Workshop on Determining Antioxidants as Reactive Species Scavengers

27-28 October 2014

BOOK OF ABSTRACTS

27-28 October, 2014

Istanbul, TURKEY

FOREWORD

Dear Colleague,

It is a pleasure to invite you to the "International Workshop on Determining Antioxidants as Reactive Species Scavengers". The workshop will be held in Istanbul, Turkey, in October 27-28, 2014.

This special workshop is associated with the IUPAC (International Union of Pure & Applied Chemistry) Project (Project No: 2013-015-1-500 Apak) entitled 'Methods to evaluate the scavenging activity of antioxidants toward reactive oxygen and nitrogen species (ROS/RNS)'. The workshop will bring together experts from Australia, Greece, Portugal, Israel and Turkey and provide a forum among antioxidant researchers from all fields: food analytical chemistry, food technology, biochemistry and medicinal chemistry, and is expected to enable sharing the achievements of the IUPAC project with other participants.

We look forward to welcoming you in Istanbul for this special event.

Prof. Dr. Reşat APAK

Task Group Chairman of the Project and Workshop

October 2014, Istanbul, Turkey

IUPAC PROJECT INFO

Project name: Methods to evaluate the scavenging activity of antioxidants toward reactive oxygen and nitrogen species (ROS/RNS)

Project No: 2013-015-1-500

Objective: To identify the quenching chemistry of biologically important reactive oxygen and nitrogen species (ROS/RNS, including free radicals); to show antioxidant action against reactive species through H-atom and electron transfer reactions, and to evaluate the ROS/RNS scavenging activity of antioxidants with existing analytical methods while emphasizing the underlying chemical principles and advantages/disadvantages of these methods. In addition to discussing various methodologies with respect to kinetics and thermodynamics, the project will focus on the applications and impact of existing assays on potentiating future research and innovations to evolve better methods enabling more comprehensive study of different aspects of antioxidants, and to provide a vocabulary of terms related to antioxidants and scavengers for ROS/RNS.

Description: Reactive oxygen/nitrogen species (ROS/RNS) generation is directly related to oxidative degradation in foods and biological systems. Therefore, the search for methods to determine the scavenging activity of reactive species is very important. Determination of the antioxidant/antiradical status in these systems could contribute to prevention and treatment of diseases related to aging such as cardiovascular and neurodegenerative diseases and cancer. In addition to organism defences, the intake of dietary antioxidants and influence of real contribution of foods to antioxidant status in biological systems must be evaluated [1].

The main methods comprise the scavenging activity measurement of hydroxyl radical ($HO^{\cdot}$), superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), peroxynitrite ($ONOO^-$), and peroxyl radical ($ROO^{\cdot}$). In spite of the diversity of methods, there is currently a great need to evaluate the scavenging activity of antioxidant compounds *in vivo* and *in vitro*. In addition, there are incorrect methods frequently used, such as non-selective UV measurement of H_2O_2 scavenging, producing negative errors due to incomplete reaction of peroxide with flavonoids in the absence of transition metal ion catalysts [2].

The Project Chairman's research collaborators have recently published a comprehensive review on "methods of measurement and evaluation of natural antioxidant capacity/activity" [3]. Complementary to this technical report, this project will provide chemical information about methods clarifying reaction mechanisms, thermodynamic scavenging efficiency and kinetic scavenging rates of antioxidants. Moreover, it will aid the identification of reactive species and quantification of scavenging extents of antioxidants through various assays, make the results comparable and more understandable, and bring a more rational basis to the evaluation of these assays. Modern nano-technological methods for the estimation of reactive species and their scavenging action will also be discussed.

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ABSTRACTS

PLENARY LECTURES

LUMINESCENT METHODS FOR THE EVALUATION OF ANTIOXIDANT ACTIVITY OF OLIVE OIL AND OTHER NATURAL PRODUCTS

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Luminescent methods are based on the excitation of molecules either by absorption of light (photoluminescence, fluorescence) or by a chemical reaction (chemiluminescence: CL) and are characterized by simplicity, sensitivity, low limits of detection, and relatively low cost of instrumentation. These advantages enabled the utilization of luminescence spectroscopy in numerous applications of analytical chemistry including food and edible oil analysis. However, the majority of luminescent methods in oil analysis concerns oil extracts and not untreated oils due to the fact that the sample is not miscible with water. Nevertheless, any treatment of oil prior to analysis, such as extraction, changes the chemical composition of the tested sample which might lead to erroneous results. Hence, direct application of analytical methods to oil without any pretreatment except dilution would be preferable. The CL reactions of luminol and lucigenin have been widely exploited for the determination of hydroperoxides in untreated oils using microemulsions and homogeneous solutions, naturally bringing certain problems associated with sample exposure to reagents, phase behavior, and possible interference from compounds present in oil. Additionally, two widely applied spectrophotometric methods, the Fe(III)-phenanthroline and the CUPRAC assays, have been adapted to untreated oils *via* selection of mixture of solvents (ethanol–butanol in 3:1 v/v ratio), and optimization of the reaction conditions (reagents concentration and reaction time). This presentation describes the application of luminescent methods in the analysis of edible oils without any pretreatment such as extraction prior to analysis. Emphasis has been given to applications of chemiluminescence and fluorescence assays for determining quality parameters of edible oils, such as oxidative stability, antioxidant activity, and lipid hydroperoxides content, as well as classification or adulteration of vegetable oils. The results have been evaluated successfully and extended to the development of a variety of analytical methodologies for the evaluation of the total antioxidant activity of a wide range of natural products including oils, beverages, juices, wines and other related food products without modification of the relevant matrices.

Acknowledgements: This research has been co-financed by the European Union (European Social Fund – ESF) and Greek National Funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – Research Funding Program: THALES; Investing in Knowledge Society through the European Social Fund.

AUTOMATIC FLOW BASED METHODS TO EVALUATE THE SCAVENGING ACTIVITY OF ANTIOXIDANTS AGAINST ROS AND RNS

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The formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) has been clearly implicated in the pathogenesis of several human diseases such as atherosclerosis, diabetes mellitus, chronic inflammation, neurodegenerative disorders and certain types of cancer [1]. Considering the protective effects of antioxidants against the deleterious oxidative induced reactions involved in these pathologies, interest in antioxidant research has become a topic of increasing attention in the last few years. The existence of simple, convenient, and reliable in vitro analytical methodologies for the fast determination of antioxidant capacity of pure compounds or in complex matrices is essential to this research field. In this context, flow injection techniques are an excellent tool to automate these analyses as they provide strict time control and controlled reaction conditions [2, 3].

In the present communication, the state of the art about automatic flow-based methods for antioxidant assessment will be highlighted. Special emphasis will be given to the specific features of existing flow injection based systems for determination of scavenging capacity against biologically relevant ROS and RNS. Several features will be compared, including the analytical figures of merit, the application to real samples and the chemistry behind the assays. Perspectives about novel assays and current lab work will also be presented.

Acknowledgments: This work received financial support from IUPAC (project #2013-015-1-500), from Portuguese National Funds (FCT, Fundação para a Ciência e Tecnologia) through project Pest-C/EQB/LA0006/2013 and also from the European Union (FEDER funds through COMPETE) under the framework of QREN (NORTE-07-0124-FEDER-000067, NORTE-07-0162-FEDER-000124).

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ANTIOXIDANT COMPOUNDS: STRUCTURE-CARBONIC ANHYDRASE ISOENZYMES INHIBITION STUDIES

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Antioxidant compounds can scavenge free radicals and increase shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food, medicine and pharmaceutical products during processing and storage. An antioxidant molecule has been defined as any substance when found in low concentrations compared to that of an oxidizable substrate significantly delays or inhibits the oxidation. The major antioxidant compounds are especially phenolics and flavonoids, which are responsible for their health benefits. On the other hand, carbonic anhydrase (EC 4.2.1.1., CA) is a pH regulatory/metabolic enzyme in all life kingdoms, being found in organisms all over the phylogenetic tree. It catalyzes the hydration of carbon dioxide (CO_2) to bicarbonate (HCO_3^-) and the corresponding dehydration of HCO_3^- in acidic medium with regeneration of CO_2. Also, CA isoforms are found in a variety of tissues where they participate in several important biological processes such as acid-base balance, respiration, CO_2 and ion transport, bone resorption, ureagenesis, gluconeogenesis, lipogenesis and electrolyte secretion. On the other hand, the phenyl moiety of phenol was found to lay in the hydrophobic part of the CA active site, where CO_2, the physiologic substrate of the CAs, binds in the precatalytic complex, explaining thus the behavior of phenol as a unique CO_2 competitive inhibitor. This presentation consists of two main sections. The first section is devoted to main phenolic antioxidant compounds in the foodstuffs and beverages. The second general section is about some definitions of CA inhibitory effects of the main phenolic compounds used for antioxidant activity. The phenolic compounds and acids had marked especially CA I, and II inhibition effects and might be used as leads for generating CA isoenzyme inhibitors. This class of compounds may lead to isoform-selective inhibitors targeting just one or few of the medicinally relevant CAs. In addition, there are given some chemical and kinetic basis and technical details related to phenolic antioxidant compounds and CA isoenzymes.

NUTRITIONAL AND PHARMACEUTICAL APPLICATIONS OF BIOACTIVE COMPOUNDS OF SOME EDIBLE BERRIES AND TROPICAL FRUITS

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Extracts from Chilean and Polish berries and Thai tropical fruits decreased the proliferation of HT-29 and SW48 human colorectal cancer (CRC) cells lines. This effect was concentration dependent (Fig. 1). The inhibition of cancer cell proliferation correlated with the levels of polyphenols, flavonoids and the antioxidant activities of investigated samples. DPPH kinetic measurements were used to compare, distinguish and discriminate the antiradical activity among berry extracts by multivariate analysis (Fig. 2). The interaction between two flavonoids (catechin and quercetin), human serum albumin (HSA) and polyphenol extracts of berries and fruits was investigated by 3-D fluorescence and FTIR spectroscopy (1-4). The new kind of berries and fruits has a strong ability to decrease the intrinsic fluorescence of HSA (Fig. 3) and is comparable with quercetin. Supplementation of diets with berries and fruits positively affects plasma lipid profile, and antioxidant activity in rats fed cholesterol containing diets. The main histopathological changes were detected in the liver and aorta of rats fed a high-cholesterol diet without fruit supplementation. These changes were minor in rats with fruit supplementation. In conclusion, these findings suggest that the intake of berries and fruits, as a source of natural antioxidants, may reduce colon cancer risk and coronary artery disease. The consumption of berries and fruits as a supplement to everyday human diet and for pharmaceutical applications is important for health effects.

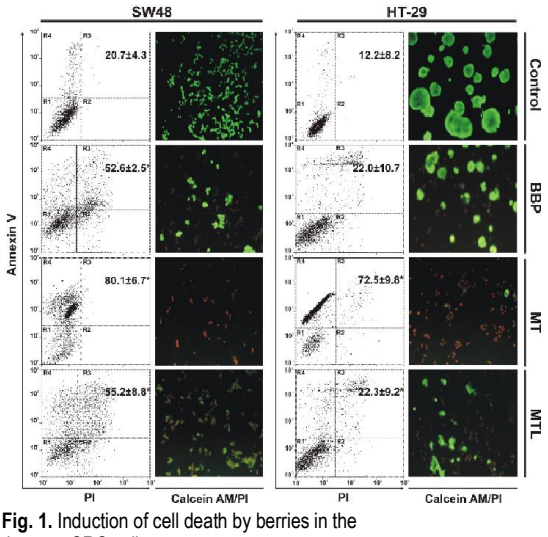
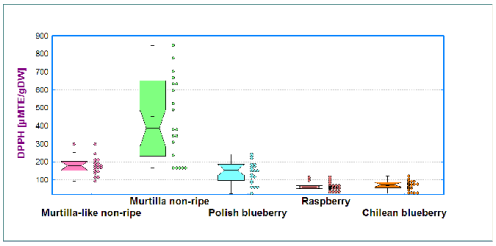


Fig. 1. Induction of cell death by berries in the human CRC cells.

Fig. 2. Box/Whisker and Dot Plots of DPPH-free radical activity of DMSO extracts of berries.



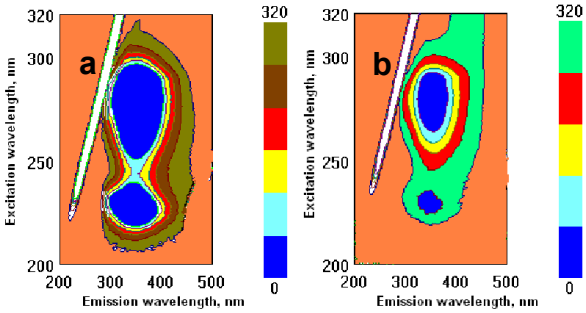


Fig. 3. Contour map (from 3 D-FL spectra) illustrates the quenching results HSA (a) and HSA+blueberry extract (b).

