Penkacik, R. Amarowicz, Poland	K.Sulewska,	Antioxidant activity of buckwheat seed extract and fractions thereof
YPP2	Katya H. Nikolova, I. T. Stoykov, P. I. Bozov, Bulgaria		Responsible structural features for cytotoxic and other kind activity of neo-clerodane diterpens from genus Scutellaria
YPP3	Yuliana Raynova, A. Pavlov, K. Idakieva, Bulgaria	Marchev,	Antioxidant activity of hemocyanins
YPP4	Maria Josefa Freiría Gandara, S. Losada-Barreiro, C. Bravo-Díaz, Spain		Partitioning of gallic acid and alkyl gallates in binary olive oil-water mixtures
YPP5	F. Mosca Juliana Villasante, X. Ramis, E. Codina, M. P. Almajano, Spain		Pla films for packaging technologies
YPP6	Simona Aleksandrova, L. Tancheva, S. Dragomanova, L. Alova, M. Stefanova, S. Abarova, B. Tenchov, R. Kalfin, Bulgaria		Antioxidant mechanisms in preventive effect of Ellagic Acid on cognitive disorders on experimental model of Alzheimer's disease
YPP7	Stela Dragomanova, L. Tancheva, M. Georgieva, A. Georgieva, R. Kalfin, Bulgaria		Neuroprotective mechanisms of combination Myrtenal and Lipoic Acid on experimental Alzheimer's disease model
YPP8	J. Ortiz, M. Toro, C. Vega, L. Ramírez, Roberta G. Barbosa, J. Barros-Velazquez, S. P. Aubourg, Brazil, Spain		Inhibition of lipid oxidation development in refrigerated salmon ( <i>Salmo salar</i> ) paste by addition of different stevia ( <i>Stevia rebaudiana</i> Bert.) extracts
YPP9	Lidiya Koleva, S. Angelova, M. A. Dettori, D. Fabbri, G. Delogu, V. D. Kancheva, Bulgaria, Italy		Antioxidant activity of selected o-methoxyphenols, benzenediols and biphenols. Theoretical and experimental studies
YPP10	A.Savova-Kazakova, Lidiya Koleva, V. Kancheva, G. Delogu, Bulgaria, Italy		Comparative study of antioxidant potential of curcumin and its degradation products – vanillin, ferulic acid and dehydrozingerone.
YPP11	Ivalina Trendafilova, M. Popova, Bulgaria		Quercetin complexes formed on initial silica and zn-modified mesoporous materials
YPP12	Stefan Dochev, P. Boranova, V. Kancheva, I.Manolov, Austria, Bulgaria		Phenolic 1,4-dihydropyridines as calcium channel blockers and antioxidants – a potential approach for effective prevention and therapy of cardiovascular diseases
YPP13	Neda Anastassova, A. Mavrova, D. Yancheva, M. Kondeva-Burdina, V. Tzankova, K. Tomovic, A. Kola-revic, G. Kocic,		Hepatoprotective and antioxidant properties of novel hydrazone derivatives of the N,N'-disubstituted benzimidazole-



	A. Smelcerovic, Bulgaria, Serbia	2-thione synthesized as melatonin analogues and study of their mechanism of action
YPP14	Caterina Rita Gemma Costanzo, M. Valcheva-Traykova, I. Kostova, Bulgaria	Effect of Pr(III) nitrate and Pr(III) complex with 5-aminoorotic acid on the accumulation of hydroxyl radicals in rat blood serum.
YPP15	I.Milkova-Tomova, Poli Radusheva, D. Buhalova, K. Nikolova, St. Krustev, Bulgaria	Fluorescence spectroscopy and antioxidant activity from Lingonberry ( <i>Vaccinium vitis-idaea</i> ) fruit juice
YPP16	Zhana Petkova, G. Antova, M Angelova-Romova, Bulgaria	Changes of lipid composition of stabilized pumpkin seed oil with different antioxidants during long-term storage
YPP17	Manol Ognyanov, N. Petkova, Bulgaria	Phytochemical characteristics and in vitro antioxidant activity of fresh, dried and processed fruits of Cornelian cherries ( <i>Cornus mas</i> L.)
YPP18	Radostina Velikova, L. Velkova, A. Dolashki, P. Dolashka, Bulgaria	Structure and stability of peptides and hemocyanin from garden snail <i>Cornu aspersum</i>
YPP19	Dessislava Gerginova, Y.Mitrev, S. Simova, I. Karadjova, Bulgaria	NMR spectroscopy of Bulgarian wine – an alcoholic beverage rich in bio-antioxidants
YPP20	Jarosław Kusio, Robert Czochara, J.Cędrawski, A. Gajda, G. Litwinienko, Poland	Synthesis and antioxidant properties of metallic nanoparticles functionalized with $\alpha$ tocopherol derivatives
YPP21	Nadya Hristova - Avakumova, L. Atanasova, St. Atanassova, T. Vangelova, V. Hadjimitova, Bulgaria	Near infra-red spectroscopy as a tool for rapid estimation of the antioxidant capacity of red wines

## IMPORTANT CONTACTS

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**Conference venue address:**

**Address** 15 Noemvri Str., 1040 - Sofia, Bulgaria

**Main accommodation place:**

Hotel of Bulgarian Academy of Sciences - Dom na uchenia – BAN

Reception: + 359 2 871 0009, Address: 50 Shipchenski prohod Str., 1113 Sofia, Bulgaria

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First International Conference on Bio-antioxidants: “Natural Bio-antioxidants – as a base for new synthetic drugs and food additives/supplements” to be held at the Bulgarian Academy of Sciences – Presidium, Sofia, Bulgaria, June 25-29, 2017 is financially supported by National Science Fund, Contract No DPMNF 01/12-20.09.2016.

# **First International Conference on Bio-antioxidants ICBA 2017**

"Natural bio-antioxidants – as a base for new  
synthetic drugs and food additives/supplements"

## **BOOK OF ABSTRACTS**

### **Young Scientists School on Bio-antioxidants YSSBA 2017**

25-29 June 2017

Bulgarian Academy of Sciences - Presidium  
Sofia, BULGARIA

<http://icba2017.orgchm.bas.bg>

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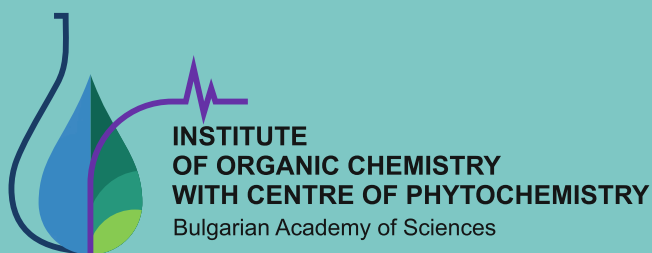
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