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COMPARATIVE EVALUATION OF SELECTED ANTIOXIDANT CAPACITY / ACTIVITY ASSAYS WITH SPECIAL REFERENCE TO CUPRAC, CERAC AND FERRICYANIDE METHODS

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Total antioxidant capacity (TAC) or antioxidant activity (AOA) levels of biological fluids and foods are measured for the diagnosis and treatment of oxidative stress−associated diseases in clinical biochemistry, for meaningful comparison of foods in regard to their antioxidant content, and for controlling variations within or between products. Since each assay has its own reaction thermodynamics and kinetics, no two assays, and even different versions of the same assay, do not yield identical antioxidant powers for antioxidants. In this presentation, the development and modification of three basic antioxidant assays in our laboratories, namely CUPRAC (CUPric Reducing Antioxidant Capacity), CERAC (Cerium(IV) Reducing Antioxidant Capacity), and ferric-ferricyanide will be summarized. The superiorities and drawbacks of the developed/modified methods compared to those of other electron-transfer (ET)-based (e.g., FRAP and Folin), hydrogen atom transfer (HAT)-based assays like ORAC, and mixed-mode (ET/HAT) assays (e.g., ABTS and DPPH) will be evaluated. The CUPRAC assay has been further modified to fit the needs of scavenging activity determinations of reactive oxygen and nitrogen species (ROS/RNS) such as hydrogen peroxide, superoxide anion and hydroxyl radicals, where either the original probe or the converted product had CUPRAC reactivity. In the assay of protein thiols, the CUPRAC assay was modified to use pH 7 urea buffer instead of the conventional ammonium acetate buffer. A colorimetric TAC sensor resembling a pH-indicator paper (named as the “CUPRAC sensor”) was developed by immobilizing the Cu(II)-Nc reagent onto a perfluorosulfonate cation-exchanger Nafion membrane. An on-line HPLC-CUPRAC method suitable for phenolic profiling of complex samples was developed with the use of a post-column reaction coil to which the CUPRAC reagent was pumped. The CUPRAC method has now evolved into an integrated series of measurements for antioxidant characterization, and is being increasingly used in world research centers performing routine antioxidant determinations.

INVITED LECTURES

IL-01

EVALUATING THE IN VITRO BIOACCESSIBILITY OF PHENOLICS AND ANTIOXIDANT ACTIVITY DURING CONSUMPTION OF DRIED FRUITS WITH NUTS

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Dried fruits and nuts are considered as healthy snacks and they are often consumed together in the Turkish diet. The purpose of the current study was to evaluate the effect of co-digestion of commonly consumed dried fruits together with nuts and to monitor the stability and recovery of total phenolics and antioxidant capacity.

The different dried fruits and nuts (figs, apricots, raisins, almonds, walnuts, hazelnuts) were collected as triplicates from a local market in Istanbul, Turkey. The recovery of total phenolics and antioxidant capacity after co-digestion of dried fruits with nuts was measured using spectrophotometric methods. For the analysis of total antioxidant capacity, four different methods were used including 2,2-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazil (DPPH), ferric reducing antioxidant power (FRAP) and cupric ion reducing antioxidant capacity (CUPRAC). Statistical differences between samples were evaluated by one-way ANOVA (p<0.05).

The results showed that, initially, walnuts contained the highest amount of total phenolics and antioxidant capacity, whereas almonds had lowest amounts. Gastric digestion enhanced the total phenolic content and antioxidant capacity determined using DPPH and FRAP assay. The dialyzed fraction after intestinal digestion represented 7-69% and 2-205% of the initial total phenolics and antioxidant capacity, respectively. For all samples, codigestion of dried fruits with nuts reduced the bioaccessible total phenolics (22-77%) and antioxidant capacity determined using ABTS and CUPRAC assays (5-68%). On the other hand, according to DPPH and FRAP assays, consumption of certain mixtures (fig-walnut, fig-hazelnut, apricot-walnut, apricot-hazelnut) resulted in higher bioaccessible antioxidant capacity (10-107%).

Current study reported the consequences of codigestion of dried fruits and nuts on total phenolics and antioxidant capacity following the *in vitro* digestion.

IL-02

CHROMAC ANTIOXIDANT CAPACITY METHOD: PRINCIPLES AND APPLICATIONS

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The CHROMAC is a novel assay as a spectrophotometric total antioxidant capacity assay based on the reaction of excessive Cr(VI) with phenolic compounds and formation of colored complex with remaining Cr(VI) and diphenylcarbazide. Phenolic compounds react with excessive amounts of Cr(VI) at low pH values, causing reduction of Cr(VI) to Cr(III) and conversion of phenols to oxidized products. The assay comprises of the antioxidant with a chromium(VI) solution, a 1,5-diphenylcarbazid in acidic medium and subsequent measurement of the developed absorbance at 540 nm after 50 min. The developed assay was successfully applied to the measurement of antioxidant capacity of different plant and fruit samples and comparable results were obtained by CUPRAC and ABTS assays. The CHROMAC assay was applied to individual phenolic acids and flavonoids, and their combinations with different classes. The results show structure-activity relationship with the position and number of hydroxyl group in the phenolic compound. Additivity of different classes of phenolics was above 93% which implies that CHROMAC assay is not influenced by other phenolics and interfering compounds. The CHROMAC assay is simple, inexpensive, sensitive and selective indirect spectrophotometric method for the determination of total antioxidant capacity of different types of food antioxidants.

IL-03

ANTIOXIDANTS BOUND TO INSOLUBLE FOOD MATRIX: THEIR MEASUREMENT, REGENERATION BEHAVIOR AND NUTRITIONAL RELEVANCE

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Antioxidants bound to insoluble food matrices, i.e. dietary fiber constitutes a major part in dietary antioxidants and known to exert significant antioxidant activity. Dietary fibers affect biological properties of these bound antioxidants by reducing the rate of their release. The slow and continuous release dietary fiber bound antioxidants lead them to survive a considerable time in the human gastrointestinal tract. It is challenging to take the advantage of longer survival of bound antioxidants in designing new generation of healthy foods. This needs deeper investigation of the activity of bound antioxidants and their interaction with soluble antioxidant compounds. However, literature related to food antioxidants is largely based on the measurement of solvent extractable and or acid/alkaline hydrolysable forms present in foods. Recently, we have introduced the QUENCHER procedure to measure the antioxidant activity of functional groups, which are bound to insoluble fraction of food materials. Working hypothesis of this procedure is the reaction between bound antioxidants and freely soluble radical species by a surface reaction phenomenon. The procedure has been successfully applied to a variety of food matrices including cereals and bakery products, fruits and vegetables, nuts and seeds, and meat products using various measurement methodologies including ABTS, DPPH, FRAP, ORAC, and CUPRAC. Recently, we investigated the regeneration behaviors of depleted antioxidants bound to insoluble dietary fibers from different sources. Results indicate that the antioxidant capacity of compounds chemically bound to the insoluble moiety can be reconstituted in the presence of other hydrogen-donating substances in the liquid phase. Beverages like green tea, black tea, espresso and instant coffee are very effective in the regeneration of bound antioxidants.

ORAL PRESENTATIONS

OP-01

ANTIOXIDANT EFFICIENCY OF SEABUCKTHORN (*Hippophae rhamnoides* L.) EXTRACT IN EHRLICH ASCITES CARCINOMA (EAC) OF BALB/C MICE MODEL

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The majority of the world's population in developing countries still relies on herbal medicines to meet their health needs. There is a growing interest in the pharmacological evaluation of medicinal plants. Seabuckthorn (SBT), which belongs to the family *Elaeagnaceae*, a unique and valuable plant has recently gained worldwide attention, mainly for its medicinal and nutritional potential. SBT has an extremely wide distribution but fragmented in Europe and Asia, from China, Mongolia, Russia, Kazakhstan, Turkey, Romania, Switzerland, France to Britain and north to Finland, Norway and Sweden (Bartish*et al*., 2002). This plant has been distributed over Turkey at mainly North and East regions from the sea level up to high elevations of about 3000 m (McKean, 1982). Almost all of parts of SBT contains vitamins (A, C, E, K, riboflavin, folic acid), mineral elements, organic acids, free amino acids, monosaccharides sugars, large amount of carotenoids (α, ß, δ-carotene, lycopene), different flavonoids (quercetin, isorhamnetin, myricetin, kaempferol), fatty acids, triacylglycerol, glycerophospholipids, phytosterols (ergosterol, stigmasterol, lansterol, amyrins), zeaxanthin esters, phenolic compounds and alpha-tocopherol acids.

Like SBT, these compounds are of our interest from the chemical point of view, and also because many of them possesses biological and therapeutic activities including antioxidant, cardiovascular, cancer therapy, healing, anti-inflammation, antiradiation effect, treatment of gastrointestinal ulcers, as a liver protective agent, antioxidant, platelet aggregation, and immunomodulator. Another aspect of antioxidant administration in cancer patients is that these could affect antineoplastic efficacy or the development of side effects of anticancer drugs.

The aim of the present study was to evaluate the antioxidant activity of SBT against EAC cells by measuring primer antioxidant enzyme superoxide dismutase (SOD), and lipid peroxidation products malondialdehyde (MDA). The seed oil of SBT was obtained from Inner Mongolia Yuhangren Hi-Tech Industrial Co., Ltd.

Male Balb/c mice (3-4 month) were obtained from Experimental Animal Research Center, Cerrahpasa Medical Faculty. Experiments were carried out in accordance with Ethical Committee Guidelines laid down by the local committe regarding the care and use of animals for experimental procedures. A line of EAC obtained from the Faculty of Pharmacy. The tumor line was maintained in male Balb/c mice once by interperitoneal injection of $1.5x10^6$ cells/mouse. Mice were divided into three groups. Control, interperitoneal injected EAC and the animals once interperitoneal injected EAC also interperitoneal injected SBT extract daily 10 Unit for three weeks. At the end of three weeks, all groups were sacrificed under ether anesthesia, the accumulation of ascithes ejected,

whole blood samples were collected from the heart of the animals and 5 ml liquid of ascithesmaiwas collected separately. In both experimental groups, SOD activity were determined by the method of Sun *et al* (1988). MDA levels were determined by the method of Budge and Aust. In EAC cells, protein contents were determined by the method of Lowry *et a* (1951).

In EAC group, both in ascetic fluid and EAC cell oxidative stress parameters were significantly high when compared with EAC threaded with SBT group. It is because MDA as an oxidative stress marker was decreased when EAC group was treated with SBT ($p<0.001$). Although, the most important primer antioxidant SOD enzyme activity was found decreased in ascetic fluid ($p<0.001$), same enzyme activity was found increased in EAC cells ($p<0.001$) (Table 1 and 2). From these results, we can conclude that increased SOD activity in EAC cell is more important to decrease oxidative stress and growth of EAC cells. It is clear that SBT has antioxidant efficiency with vitamins and specific benefical biomolecules combinations both on ascetic fluid and also on EAC cells. On the other hand, a significant decrease of SOD activity of ascetic fluid in EAC+SBT group but increase of SOD activity in the same group EAC cells seems to be contradictory but SOD expression and activation may be the result of cell response of EAC cells (Table 1). So, high SOD activity of EAC cells and antioxidant efficiency of SBT might be the result of decreased MDA levels when EAC cells treated with SBT (Table 2).It is clear with these results that SBT vitamins and specific benefical molecules has an important advantage on cancer treatment by lowering oxidative potential.

Table 1: Results and comparison of ascetic fluid MDA levels and SOD activity of mice in EAC and EAC treated with SBT groups

	EAC	EAC + SBT
MDA (nmol/mg protein)	93.27 ± 5.63	26.61 ± 4.18*
SOD (U/mg protein)	63.64 ± 3.54	32.64 ± 3.35*

*$p<0.001$; against EAC group.

Table 2: Results and comparison of EAC cells MDA levels and SOD activity of mice in EAC and EAC treated with SBT groups

	EAC	EAC + SBT
MDA (nmol/mg protein)	220.31 ± 13.91	121.59 ± 5.24*
SOD (U/mg protein)	69.46 ± 5.20	101.05 ± 5.10*

*$p<0.001$; against EAC group.

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OP-02

A COMPARISON OF ANTIOXIDANT CAPACITY, PROTEIN PROFILE AND CARBOHYDRATE CONTENT OF WHEY PROTEIN FRACTIONS

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Whey is used as an additive in food industry and a dietary supplement in nutrition. In our study we performed a comparative analysis of antioxidant potential of whey and its fractions. Fractions were obtained by size exclusion chromatography, before and after enzymatic digestion with pepsin or trypsin. Superoxide radical scavenging, lipid peroxidation inhibition and cupric ion reducing activities of different fractions were checked. Peptides were detected by SDS–PAGE and GC–MS was used to determine carbohydrate content of the fractions. All samples showed antioxidant activity and the second fraction of the trypsin hydrolysate showed the highest superoxide radical scavenging activity. CUPRAC value of this fraction was two times higher than that of whey filtrate. The first fraction of the pepsin hydrolysate was the most effective inhibitor of lipid peroxidation. Each sample exhibited a different polypeptide profile. Different percentages of carbohydrates were identified in whey filtrate and in all second fractions, where galactose was the major component.

OP-03

RADICAL SCAVENGING COMPOUNDS FROM LAMIACEAE FAMILY PLANTS GROWING IN ANATOLIA

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Lamiaceae (=Labiatae) family plants were represented by over 7500 species within 236 genera in the world. Its largest genera are *Salvia*, *Scutellaria*, *Stachys*, *Plectranthus*, *Hyptis*, *Teucrium*, *Vitex*, *Thymus*, and *Nepeta*. Anatolia is a rich source for the Lamiaceae which is the most endemic species containing family in Turkey, represented by 45 genera and 550 species with over 735 taxa [1]. Since the family plants are rich in terpenoids and flavonoids and other phenolic compounds they have high potential as antioxidant and anti-inflammatory and cytotoxic agents. In our continuing studies since over 30 years on Lamiaceae family plants, 65 *Salvia* species, 18 *Sideritis* species, 20 *Nepeta* species, 10 *Teucrium* species, 3 *Ajuga* species, 2 *Lavandula* species, 2 *Stachys* species, and 5 *Origanum* species have been investigated for their chemical components and or biological activities, and particularly antioxidant, anticholinesterase and cytotoxic activities [2-4]. Many Lamiaceae plant extracts showed high anti-radical activities in various antioxidant test systems [4-5]. Also, from most of them, such as *Salvia, Sideritis, Teucrium and Stachys* species, a number of flavonoids isolated which were showed high antioxidant activity. From *Salvia* species isolated triterpenoids, such as ursolic acid, oleanolic acid, and their derivatives and also some diterpenoids, particularly abietane diterpenes with phenolic ring C exhibited high antioxidant properties including carnosic acid, carnosol, ferruginol, taxodione and 6-hydroxysalvinolone besides anticholinesterase effects [4]. *Sideritis, Teucrium*, *Nepeta* and *Stachys* species have many phenolics and flavonoids, among them especially flavonols and phenolic acids showed high antioxidant properties in a complementary test assays (lipid peroxidation inhibitory, DPPH free radical, ABTS cation radical, superoxide anion radical and CUPRAC tests). As a conclusion, a number of Lamiaceae plants that have been investigated exhibited medium-high antioxidant properties besides their anticholinesterase, anti-inflammatory and/or cytotoxic effects, which might be considered as potential food additives, nutraceuticals and phototherapeutics. But they require immediately further clinical trials.

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OP-04

CAPILLARY ELECTROPHORETIC METHODS FOR THE ANALYSIS OF ANTIOXIDANT COMPOUNDS IN FOOD AND PLANT MATERIAL

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Capillary electrophoresis (CE) is a separation method known for easy method development, low sample consumption, very fast analysis times, and high separation efficiency. Recently, CE has been widely employed for the separation and determination of active components in various plants and food. This presentation will review some capillary electrophoretic methods developed by our group for the analysis of antioxidant compounds in food or in plants.

New capillary electrophoretic methods with laser induced fluorescence detection (LIF) were developed for the determination of two important antioxidant compounds, namely berberine and curcumin. Though berberine and curcumin have native fluorescence, their intensities are very low in aqueous medium. In the present study, by the addition of 2-hydroxy propyl β-cyclodextrin (2-HP-beta-CD) to the separation electrolyte, the fluorescence intensities of both molecules were enhanced and very low detection limits were obtained. The developed method was successfully applied for the determination of the berberine contents in Chinese medicinal plants and herbal supplemental tablets [1]. The developed method for curcumin was applied to turmeric samples. Horminone and 7-O-acetylhorminone are abietane diterpenoids found in Salvia species. Both isomers exhibit antioxidant properties. The main problem in their isolation is to separate them from each other. They have been often isolated simultaneously from plant extracts. A simple and fast micellar electrokinetic method (MEKC) was developed for the quantitative analysis of horminone and 7-O-acetylhorminone in *Salvia* species [2]. Though the sage (*Salvia officinalis*) plant is a rich source of di- and triterpenoids, phenolic acids, and flavonoids, the main antioxidative effect of sage has been reported to relate to the presence of carnosic acid and rosmarinic acid. A simple and rapid capillary electrophoresis method was developed for the identification and quantitative determination of these two antioxidant compounds in the extracts of commercial sage tea bags [3]. Three rarely found 1,5-hydroxy isomers of anthraquinones in the plant root were completely separated in 6 min and their amounts were determined [4].

In two of our studies, the amount of food ingredients determined by capillary electrophoretic methods were interpreted together with the antioxidant activity and total phenolics of food in order to detect food adulteration or the change in nutritional value of food. From the fructose/glucose ratio, organic acid profiles, total phenolics, and antioxidant capacity values, a possible adulteration was detected in one of the commercial pomegranate juices investigated [5]. Chemical and biochemical properties of standard, hybrid, and grafted melons cultivated under

the same agricultural conditions in adjacent fields were investigated and compared based on pH, Brix, antioxidant activity, total phenolics, ascorbic acid, individual phenolics, sugar, and organic acid values. It was thus concluded that the nutritional value of melons changed by the application of hybridization, grafting, or standard (open pollinated) production methods [6].

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OP-05

DEVELOPMENT OF NOVEL METHODS FOR THE DETERMINATION OF SYNTHETIC FOOD COLORANTS

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Color has been added to our foods for centuries in order to attract consumers. Colorants are basically divided into two main groups as synthetic and natural food colorants. As a result of the toxic and carcinogenic effects of some synthetic food colorants, their utilization is limited by governmental regulations. Therefore, the identification/determination of these substances is of great importance. The aim of this study is to investigate fast, accurate and applicable methods for the determination of synthetic colorants. In this study, the determination of synthetic food colorants was investigated in three different ways. In the first investigation, CUPRAC [1] and CERAC [2] antioxidant capacity assays were adapted to the determination of synthetic food colorants for the first time. For the second step, the results of the proposed methods were correlated with HPLC findings. Individual standard solutions, synthetic mixtures of synthetic colorants, and colorant extracts were identified and quantified with HPLC on a C18 column equipped with a diode array detector, and slight modifications on the existing HPLC method were made to analyze synthetic colorant mixtures. In this part of the study, the chromatographic conditions were adjusted for the seperation of synthetic food colorants. The third part of the study, which may be seen as a combination of the first and second parts, consists of post column derivization experiments. On-line HPLC-CUPRAC technique introduced in 2010 [3] was applied, and on-line HPLC-CERAC assay was developed for the first time to synthetic food colorants. By adapting the novel spectrophotometric CERAC and CUPRAC assays of total antioxidant capacity to the determination of total food colorant content, certain beverage samples were easily and accurately analyzed. The total colorant content was found at low reagent and instrumentation costs with the use of a UV–vis spectrophotometer easily found in a conventional laboratory equipped with simple instruments. Moreover, CUPRAC and CERAC methods have the potential to work with HPLC technique in post column derivization experiments.

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OP-06

COMPARISON OF TOTAL ANTIOXIDANT CAPACITIES OF HUMIC ACIDS BY USING CONVENTIONAL AND QUENCHER-CUPRAC METHODS

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Humic acids are the class of high molecular weight aromatic polyhydroxypolycarboxylic acids-natural polyelectrolytes of humic substance [1]. Antioxidant properties of humic acids are sourced from the structures having carboxyl and phenolic hydroxyl groups (-COOH, Ar-OH) which are also responsible for acidic properties of humic acids. These structures have lower molecular weight than other fractions of humic acid such as aliphatic chains or polyols [2]. It is assumed that electron transfer take places via hydroquinoid moieties and/or phenolic hydroxyls to give free radicals [3, 4]. In order to prevent loss on antioxidant capacity, QUENCHER method, which is recently introduced for measuring the antioxidant capacity of cereals, was chosen. This method is based on direct measurement of solid samples by mixing them with the free radicals. Thus, bound phenolics are forced to dissolve by oxidizing TAC (Total Antioxidant Capacity) reagents. Antioxidant capacities sourced from nonextractable polyphenols and polysaccharides can be determined through this method [5, 6]. In this study, antioxidant properties of humic acids samples, obtained five different sources, were investigated. Total antioxidant capacities (TAC) of them were calculated as gallic acid equivalent antioxidant capacity per kg of humic acid by using conventional and QUENCHER-CUPRAC method. 10 mg samples were shaked with CUPRAC reagent for 30 minutes at room temperature. The incubated samples were studied at 50 ^{o}C. Different solvent mixtures such as ethanol:distilled water and dimethyl sulfoxide:distilled water with varying ratios were used in the experiments. Conventional measurement of antioxidant capacity was performed in the samples prepared by dissolving of humic acid in convenient solvent. All experiments were sequentially tried for four days and three parallels in each day. The maximum total antioxidant capacity was obtained by using 1:3 dimethyl sulfoxide:distilled water solvent mixture, which is followed by 1:3 ethanol:water. TAC values gained from conventional method were significantly lower than obtained from QUENCHER-CUPRAC Method. This method provides calculation of real TAC values of humic acids without oxidation process, which causes loss on antioxidant groups, by extraction of phenolic groups simultaneously with redox reaction by CUPRAC reagent.

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OP-07

APPLICATIONS AND DEVELOPMENT OF CERAC (Ce(IV)-based antioxidant capacity) METHODS

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Antioxidants are health-beneficial compounds fighting against reactive oxygen and nitrogen species (ROS/RNS) and free radicals that may eventually give rise to various diseases. The chemical diversity of antioxidants makes it difficult to separate and quantify antioxidants from the vegetable, fruit and food matrix. Therefore it is desirable to establish methods that can measure the total antioxidant capacity (TAC) level directly from vegetable and fruit extracts. After reaction with antioxidant test compounds, cerium(IV)-based antioxidant capacity (CERAC) methods either measure produced Ce(III) ions fluorometrically [1] or unreacted Ce(IV) ions spectrophotometrically [2]. The reaction conditions for both CERAC methods were first optimized to produce meaningful results for antioxidants. In this regard, 0.3 M H_2SO_4 and 0.7 M Na_2SO_4 in aqueous medium maintained the required redox potential for Ce(IV) ions to oxidize true antioxidant compounds but not citric acid, simple sugars and pharmacological ingredients. The developed procedure was successfully applied to the TAC assay of antioxidant compounds such as trolox, quercetin, rutin, gallic acid, ascorbic acid, catechin, naringin, naringenin, caffeic acid, ferulic acid, glutathione, and cysteine. Since the TEAC (trolox-equivalent antioxidant capacity) coefficients found with the proposed method of naringin–naringenin and rutin–quercetin pairs were close to each other, this Ce(IV)-based assay presumably caused the simultaneous hydrolysis of flavonoid glycosides to the corresponding aglycones and their subsequent oxidation such that the hydrolysis products exhibed antioxidant capacities roughly proportional the number of –OH groups contained in a molecule. After the investigation of experimental results, it can be concluded that the redox potential of reaction between Ce(IV) and antioxidant molecules disables the interference effects of simple sugars, citric acid and amino acids (without thiol groups). This property of the improved method increases its importance when compared to widely used alternative electron transfer-based methods (Fe(III)- phenanthroline with E^0 = 1,06 V, and Folin-Ciocalteu method with an unknown potential) showing high redox potentials. Spectrophotometric and spectrofluorometric CERAC methods have advantages over other ET−based assays, namely simplicity, availability and stability of reagents, reproducibility over a wide concentration range, completion of the redox reactions for most common flavonoids within reasonable time. Significant differences between the methods were calculated by analysis of variance (ANOVA) procedures; $p < 0.05$ were regarded as significant. Moreover, in order to prove the additivity and accuracy of the method, linear calibration graphs of trolox in ultra-pure water and 3 different plant tea samples were drawn with the aid of standard addition method. The slopes of parallel calibration lines were found to be identical with a value of $(1.86 \pm 0.02) \times 10^6$. This result shows that the method does not chemically deviate from Beer's law due to interferences

from other species found in complex chemical matrices, and improved spectrofluorometric CERAC assay can measure the total antioxidant capacities of sample mixtures. Methyl alcohol extracts and traditional hot water infusion of curative plants such as sage, nettle, green tea, chamomile tea, linden, mint and rose hip were anayzed with spectrophotometric CERAC, spectrofluorometric CERAC and other electron-transfer based total antioxidant capacity determination methods.

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OP-08

ANTIOXIDATIVE SECONDARY METABOLITES AND EXTRACTS FROM MEDICINAL PLANTS

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Free radicals such as superoxide anion, and hydroxyl and peroxyl radicals, which are produced in biological systems and foods, are responsible for oxidation of cell lipids and DNA damage, and they may cause serious diseases (e.g. cancer, coronary arteriosclerosis, diabetes mellitus). Dietary antioxidants may be effective in prevention of oxidative damage. Many scientists have focused on medicinal plants to discover natural antioxidants since some synthetic antioxidants have toxic effects. In addition, natural antioxidants may have an important role in protecting human health. Twenty Turkish medicinal plants (*Jurinea*, *Iris*, *Achillea*, *Salvia*, *Plantago*, Micromeria) were investigated phytochemically and biologically by our research group (1-5). DPPH free radical, ABTS cation radical, superoxide anion radical scavenging, metal chelating, FRAP, β-carotene bleaching and CUPRAC methods were used to determine the antioxidant activity of the plant extracts and their secondary metabolites.

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OP-09

BIOMARKERS OF OXIDATIVE STRESS IN THE MUSCLE OF AGING MICE

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Oxidative stress caused by reactive oxygen species has been proposed to cause age related muscle wasting (sarcopenia). I used three biomarkers to evaluate the relationship between oxidative stress and muscle wasting. To evaluate the extent to which irreversible oxidative damage of macromolecules could be contributing to sarcopenia, lipofuscin was quantified as a cumulative measure of oxidative damage. Reversible and irreversible oxidative damage to proteins was measured using a protein thiol oxidation and carbonylation assay, respectively. The result showed that there was also a strong age-related increase in lipofuscin content of the quadriceps limb muscles. A correlation (R^2=0.698) was observed between increasing protein carbonylation and loss of mass of gastrocnemius muscle of aging mice. However, reversible thiol oxidation of total and individual proteins did not change significantly with age. These data indicate a strong association between age-related skeletal muscle wasting (sarcopenia) and increased levels of irreversible oxidative damage of proteins and lipofuscin accumulation. However, there was no marked relationship between sarcopenia and reversible protein thiol oxidation.

POSTER PRESENTATIONS

PP-01

ANALYSIS OF VOLATILE COMPONENT AND TOTAL ANTIOXIDANT CAPACITY OF *PROSOPIS FARCTA*

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Plants have been used for medical purposes since many centuries. Prosopis includes nearly 50 species which are generally spiny trees or shrubs and well adapted to warm weather. *Prosopis farcta* is a small, prickly shrub, 30-80 cm tall species of prosopis and widespread in Northern Africa and much of southwestern Asia, from Kazakhstan south to the Indian and west to the Middle East. It has an important ecelogical role for the protection and improvement of soils since has an high degree of salt toleration. On the other hand leaves and beans of *Prosopis farcta* have been used as a traditional medicine for treatment of some diseases and disorders in Turkey. For example, after boiling P. Farctas leaves used for epidemic disase order. Beans of *prosopis farcta* used for treatment of diarrhea among the people in Turkey. There have been some academic works searching the effect of *Prosopis farcta* on cholesterol and diabets hurts. Besides these effects, it's antitumour activity, antiparasitic and antimicrobial have been noted recently. There has not been academic work for antioxidant capacity of *prosopis farcta*, therefore to research it's total antioxidant capacity the beans of *prosopis farcta* collected in september. The beans are kept in proper conditions during experiments. In this study, firstly the beans of prosopis farctas volatile components are identified with two-dimensional gas chromatography time of flight mass spectrometry (GC*GC-TOF/MS). Accordingly GC*GC-TOF/MS chromatography, volatile components of *prosopis farcta* listed through high percent to the lowest percent. Glicerin is the second percent of volatile components which effective on diabets disordes. Secondly, we investigated total antioxidant capacity of *prosopis farcta* after applying extraction methods to beans of *prosopis farcta*. Spectrophotometric and spectrofluorometric CERAC [1, 2], CUPRAC [3] and Modified Folin-Ciocalteu [4] methods used for antioxidant capacity analysis.

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PP-02

SEPARATION OF CAROTENOIDS FROM PASTE (TOMATO) INDUSTRY WASTES

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It is known that there is a reverse relationship between the consumption of fruits and vegetables and the incidence of certain types of cancer and cardiovascular diseases. This protective effect of foods is attributed to bioactive compounds such as carotenoids, phenolics and ascorbic acid. Since carotenoids are commonly found bioactive compounds in fruits and vegetables and have antioxidant effect, the interest on carotenoids has increased. Tomato and tomato products are important sources of carotenoids. Lycopene is the main tomato carotenoid [1, 2].

Due to gained importance in the use of natural antioxidants instead of synthetic ones, recovery studies of natural antioxidants from food processing by-products (wastes) have increased [3]. Tomato paste industry wastes also have a potential to become a source of important compounds having antioxidant properties.

In this study, total antioxidant capacity of paste (tomato) industry waste by easy and cheap CUPRAC (Cupric Ion reducing Antioxidant Capacity) [4] assay which was developed in our laboratories, total phenolic content by Folin-Ciocalteu method [5] and also total carotenoid content by Minguez-Mosquera and Hornero-Mendez method [6] were determined. HPLC method was developed for the determination of phenolic and carotenoid components. For the separation of lycopene and β-carotene from phenolic components and from each other, 100% acetone extract of tomato waste was loaded on alumina sorbent and eluted by different volume ratios of ethyl acetate-hexane mixture. Recoveries of lycopene with 38% yield and β-carotene with 25% yield were achieved.

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PP-03

SEPARATION OF FLAVANONES FROM ORANGE JUICE INDUSTRY WASTES

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Because of their antioxidant properties, phenolic compounds help to neutralize free radicals, which are unstable species that are linked to the development of a number of degenerative diseases including cancer, cardiovascular disease and cataracts. Orange is an important source of polyphenolic compounds. A major part of phenolic compounds in orange are hydroxycinnamic acids and flavonoids, among which flavanones are predominant [1].

Recovery studies of antioxidants from by-products (wastes) of food processing plants have increased due to the fact that replacement of synthetic antioxidants by natural ones has gained importance [2]. Citrus industry produces large quantities of by-products which may account for up to 50% of the total fruit weight. The peels are an abundant source of natural flavonoids, and contain higher amount of phenolics compared to the edible portions [3].

In this study, the orange juice industry waste was primarily evaluated in terms of total antioxidant capacity and phenolic compounds. For this purpose, CUPRAC method [4] for the total antioxidant capacity and reversed-phase HPLC method for identification and quantitation of phenolic compounds were used. Total antioxidant capacity of methanol:water phase of orange juice industry waste extract was found as 78.1±3.0 μmol trolox g^{-1} dry weight, and of acetone phase as 2.0±0.1 mmol trolox g^{-1} dry weight. Mainly phenolic compounds, naringin and hesperidin were determined by HPLC as 0.144±0.012 mg g^{-1} dry weight and 0.177±0.013 mg g^{-1} dry weight, respectively.

Separation (preconcentration) studies of naringin and hesperidin from the other phenolic compounds with small quantities and also from each other were carried out on commercial DPA-6S SPE cartridge. While naringin and hesperidin were completely separated from other phenolic compounds with acidic and non-acidic methanol elutions, the separation of the two compounds from each other was significantly achieved with 22% (v/v) dimethyl formamide elution.

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PP-04

EVALUATION OF PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF ACACIA HONEY-BASED PRODUCT

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Honey is one of the oldest and widely used food product which is well known for its high nutritional and medicinal value. It serves as a source of natural antioxidants, effective in reducing the risk of heart disorder, cancer, immune-system decline, etc. The antioxidant activity of honey is mainly contibuted to the presence of various bioactive components (enzymes, amino and organic acids, polyphenols, carotenoid-like substances). In Serbia, honey is usually used in the original, unprocessed form, and also enriched with pollen, propolis, royal jelly, or other primary bee products. Besides these types of honey, honey-based product (honey with nuts and dried fruits) is also prepared and consumed as a tasty dessert.

The total phenol (TPh) content and antioxidant properties of honey-based product (acacia honey with 20, 30 and 40% apricots; AH, AH20, AH30 ahd AH40) were evaluated after one-year storage. TPh increased with increasing concentration of apricot in honey, from 20.04 for AH to 92.79 mg GAE/100 g for AH40. In both antioxidant assays (DPPH test and reducing power, RP), the antioxidant activity increased with increasing the concentration of apricots in honey. Antioxidant activities were expressed as EC_{50}^{DPPH} value (the amount of antioxidant necessary to decrease the initial concentration of DPPH radicals by 50%) and $RP_{0.5}$ value (the effective concentration assigned at 0.5 value of absorption). The EC_{50}^{DPPH} value ranged from 164.09 for AH to 14.26 mg/ml for AH40, while $RP_{0.5}$ value varied from 100.80 for AH to 11.27 mg/ml for AH40. Based on the correlation analysis, it is obvious that TPh were associated with the antioxidant activity of honey-based product (R^2 = 0.862 for DPPH and R^2 = 0.94 for RP). Obtained results indicated very good quality of acacia-honey based products.

PP-05

COMPARISON OF TOTAL PHENOLIC CONTENT AND TOTAL ANTIOXIDANT ACTIVITY IN LOCAL RED WINES DETERMINED BY SPECTROPHOTOMETRIC METHODS

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The interest of natural antioxidants has increased in recent years because of their presumed safety and potantial nutritional and therapeutical effects. Among natural antioxidants, red wine has attracted particular interest due to a high content of biologically active compounds [1]. Phenolic compounds are one of the most important quality parameters of wines, and they contribute to organoleptic characteristics such as color, astringency, and bitterness [2].

Several *in vitro* methods have been developed to measure antioxidant capacities of food, beverages and biological samples. The most commonly used antioxidant capacity assays were 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·) assay, 2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid) (ABTS) assay, ferric ion reducing antioxidant power (FRAP) assay, cupric ion reducing capability (CUPRAC) assay and oxygen radical absorbance capacity (ORAC) assay. These methods differ from each other in terms of their assay principles and reaction conditions. Single assay will not accurately reflect all antioxidants. Therefore it is recommended that at least two or all of these assays be combined to provide a full profile of antioxidant capacity of foodstuff [3].

The main objective of this study was to determine the polyphenolic content and antioxidant capacity of local wine samples and to compare the antioxidant capacity of these samples applying four most widely used spectrophotometric methods: DPPH, ABTS, FRAP, CUPRAC and to estimate correlation of antioxidant capacities with total phenolics.

The total polyphenol concentration was found to vary from 2599.90 to 4846.57 mg/L gallic acid equivalents (GAE). The total antioxidant activity determined by DPPH, ABTS, FRAP and CUPRAC methods was found to vary from 7.49 - 15.93 mmol/L, 12.02 - 24.73 mmol/L, 12.65 - 27.68 mmol/L and 13.19 - 31.07 mmol/L, respectively. The total phenolic contents of red wine samples exhibited a good correlation ($p < 0.01$) with antioxidant properties.

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PP-06

ANTIOXIDANT EFFICIENCY OF SEABUCKTHORN EXTRACT IN EHRLICH ASCITES CARCINOMA OF BALB/C MICE MODEL

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Seabuckthorn (SBT; *Hippophae rhamnoides* L.), a unique and valuable plant has recently gained worldwide attention, mainly for its medicinal and nutritional potential [1]. The aim of the present study was to evaluate the antioxidant activity of SBT against Ehrlich Ascites Carsinoma (EAC) cells by measuring primer antioxidant enzyme superoxide dismutase (SOD) and lipid peroxidation products malondialdehyde (MDA).

Mice were divided into three groups. First group was control. Second group was injected EAC interperitoneally. Third group was received both EAC and SBT extract daily 10U for 3 weeks. All groups received diet and water *ad-libitum*. Both control and experimental groups SOD activities, MDA levels and EAC cells protein contents were determined by the methods of Sun, Buege JA and Lowry, respectively [2-4].

Both in ascetic fluid and EAC cells, EAC group MDA levels (93.27±5.63, 220.31±13.91 nmol/mg protein, respectively) were significantly high when compared with EAC treated with SBT group (26.61±4.18, 121.59±5.24 nmol/mg protein, respectively) ($p<0.001$). Although, SOD activity was found decreased in ascetic fluid ($p<0.001$), same enzyme activity was found increased in EAC cells ($p<0.001$). When plasma EAC group compared with control, high levels of EAC plasma MDA levels were found ($p=0.001$). But, SBT application on EAC showed that plasma oxidative stress were found decreased by MDA levels ($p<0.001$).

Conclusion: According to these results, increased SOD activity by SBT implementation in EAC cells shows that radical metabolism is still active in EAC cells. SBT has antioxidant effect in ascetic fluid when significantly decreased SOD activity is considered. It is the case that plasma MDA levels significantly deplete and also SBT indicates antioxidant effect to whole body by circulation.

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PP-07

ANTIOXIDANT PROPERTIES OF SEABUCKTHORN (*HIPPOPHAE RHAMNOIDES* L.) LEAVES AND BRANCHES

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Present study focused on the antioxidant properties of leaves and branches of *Hippophae rhamnoides* (Seabuckthorn). Dried leaves, branches and processed branches of Seabuckthorn (SB) were extracted with methanol and also prepared in forms of tea infusions. All samples were analyzed for their contents of total phenolics (TPC) and total flavonoids (TFC) as well as their total antioxidant capacity by using four different methods including 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis(3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt (ABTS), CUPRAC (Copper Reducing Antioxidant Capacity), FRAP (Ferric Reducing Antioxidant Power). The TPC of SB leaves, branches and processed branches were found in the range of 12.0±5.0 to 99.0±2.0 mg GAE/g. The TPC of leaves from methanolic extracts (ME) were significantly higher than branch and processed branch extracts. The TFC of SB leaves, branches and processed branches changed in between 8.0±1.6 to 27.7±3.7 mg CA/g. The highest amount of flavonoids was found in ME of processed branches (27.7±3.7 mg/g). The DPPH scavenging activity of extracts ranged from 19.9±0.5 to 78.9 ± 2.2 mg TE/g. The FRAP value of extracts were found to be in between 15.6±0.2 to 62.9±0.8 mg TE/g. The ABTS of extracts ranged from 8.1±0.2 to 78.1±10.0 mg TE/g. On the other hand, the highest antioxidant capacity values were obtained with the CUPRAC method, indicating values ranging from 89.9±8.3 to 530.5±25.1 mg TE/g. In conclusion, higher antioxidant capacity values were observed in ME of leaf, processed branch and branch samples in comparison to infused samples.

Keywords: Seabuckthorn, *Hippophae rhamnoides,* leaves, antioxidant, total phenolics

PP-08

MNSOD SERUM LEVELS AND GENETIC VARIANTS: POSSIBLE ASSOCIATIONS BETWEEN THE RISK OF COLORECTAL CANCER AND OXIDANT-ANTIOXIDANT STATUS

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Colocteral cancer (CRC) is one of the most common cancers worldwide. There are numerous genetic and lifestyle factors that can affect reactive oxygen species (ROS). ROS are involved in the cell growth, differentiation, progression, and death. It is known that there is an evidence for the importance of selenium's, copper's and zinc's antioxidant role in protecting cells against free radical-induced oxidative damage. The levels of zinc (Zn), copper (Cu) and ceruloplasmin have been found to be critical parameters. The aim of this scientific work is to find CRC and to provide essential background information for oxidative stress and CRC risk.

Our study groups were selected from Istanbul Education and Research Hospital, General Surgery Clinic, Istanbul, Turkey. MnSOD Ala-9Val genotypes were determined by using polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) method. We used atomic absorption spectrophotometer to detect copper (Cu), Zinc (Zn) and Selenium (Se) serum levels. On the other hand we evaluated serum mnSOD levels by ELISA

According to our results, zinc levels was found significantly decreased in patients than controls ($p<0,001$). We found serum Se levels of patients less than controls. However, we didn't find any significant difference between Cu and Se serum levels of patients and controls. In addition, there is a significant difference between Zinc levels of patients carrying Val/Val and Ala/Ala genotypes compared to controls. ($p<0,001$). However, when we look at MnSOD polymorphism there is no significant difference between the patients and control groups. Also there was no correlation between MnSOD genotypes and MnSOD serum levels. ($p>0,05$)

As a result, concentrations of redoxal enzymes and their cofactor elements appear to change. Trace element levels may contribute to the pathogenesis of colorectal cancer, and free-radical-mediated damages may play an important role during etiopathogenesis.

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PP-09

A POSSIBLE ASSOCIATION BETWEEN MANGANESE SUPEROXIDE DISMUTASE GENOTYPES AND ANTIOXIDANTS MARKERS IN ACROMEGALY PATIENTS

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Acromegaly is an endocrine disorder which is an excessive production of growth hormone (GH) and in the majority of the patients it is caused by a pituitary macroadenoma. It is known that developing acromegaly complications, like hypertension and diabetes mellitus and hypercholesterolemia, have degradation in endothelial function. Recent scientific works suggest that oxidative stress may be the mechanistic connection between acromegaly and related risk for complications. The aim of this scientific work is to determine the measurement of total antioxidant capacity (TAC) in serum and to evaluate genetic phenotypes for Manganese superoxide dismutase (MnSOD) enzyme in patients with acromegaly, comparing the results with a group of healthy individuals and correlated with clinical information of patients. Clinical features determine the diagnostic of acromegaly diseases confirmed by GH unsuppression to < 0,4 ng/ml after an oral glucose tolerance test or high IGF1 levels age matched. Acromegaly patients are classified in different subgroups which are naïve-uncontrolled (Active), medically uncontrolled (Active-MT), medically controlled (Controlled-MT) and surgically cured (Controlled). Blood GH and IGF1 levels were assayed using a chemiluminescence immunometric assay. There are 51 acromegaly patients and 57 healthy controls that are included in this study. MnSOD Ala-9Val genotyping was found out by PCR-RFLP method. Total antioxidant capacity TAC as evaluate by Oxygen Radical Antioxidant Capacity (ORAC), trolox equivalent antioxidant capacity (TEAC) method and serum SOD levels as evaluate by ELISA. We have found that SOD, TAC cys and TAC aa levels are significantly decreased in patient groups (controlled and uncontrolled) compared to healthy subjects ($p<0,001$). Besides, no significantly decreased between controlled and uncontrolled acromegaly groups. In addition, when we look at MnSOD polymorphism there is no significant difference between the patients and control groups. Also there was no correlation found between MnSOD genotypes and its characteristics such as GH, GF1, IGF1. However, total cholesterol and LDL-C levels was found significantly increased in patients carrying VV genotype compared to the patients with VA genotype ($p=0,048$, $p=0,05$ respectively).

In literature there are not much studies conducted on measuring the oxidant and antioxidant levels of acromegaly patients. Our results showed that the evaluation of serum TAC, SOD levels and MnSOD genotypes are considerable to better understand the pathophysiological mechanisms of acromegaly disease.

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PP-10

DETERMINATION OF ANTIOXIDANT PROPERTIES AND METAL CONTENTS IN COMMERCIAL CORNELIAN CHERRY MARMALADES

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Cornelian cherry fruit have taken great attention for their health benefits in the last years. In this study, antioxidant activity of different commercial cornelian cherry marmalades were investigated by 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferrous ion-chelating activity. β-carotene and lycopene contents were analysed. Total phenolic, flavonoid and anthocyanin contents were measured spectrophotometrically. In addition, Inductively coupled plasma mass spectrometry (ICP-MS) was used for the determination of minor and major elements present in Cornelian cherry marmalades. Prior to ICP-MS measurement, the samples were digested in a wet digestion system. The ICP-MS method was validated and optimized for the determination of Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sr, Zn, Mg, K, B, Ca, Na, P in Cornelian cherry marmalades. Extraction experiments were carried out with 100% methanol and were mixed by magnetic stirrer during 1h. The values ranged between 1.13 and 1.27mg/ml gallic acid for total phenolic content, and between 0.068 and 0.093 mg/ L Cyd-3-glu for total flavonoid content, and between 52,4 and 65,5mg/L catechin for total anthociyanin content. The DPPH Method and 's % values ranged from 46 to 67. The chelating activities of marmalade extracts on ferrous ions were from 42% to 29%.

PP-11

PREPARATION AND IN-VITRO ANTIOXIDANT ACTIVITIES OF SOME NOVEL 4-[3,4-DI-(4-NITROBENZOXY)-BENZYLIDENAMINO]-4,5-DIHYDRO-1*H*-1,2,4-TRIAZOL-5-ONE DERIVATIVES

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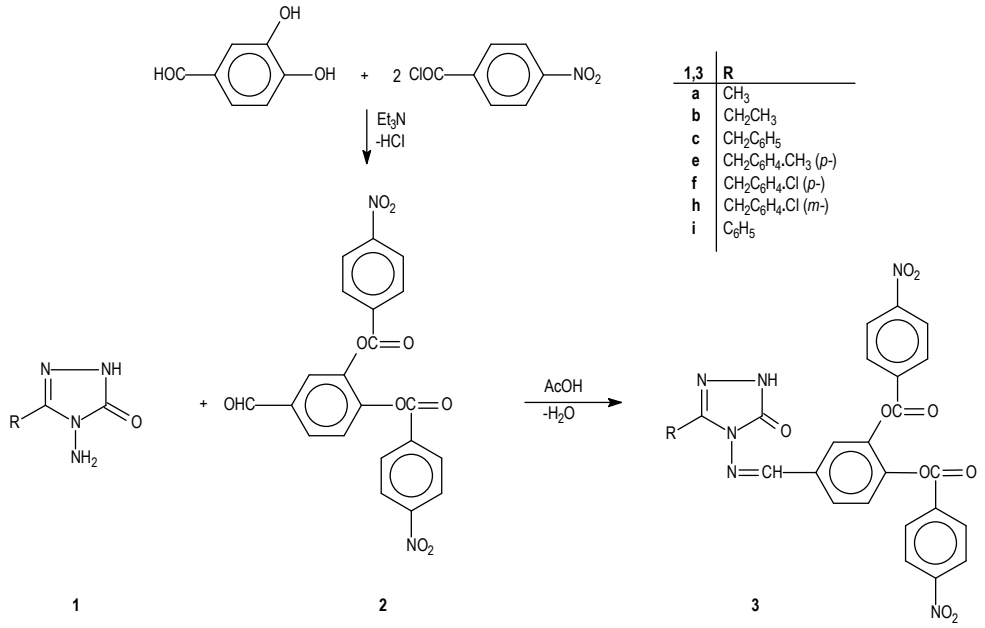
1,2,4-Triazole derivatives have drawn considerable attention for the past few decades because of their diverse biological properties. Many 1,2,4-triazole derivatives are found to be potent antioxidant, anti-inflammatory, antimicrobial and antiviral agents. The identification of triazoles and determination of their antioxidant activities are of considerable interest because of the role they play in pharmacological actions.

This study was planned as two parts; in the first part seven novel 3-alkyl(aryl)-4-[3,4-di-(4-nitrobenzoxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**3**) having 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring were synthesized from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**1**) with 3,4-di-(4-nitrobenzoxy)-benzaldehyde (**2**). The structures of seven new compounds are established from the spectral data.



In the second part of the study, the antioxidant properties of the compounds **3** were studied and evaluated using different three antioxidant assays; including reducing power, free radical scavenging and metal chelating activity. For the measurement of the reductive ability, Fe^{3+} - Fe^{2+} transformation was investigated in the presence of compound by the method of Oyaizu [1]. The hydrogen atoms or electrons donation ability of the synthesized compound was measured by DPPH· by the method of Blois [2]. The chelating effect of ferrous ions by the compound was determined according to the method of Dinis et al [3].

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PP-12

IN VITRO FREE RADICAL SCAVENGING ACTIVITY AND CYTOTOXIC EFFECTS OF THE RASPBERRIES (*RUBUS IDAEUS* L.) POMACE EXTRACT

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Raspberry waste, a by-product in juice processing, obtained from two cultivars, Meeker (ERM) and Willamette (ERW) was subjected to evaluation as potential source of phenolic antioxidants and anticancer agents. Some individual phenolic compounds including phenolic acids and flavonoids were identified and quantified by HPLC. The antioxidant activity of extracts was tested by measuring their ability to scavenge DPPH and hydroxyl radicals by electron spin resonance (ESR) spectroscopy. The $IC_{50}^{DPPH\bullet}$ varied from 0.67 for ERM to 0.54 mg/ml for ERW, while the $IC_{50}^{\bullet OH}$ values varied from 3.73 for ERM to 1.23 mg/ml for ERW. Cytotoxic activity was investigated using the *in vivo* model of Ehrlich carcinoma cells (EAC) in mice. Extracts applied prior to EAC implantation exhibited the potent cytotoxic activity against EAC cells (up to 60%) and both extracts inhibited the tumour growth in a dosage dependent manner. No significant differences between extracts were noticed, although ERM showed slightly stronger effect. The antioxidant system of EAC cells was significantly disturbed upon treatment with the raspberry extracts, so the activity of XOD was extremely increased in pretreated animals (from 0.123 μmol/ml to 6.140 μmol/ml). Furthermore, the activity of enzyme complex GR and GSHPx, involved in the regeneration of GSH, was significantly increased (3-fold and 4.6-fold respectively) in pretreated female groups, thus indicating that the possible mechanism of cytotoxic activity of raspberry extracts against EAC cells *in vivo* might be selective induction of oxidative stress.

PP-13

EFFECTS OF SALINITY ON ALTERNATIVE ELECTRON SINKS AND ANTIOXIDANT DEFENSE IN CHLOROPLASTS OF EXTREME HALOPHYTE *Thellungiella parvula*

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The main aim of this study was to reveal how an extreme halophyte *Thellungiella parvula* tolerates salinity in the manner of reactive oxygen species (ROS) production, antioxidant system and redox regulation of electron transport system in chloroplasts. *T. parvula* were grown for 30 d and then treated with 50-200-300 mM NaCl. Activities of antioxidant enzymes were measured in whole leaf and isolated chloroplasts. In addition, the expressions of chloroplastic redox components such as ferrodoxin thioredoxin reductases (FTR), NADPH thioredoxin reductases (NTRC), thioredoxins (TRXs) and peroxiredoxins (PRXs) were measured. Gradually increased salt treatment affected water relations negatively and the accumulation of osmolyte proline was increased by salinity. *T. parvula* could cope with 300 mM NaCl in long term as evident by H_2O_2 content and lipid peroxidation. While Ca^{+2} and K^+ contents decreased by salinity, Na^+ and Cl^-contents were enhanced. Efficient induction of water-water cycle enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) prevented accumulation of excess ROS in chloroplasts and therefore the photosynthetic machinery could be protected in *T. parvula*. The redox homeostasis in chloroplasts was achieved by efficient induction of expressions of redox regulatory enzymes such as FTR, NTRC, TRXs, PRXs under salinity.

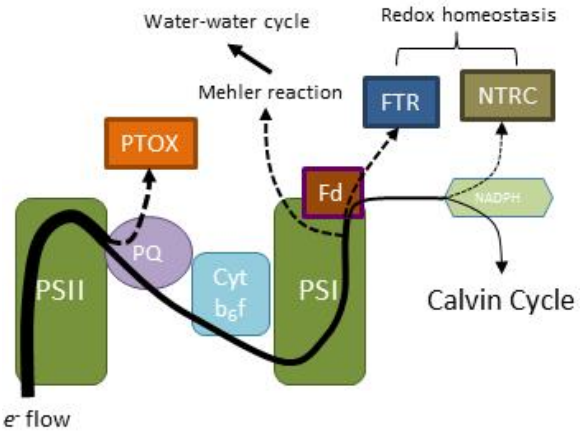

Figure 1. Scheme summarizing the alternative electron sinks and mechanisms that can relax the electron load on chloroplastic electron transport chain.

Keywords: Alternative electron sink, antioxidant enzymes, chloroplastic redox, halophyte, water-water cycle

PP-14

INVESTIGATION OF ANTIOXIDANT CAPACITY OF HACKBERRY (*Celtis australis L.*)

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Antioxidants become oxidized while preventing the oxidation process by deactivating free radicals. Therefore, our body has a continuous need for antioxidants during a lifetime. Earlier studies are mostly related to effects of air quality on hackberry leaves and the use of hackberry peel and leaves as bio monitor on determination of heavy metal concentration of air. There are a few assays about the antioxidant capacity of hackberry fruit and as the hackberry species are widespread in the geography of our country it is influential to actualize this study. Antioxidant determination methods were applied to green, yellow and black cases of hackberry fruit during the ripeness stage, moreover it is aimed to determine the change of the antioxidant capacity of hackberry along the growing process and the causes of the current changes. The antioxidant capacities of Hackberry fruit extracts and infusions were determined by CUPRAC [1], CERAC [2], Folin-Ciocalteu [3] and Modified Ferricyanide methods [4]. Hackberry fruit samples were collected from ITU Ayazaga Campus in order to prepare hackberry extracts. Kernel and fruit parts of hackberry were separated and triturated in a mortar. 80% (v / v) methanol is used as the extraction solvent and used by shaking at 350 rpm in the shaker. According to the results obtained using CUPRAC method, in the kernel part of hackberry, much lower antioxidant capacity than the fruit part of hackberry was observed and further studies were proceeded with the fruit fraction of hackberry. Appropriate type of solvent were tried to determine for the extraction of hackberry and for this purpose ethanol, methanol and acetone were selected as extraction solvents. Before preparing extracts, hackberry fruit was separated from its kernel part and allowed to dry at room temperature for 3 days, after then drying the sample was prepared by trituration. In solvent optimization studies, hackberry extracts were prepared using absolute, 80%, 70%, 50%, 40% and 25% (v / v) methanol solutions and applying CUPRAC method to these extracts it was found that the extracts prepared with 50% (v / v) with methanol had the highest antioxidant capacity with the value of 0.072 (mmol TR/g). CUPRAC, CERAC, Folin-Ciocalteu, and Modified Ferricyanide methods are applied to 50% (v / v) methanol extracts and infusions and, as the results of each method, antioxidant capacity amounts were determined in that: green > yellow > black. Besides, antioxidant capacity amounts for all four methods were seen that 50% hackberry methanol extract > hackberry infusion as mmol TR/g.

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PP-15

DETERMINATION OF TRACE AMOUNT OF ANTIOXIDANTS BY USING Fe(III)-BASED ANTIOXIDANT CAPACITY ASSAYS

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A ferric-ferrozine method of antioxidant capacity measurement has been developed for simple, low- cost and versatile assay of food antioxidants. As the conditional stability constant of the ferrous-ferrozine (Fe(II)-FZ) complex is very high (*i.e*. at the order of Log β_3 = 15.5), FZ preferentially stabilizes the ferrous state compared to ferric ion, resulting in increased redox potential of Fe(III)/Fe(II) couple capable of oxidizing antioxidants. Solid-phase spectrophotometry (SPS) in the visible region was used for the determination of antioxidants based on their reducing effect on iron(III), followed by formation of the iron(II)-ferrozine chelate. In this work, a sensitive and selective SPS method for determination of total antioxidant capacity is developed. The anionic Fe(II)-FZ complex can be quantitatively sorbed on an ion-exchange resin showing an absorption peak at 562 nm. The fixation of the coloured complex on the resin results in a noticeable increase in sensitivity because of the concentrating capability of the resin. Influence of pH on the formation and fixation of the Fe(II)-FZ complex was tested from pH 1.0 to 10, and it was found that the absorbance obtained is independent of pH over the range 3-8. A secondary antioxidant assessment method studied in this work is the ferric-ferricyanide assay, which was capable of oxidizing thiol compounds acting as important antioxidants (such as cysteine and glutathione), based on Prussian blue formation. It should be remembered here that the widely used FRAP assay, based on Fe(III) reduction by antioxidants, cannot measure thiols effectively, due to kinetic reasons. Prussian blue complexes sorbed on the resin showed an absorption peak at 725 nm, enabling successful adaptation to SPS. The apparent molar absorptivity, linear concentration range and TEAC (trolox equivalent antioxidant capacity) values of certain antioxidants were found in the proposed assays. The SPS method can be potentially used for flow-through renewable surface optosensing and allows the determination of antioxidants at the ng ml^{-1} level with higher reproducibility and molar absorptivity than those of the original methods. Antioxidants in herbal teas and fruit juices were tested with the proposed method.

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PP-16

NOVEL *N*-HYDROXYCINNAMOYL AMIDES OF OSELTAMIVIR WITH POTENT RADICAL SCAVENGING ACTIVITIES

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During the recent years, hydroxycinnamic acids and their derivatives have received considerable attention, due to the multiple biological and pharmacological activities they exert [1]. Amongst them, our research is focused on evaluation of antioxidant effect *in vitro*.

The newly amides were obtained by combining previously synthesized *N*-Hydroxycinnamoyl amides of fluorinated amino acids [2] and fragment of anti-influenza drug-oseltamivir. Futhermore, their ability to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was determined, based on Nenadis' method [3]. All amides behave as potent DPPH scavengers with significant activity level.

Acknowledgments: This work was supported by grants from the South-West University "Neofit Rilski"(Project SRP-A11/14)

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PP-17

PHENOLICS, ANTIOXIDANT AND ANTIMICROBIAL POTENTIALS OF SHERBETS FROM FLOWERS OF *ALTHAE ROSEA* AND *HIBISCUS SYRIACUS*

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Althae rosea and *Hibiscus syriacus* are members of the *Malvaceae* family as flowering plants and their common name is "Gulhatmi" in Turkey. It is known that Gulhatmi sherbet (GS) were frequently consumed by people living in the Ottoman Period due to their therapeutical benefits. They are considered especially good against cough and minor respiratory problems. In this study, Gulhatmi sherbets were prepared from dark red (GS1) and lilac (GS2) flowers from *Althae rosea* and *Hibiscus syriacus*, respectively. In order to assess antioxidant and radical scavenging properties of GS1 and GS2, we performed some *in vitro* methods such as N,N-dimethyl-p-phenyl-enediamine dihydrochloride ($DMPD^{\bullet+}$) radical scavenging activities, the determination of reducing capacity assays (FRAP and CUPRAC) and the results were compared with standard antioxidants such as BHA, BHT and α-tocopherol . Also, we determined amount of the total phenolic contents of GS1 and GS2 as 417.8 and 173.8 mg GAE/L, respectively, and the total flavonoid contents of GS1 and GS2 as 182.0 and 134.2 mg QE /L, respectively. Total monomeric anthocyanin contents of GS1 and GS2 were 155.7 and 30.8 mg cyanidin 3-glucoside/L, respectively. Their antimicrobial activities were also considered against to *Echerichia coli* 25922 as a surrogate microorganism of *E. coli* O157:H7. $DMPD^{\bullet+}$ radical scavenging activities of GS1 and GS2 were determined as IC_{50} values. $DMPD^{\bullet+}$ radical scavenging activity of these sherbets were 90.5% for GS1 and 89.9% for GS2, respectively. At the same concentration IC_{50} values of BHA, BHT and α-tocopherol was 84.6, 76.9 and 91.0%, respectively. After examining the results of FRAP and CUPRAC assays, we deduced GS1 (1.898±0,237-1,012±0,029, respectively) has better reducing activity than GS2 (0,956±0,066-0.825±0,006, respectively) and GS1 exhibited similar activity to the standard antioxidants.

PP-18

ANTIOXIDANT POTENTIAL AND PHENOLICS OF LICORICE ROOT (*GLYCYRRHIZA GLABRA* L.) SHERBET AS A TRADITIONAL BEVERAGE

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Licorice (*Glycyrrhiza glabra* L.) root is known as a remedial plant since ancient times. Therapeutic and biological activities of the root includes anti-inflammatory, anticancer, antimicrobial activities, cardioprotective, hepatoprotective and immunomodulatory effects. Also, their food applications as natural sweetener and flavor enhancer includes candies, chewing gum and drinks.

In Eastern and Southeastern Anatolia of Turkey, licorice roots are commonly used for production of a traditional beverage called "Licorice Root Sherbet". This sherbet is favorably consumed on a daily basis during the summer months due to its refreshing effects. Sherbet production is simple and made by infusion of dried and fringed roots in cold water.

In this study, glycyrrhizic acid (GA), total phenolics and flavanoid contents of sherbet made by roots harvested from Diyarbakır, Turkey (n=7) were done. Glycyrrhizic acid as a triterpene glycoside is the most important active compound found in the licorice root. GA content of sherbet was found as 748.4 mg glycyrrhizic acid/L. Total phenolics and flavanoid contents were determined as 2086.3 mg GAE/L sherbet and 196 mg QE/L sherbet, respectively. Antioxidant activity of sherbet by TEAC (Trolox equivalent antioxidant capacity) method was also determined to compare with antioxidant potential of some fruit juices such as grape, orange and pomegranate juices.

PP-19

PREVENTION AND POTENTIAL RISK OF THERAPEUTICALLY USED NANOPARTICLES IN OXIDATIVE STRESS

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Increased exposure of humans to nanomaterials introduced in the diagnosis and treatment of various diseases leads us to the question of the potential risks of nanomaterials. The size of nanoparticles enables them to pass through the biological membranes and thus influence the function of any cells of the organism. A variety of *in vitro* and animal experiments (Singh and Nalwa, 2007) confirmed the toxicity of nanoparticles in oxidative stress and inflammation.

Our goal was to monitor the effect of the commercially used nanoparticles with different physico - chemical properties (TiO_2, silver) on the oxidative status presented by total antioxidant capacity, markers of oxidative damage to proteins (protein carbonyls) and markers of oxidative damage to lipids (8 – isoprostanes) *in vitro* and to research the ability of substances with hormonal properties (FSH) to prevent the negative impact of nanoparticles.

Total antioxidant status was measured by TEAC method, concentration of protein carbonyls and 8-isoprostanes were detected by commercial kits. Measurements were performed on primary ovarial granulosa cells isolated from ovaries of pigs incubated in the presence of TiO_2 (100, 50, 10 and 1 mg/ml) or Ag (100, 10 and 1 mg/ml) or FSH (1 µg/ml) + TiO_2 and FSH (1 µg/ml) + Ag at the same concentrations for 48 hours.

Compared to control cells (without TiO_2, Ag orFSH) and cells affected by FSH, incubation of ovarial cells with Ag or Ag + FSH has increased their total antioxidant capacity. In cells incubated with TiO_2 or TiO_2 + FSH differences in TEAC between groups were not observed. TiO_2 in concentration 100 mg/ml increased levels of protein carbonyls significantly in comparison to control cells. Incubation of ovarial cells with TiO_2 caused significant increase of 8-isoprostanes concentration when compared to control cells.

A better understanding of interactions between therapeutically used nanoparticles and cells helps us to prevent their negative side effects such as oxidative stress and inflammation in cells, tissues and organism.

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PP-20

ANTIOXIDANT ACTIVITY AND POLYPHENOL CONTENT OF LEAVES OF CHARD (*BETA VULGARIS VAR. VULGARIS*) MONITORED BY LC-MS/MS

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Chard is a biennial medicinal vegetable that has large shiny green leaves. Fresh leaves of chard are being used in cookery in many parts of the world, especially in Turkey, the Mediterranean, and Europe. The leaves and stalks of chard contain nutritionally significant concentrations of some vitamins and minerals(1). We investigated Chard (*Beta vulgaris var. vulgaris*) for phenolic contents and antioxidant activities.

The antioxidant activities of ethanol and aqueous extracts of Spinach (Spinacia oleracea) were determined by different in vitro methods such as DPPH·radical scavenging, reducing power by FRAP and CUPRAC methods, separately. In addition, total phenolic and total flavonoid contents were determined as gallic acid equivalent (GAE) and quercetin equivalent (QE), respectively. Finally, the quantities phenolic compounds such as of caffeic acid, ferulic acid, ascorbic acid, p-coumaric acid, p-hydroxybenzoic acid and vanillin were detected by high performance liquid chromatography and tandem mass spectrometry (LC-MS/MS).

The both plant extracts revealed significant antioxidant activity in all used antioxidant assays. The total phenolic compounds in AES and EES were 44.35 and 52.35 µg GAE/mg extract, respectively.

In this study, for the first time, we determined phenolic contents, investigated antioxidant potential of Chard. The results indicate that Chard (*Beta vulgaris var. vulgaris*) is a good dietary source with phenolic properties.

Keywords: Antioxidant activity; LC-MS-MS; Phenolic compounds; Chard; *Beta vulgaris var. vulgaris*

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PP-21

PHENOLIC ACIDS AND FLAVONOIDS OF FIG FRUITS (*Ficus carica* L.) FROM CORUH VALLEY IN TURKEY

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Coruh valley located Northeastern part of the Turkey is accepted one of the 34 hot spots for plant biodiversity in the world. Wild edible fig trees cover throughout the valley. There were mainly yellow-green, light purple and dark purple (black) fig fruits in the valley. Phenolics are widespread constituents of plant foods (fruits, vegetables, cereals, olive, legumes, chocolate, etc.) and beverages (tea, coffee, beer, wine, etc.), and partially responsible for the overall organoleptic properties of plant foods. For example, phenolics contribute to the bitterness and astringency of fruit and fruit juices. They are important for plant metabolism and have also becoming important for humans due to their health characteristics, particularly related to their antioxidant properties. In this study we have tried to evaluate the phenolic profile of different colored (yellow-green, light purple and dark purple=black) fig fruits. Light purple and dark purple fig fruits exhibited higher total level of analysed phenolics when compared to yellow-green fruit. According to the HPLC results, gallic acid, chlorogenic acid, syringic acid, (+)-catechin, (-)-epicatechin and rutin were dominant in different colored fig fruits. We determined rutin (up to 22.9 mg per 100 g FW), followed by (+)-catechin (up to 5.29 mg per 100 g FW), chlorogenic acid (up to 2.14 mg per 100 g FW), (-)-epicatechin (up to 1.11 mg per 100 g FW), gallic acid (up to 0.33 mg per 100 g FW) and, finally, syringic acid (up to 0.08 mg per 100 g FW). As a results in particular light purple and dark purple fig fruits are natural phenolic sources and could be important as antioxidant action.

PP-22

ANTIOXIDANT AND ANTIVIRAL ACTIVITY OF THE ARGININE DERIVATIVES OF AMANTADINE AND RIMANTADINE

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Amantadine is a drug both as an antiviral and an antiparkinsonian drug. It is the organic compound 1-adamantylamine or 1-aminoadamantane, meaning it consists of an adamantane backbone that has an amino group substituted at one of the four methine positions. Rimantadine is a closely related derivative of adamantane with similar biological properties. Influenza virus infection is associated with development of oxidative stress.

Arginine, is one of the 20 most common amino acids, and a limited amount is synthesized within the human body. In order to make up for the difference between the amount of arginine needed and the amount a person is able to produce, additional arginine must be consumed. Arginine is involved in a number of important processes, including healing, the removal of waste from the body, and cell division. Arginine involved in drugs and is used for symptomatic treatment of fatigue (sarjenor).

The aim in this study is synthesis of analogues of amantadine and rimantadine with arginine and evaluated their antiviral and antioxidant activity.

PP-23

EVALUATION OF DPPH SCAVENGING ACTIVITY OF LIPOYL AMIDES OF ANTI-INFLUENZA DRUGS

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For all living beings, free radicals are dangerous substances generated in the body as a result of the natural metabolic processes. Oxidative stress, associated with the increased reactive oxygen species is suspected to be liable for most of diseases such as diabetes, hepatitis, influenza, atherosclerosis and etc. Therefore, to neutralize these oxidative compounds, antioxidants could be used successfully as therapeutic agents.

α- Lipoic acid (LA; thioctic acid) known as a powerful antioxidant in living cells contains two oxidized or reduced thiol groups. The antioxidant properties of both forms (LA and dihydrolipoic acid (DHLA)) are associated with scavenging of reactive oxygen species, regeneration of endogenous antioxidants, metal helating activity, and repairing oxidative damage in macromolecules. Being amphiphilic molecules, LA and DHLA can penetrate easily through the cell membrane and thus can reach all cells [1].

Earlier findings have revealed that polyphenols are effective in alleviating the oxidative stress induced during influenza virus infection [2,3]. This urges us to synthesise lipoyl amides of anti-influenza drugs (rimantadine, amantadine and oseltamivir) and to evaluate their radical scavenger activity.

The estimation of antioxidant (against DPPH radical) activities of newly synthesized compounds *in vitro* is in progress.

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PP-24

ANTIOXIDANT ASSAYS AGAINST LIPID PEROXIDATION

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Lipid peroxidation in foods causes rancidity and nutritional deterioration, which can be delayed or inhibited by antioxidant compounds. Lipid oxidation measurement methods are usually concerned with absorption of oxygen, loss of initial substrates, generation of free radicals, and formation of primary and secondary oxidation products. If oxygen is passed through a linoleic acid (LA) emulsion by adding copper (II) salts, primary oxidation products (*i.e.* hydroperoxides) and secondary products (*i.e.* aldehydes or ketones) are formed [1]. The peroxidation of LA in the absence and presence of Cu(II) ion alone or Cu(II)-ascorbate combinations was investigated in aerated and incubated emulsions at 37°C and pH 7 [2]. LA peroxidation induced by copper (II) was observed to follow pseudo-first-order kinetics with respect to the formation of hydroperoxides and aldehydes, which were monitored by ferric thiocyanate (Fe(III)-SCN) and thiobarbituric acid-reactive substances (TBARS) methods, respectively. As total antioxidant capacity (TAC) against lipid peroxidation was not quantified before, both methods were adapted to an 'area under curve (AUC)' approach so as to determine the TAC values of some antioxidants and one plant extract (raw garlic). Changes of absorbance due to ferric thiocyanate and TBARS as a function of incubation time exhibited sigmoidal curves [1]. As the maximum absorbance of the oxidation products approached 1 [*i.e.* $A_{max} \approx 1$], the following parameters could be found: (1) Area under kinetic curve (AUC) and 'net AUC' [*i.e.* ($AUC_{sample} - AUC_{blank}$)], (2) Standard calibration curve by plotting net AUC against the concentration of Trolox (or of other antioxidants) (3) Trolox-equivalent antioxidant capacity (TEAC) of the tested compounds. As a result of this new AUC approach, the TEAC coefficients of antioxidants in LA emulsion were: hydroquinone (HQ) > *tert*-butyl hydroquinone (TBHQ) > morin (MR) > ascorbyl palmitate (AP) > tocopherol (TocH) > ascorbic acid (AA) > catechin (CT) > quercetin (QR). For Cu(II) ion-ascorbate oxidizing combinations, the TEAC coefficients of defensive flavonoids; morin, catechin and quercetin, were calculated as 1.83, 1.50 and 1.06, respectively, and this finding conformed to the observed antioxidant protection order without AUC account for the same system [2]. In accordance with theoretical expectations, garlic extract showed a distinct dose-dependent antioxidant effect on inhibition of LA peroxidation with respect to hydroperoxide formation, as measured by Fe^{III}-SCN colorimetry. Surprisingly, TBARS results were contradictory to this finding, showing the prooxidative effect of garlic extract on secondary product formation during peroxidation. This unexpected result may stem from the generation of reactive sulfur species (RSS) from garlic extract under the Cu(II)-induced oxidizing conditions of the TBARS assay, enhancing (rather than retarding) sulfhydryl and LA oxidation in model systems of lipid oxidation which is better expressed in secondary product formation [3].

Keywords: Antioxidant Capacity, Ferric Thiocyanate Method, TBARS, Area Under Curve (AUC) Approach

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PP-25

EVALUATING THE ANTI-OXIDANT CAPACITY OF COMMERCIAL AND FRESH ORANGE JUICES AND CLASSIFYING THE SAMPLES WITH CHEMOMETRIC APPROACH

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Intense increase in world population cause a high demand on food industry. Thus, not only production of food and beverages but also analyze and classify them become a very important issue for scientists. In this study, it is aimed to determine the anti oxidant capacity of both commercial orange based beverages as orange juice, orange nectars and freshly prepared orange juice.

The proposed study was maintained by purchased different commercial orange based beverages. This beverages were diluted in between 1 and 1/32 (v:v) and then their anti oxidant activity were calculated by FRAP, CUPRAC, DPPH and ABTS/TEAC methods. The same procedure was also applied onto freshly prepared orange samples. Different concentration of Trolox in between 1-30 µg/ml were prepared to determine the trolox equivalent antioxidant capacity of each samples. All of the measurements were carried out with spectrophotometer which was a trademark of ThermoQuest and data was evaluated on XLSTAT statistic programmer.

Results were successively conveyed to chemometric calculations. By the help of chemometric approach two clusters are obtained from the samples and freshly prepared orange juice samples completely separated from the commercial samples.

According to the results a fast, simple and reproducible classification method for freshly prepared orange juice and commercial products in Turkey was developed in terms of their power of anti-oxidant capacity. This simple, fast and reproducible method can be used to make decision on food samples whether they are originally prepared or not and can give information on forgery and other business tricks.

PP-26

A NOVEL DIFFERENTIAL PULSE VOLTAMMETRIC (DPV) METHOD FOR MEASURING THE ANTIOXIDANT CAPACITY OF TROLOX

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A novel differential pulse voltammetric (DPV) method was presented, using a chromogenic oxidizing reagent, cupric neocuproine complex ($Cu(Nc)_2^{2+}$), for the assessment of antioxidant capacity of trolox and real samples for the first time. The electrochemical behavior of $Cu(Nc)_2^{2+}$ complex was studied by cyclic voltammetry at a glassy carbon (GC) electrode. The electroanalytical method was based on the reduction of $Cu(Nc)_2^{2+}$ to $Cu(Nc)_2^{+}$ by antioxidants and electrochemical detection of the remaining Cu(II)–Nc (unreacted complex), the difference being correlated to antioxidant capacity of the analytes.

$$n\ Cu(Nc)_2^{2+} + n\text{−electron reductant (AO)} \rightarrow n\ Cu(Nc)_2^{+} + n\text{−electron oxidized product} + n\ H^{+} \qquad \text{(Eq.)}$$

The proposed method was successfuly applied to the measurement of total antioxidant capacity (TAC) in some herbal tea samples such as green tea, marjoram, sage and alchemilla. Results demonstrated that the proposed voltammetric method has comparable precision and accuracy to that of the spectrophotometric CUPRAC assay.

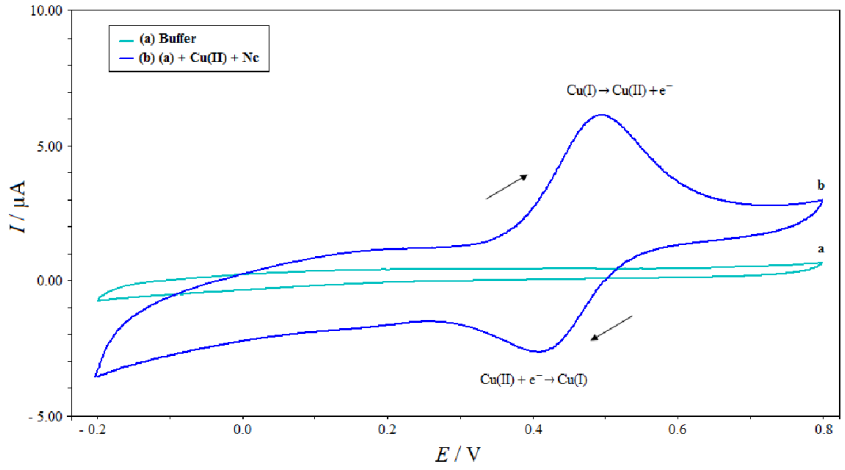

Figure. Cyclic voltammogram of $Cu(Nc)_2^{2+}$ reagent (scan rate 40 mV/s). ((a) acetic acid/acetate buffer solution (0.1 M, pH 4.76) (supporting electrolyte), and (b) acetic acid/acetate buffer solution + 0.4 mM Cu(II) + 0.8 mM Nc) (CV scan was made negatively from +0.8 to -0.2 V (one cycle), and at a rate of 40 mV/s).

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PP-27

DETERMINATION OF POLYPHENOLS IN METHANOLıC EXTRACTS OF PENNYROYAL (*MENTHA PULEGIUM* L.), MARJORAM (*ORIGANUM MAJORANA* L.), LAVENDER (*LAVANDULA OFFICINALIS*) BY UPLC-ESI-MS/MS

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This study reports the characterization of polyphenolic compounds in pennyroyal (*Mentha pulegium*), marjoram (*Origanum marjoram*) and lavender (*Lavandula vera*) extracts by using ultra performance liquid chromatography (UPLC) coupled with tandem mass spectrometry (MS-MS) in negative mode of electron spray ionization (ESI). 9 phenolic acids (gallic acid, protocatechuic acid, vanillic acid, caffeic acid, ferulic acid, sinapic acid, syringic acid, *p*-coumaric acid, rosmarinic acid) and 9 flavonoids (catechin, rutin, hesperidin, morin, fisetin, quercetin, naringenin, kaempferol and apigenin) were monitored within 12 min gradient elution program. The developed method was validated with good precision (RSD % 0.54- 2.72 for intra-day, 1.71-4.64 for inter-day), reproducibility (REC% 95.1-104.6) and linearity (r=0.9989-0.9999). Polyphenols were quantitatively analyzed in the plant extracts. Total antioxidant capacity and total phenolic content of samples were measured by using CUPRAC (cupric reducing antioxidant capacity) and Folin assays. Microwave assisted extraction (MAE) technique was used to extract phenolic antioxidants in herbal plants. The extracts exhibited high antioxidant capacity (ranged from 0.33 mmol T/g to 0.70 mmol T/g). The proposed method was found to be easy, fast, reproducible and convenient for quantitative analysis of polyphenols.

PP-28

ANTIOXIDANT ACTIVITY OF THREE MEDICINAL PLANTS COMMERCIALIZED IN LIBYA

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Antioxidants possess the ability to prevent damage caused by free radical-induced oxidative stress. This feature makes them vital substances. The body uses a variety of free radical scavenging antioxidants. Most of those antioxidants are derived from dietary sources like fruits, vegetables and teas.

This research reports the measurement of the antioxidant activity of methanolic extract and the water extracts of three medicinal plants commercialized or grown in Libya (Ginger, green tea, and Hyousumes albus). These measurement were obtained using DPPH (1,1-diphenyl-2-picrylhydazyl) method. The values obtained were compared and the effect of boiling of water to the antioxidant activity will be discussed. Also, the anti-Parkinsonian activity of the Hyousumes albus extract was determined by using haloperidol-induced catalepsy method in mice, preliminary results of the anti-Parkinsonian activity will be discussed.

PP-29

FLAVONOIDS IN FLOWERS OF HEATHER *CALLUNA VULGARIS* (L.) HULL.

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Calluna vulgaris (L.) Hull (heather) can be found in most parts of Europe. The plant material is a part of traditional folk medicine for treating urinary tract disturbances and inflammatory related disorders [1, 2]. The aim of this study was to investigate the content of some flavonoids and antioxidant capacity of the extracts of the aerial parts of *Calluna vulgaris* (L.) Hull.

Water, ethanol and its mixture as well as ethyl acetate were used for extraction. The influence of extraction temperature and ultrasonic were also studied. Antioxidant (reducting) capacity of the prepared extracts were screened by assays of cupric ion reducing antioxidant capacity (CUPRAC) and Folin-Ciocalteau (so-called total phenolic content). The content of some flavonoids was determined by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS).

Phytochemistry of heather extracts and its antioxidant capacity strongly depend on the extractant. The highest content of flavonoids were found in the ethyl acetate fraction followed by ethanol. The lowest results, especially for the quercetin and rutin glycosides, were obtained when the ultrasound was used. Probably these polyphenols were degraded during ultrasonic extraction [3, 4]. The temperature can increase the efficiency of extraction process. Total phenolic content of the samples (Folin-Ciocalteau assay) was in range 20 – 75 mg gallic acid equivalent per g dry material. The strongest antioxidant capacity of the extracts, determined by CUPRAC method, was found in 60% ethanolic and ethyl acetate fractions. Better results were obtained when the extracts were incubated at 55° C.

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PP-30

ELECTROCHEMICAL AND COLORIMETRIC DETERMINATION OF NITRITE WITH GOLD NANOPARTICLES-BASED METHODS

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Nitrite is ubiquitous in environmental, food and physiological systems because of its common use as a food additive and corrosion inhibitor. As nitrite may be a precursor of carcinogenic nitrosamines, determination of nitrite is important in environmental protection food chemistry and public health. As a result, many spectroscopic, chromatographic, and electrochemical detection. methods for nitrite have been developed in recent years. Electrochemical techniques are advantageous for nitrite determination [1]. In recent years, there has been extensive interest in the field of polymer modified electrodes. One of the most significant advantages of such electrodes is that multilayered polymer coatings yield a three-dimensional reaction zone at the electrode surface, which results in an increase in the rates of reaction occurring at the surface of the electrode. Therefore, the response sensitivity of the electrodes is improved [2]. In this study, preparation of the working electrode, *i.e*. *p*-aminothiophenol modified and gold nanoparticles-derivatized gold electrode (Au/p-ATP-Au_{nano}) was made in two steps. In the first step, surface of the Au working electrode was coated with a polymeric material (4-aminothiophenol) *via* electropolymerization method. Coating process was performed using cyclic voltammetry under (0 V) - (1,7 V) potential, 20 mVs^{-1} scan rate and 20 cycles. Ag/AgCl and Pt electrodes were used as reference and counter electrodes, respectively. In the second step Au/p-ATP-Au_{nano} working electrode was prepared by coating the surface of the Au/*p*-ATP electrode with the use of $HAuCl_4$ solution and cyclic voltammetry method under (0,4 V) - (-0,4 V) potential, 20 mVs^{-1} scan rate and 20 cycles. Determination of nitrite samples prepared in aqueous media was performed with the proposed modified electrode (Au/p-ATP-Au_{nano}) using Square Wave Voltammetry (SWV) in pH 4 buffer medium and under (0,5 V) - (1,1 V) potential. Characteristic peak potential of nitrite samples was 0.76 V, and linear calibration curve for the determination of nitrite samples at this potential was: $I = 1.305\ c_{mg\ L^{-1}} + 5.703$ ($r=0.996$), where I was current intensity and r was linear correlation coefficient. Also nitrite as a food preservative can be colorimetrically determined in sausage samples with high sensitivity by means of 4-aminothiophenol-modified AuNPs and naphthylethylene diamine as coupling agent for azo-dye formation due to enhanced charge-transfer interactions with the NPs surface. The slope of the calibration line of NO_2^- solution is not significantly different from the slope of the NO_2^- line in a sausage sample solution to which NO_2^- standards at different concentrations were added. The slopes of the calibration lines for NO_2^- were 7.66×10^{-2} and 7.74×10^{-2} L mg^{-1} in water and in sausage solution, respectively, and the NO_2^- content of sausage sample was found from the horizontal-axis intercept of the standard addition line ($A_{565}=7.4 \times 10^{-2}\ C_{NO2^-} +1.7\times 10^{-1}$; $r = 0.9998$) as 2.3 mg L^{-1}. The amount of nitrite in the case of sausages 57.5 mg / kg, respectively.

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PP-31

INVESTIGATION OF RESVERATROL-COPPER COMPLEXES USING TWO DIFFERENT TECHNIQUES

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Resveratrol, or 3,4_,5-trihydroxystilbenzene, is a secondary metabolite produced in limited plant species and is found in many natural foods (e.g., grapes, red wine, purple grape juice, and some berries) [1]. Recent studies have renewed interest in this natural compound, which seems to be responsible for a variety of biological effects, including antiinflammatory, anti-platelet, and anti-carcinogenic activities [2-4]. Oxidized resveratrol could generate complexes with others molecules, such as copper ions. Copper is one of the most redox-active metal ions present in living cells and is able to switch the resveratrol from an antioxidant to a prooxidant agent. The oxidative product of resveratrol is a dimer, and the initial electron transfer generates the reduction of Cu(II) to Cu(I), which then generate reactive oxygen species (ROS) [5-7].

It is aimed to investigate the production of Cu (I) in copper-resveratrol complex by capillary electrophoresis and voltammetry. Borate buffer (0.05M), was chosen as working electrolyte in both techniques. By capillary electrophoresis the most significant results were obtained at pH 8.5. Capillary electrophoretic experimental conditions were selected as: +23 kV applied potential, 20°C temperature while using fused silica column with 50µm inner diameter and 50cm length. Copper - resveratrol complexes were studied at pH (7.4, 9.6 and 11) by adsorptive cathodic and anodic square wave stripping voltammetry using HMDE versus Ag/AgCl.

In the electropherograms of Cu(II), resveratrol mixture three peaks (at 2.5, 3, 4 minutes) were observed. The height of resveratrol peak, at 2.5 minutes, decreased in time. While the peak of copper (II) complex get bigger until the end of an hour, then began to decrease and slide to longer retention times (approximately 4 minutes), eventually diminished. It is concluded that Cu(II)- resveratrol is converted to Cu(I)- resveratrol complex in course of time, referring to peak retention time of Cu(I)- resveratrol complex.

Conversion of Cu(II)- resveratrol complex to Cu(I)- resveratrol complex was supported with voltammetric data at pH 9.6 and 11. It was not possible to see Cu(I)- resveratrol complex at physiological pH (7.4). It is known that the prooxidant mechanism of resveratrol is based on the acidity of the phenol group, involving a proton loss from the OH in para position, to give a phenoxide anion which participates in a redox reaction with Cu(II) ion [8]. Our results also show that at higher pH values the formation of Cu (I) is accelerated.

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PP-32

A COLORIMETRIC SENSOR FOR THE SIMULTANEOUS DETERMINATION OF OXIDATIVE STATUS AND ANTIOXIDANT ACTIVITY ON THE SAME MEMBRANE: N,N-DIMETHYL-*p*-PHENYLENE DIAMINE (DMPD) ON NAFION

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A colorimetric sensor capable of simultaneously measuring oxidative status (OS) in terms of the hazard produced by reactive oxygen species (ROS) and antioxidant activity (AA) in regard to ROS-scavenging ability of antioxidant compounds was developed. The colored cationic semi-quinone derivatives, caused by ROS oxidative degradation of N,N-dimethyl-*p*-phenylene diamine hydrochloride (DMPD) in pH 5.7 acetate-buffered medium, were immobilized on a R-(-O-CF_2-$CF(CF_3)$-)$_x$-O-$(CF_2)_2SO_3^-$−functionalized perfluorosulfonate-based Nafion membrane. As ROS, hydroxyl ($^{\bullet}OH$) and superoxide ($O_2^{\bullet-}$) radicals were produced by Fenton/UV and xanthine/xanthine oxidase methods, respectively. The pink-colored, (+)-charged chromophore (referred to as DMPD-quinone or DMPDQ), resulting from the reaction between DMPD and ROS, could be completely retained on the solid membrane sensor by electrostatic interaction with the anionic sulfonate groups of Nafion. After equilibration, the Nafion membrane surface was homogeneously colored enabling an absorbance measurement at 514 nm, while the aqueous phase completely lacked color. Antioxidants, when present, caused an absorbance decrease on the membrane due to their ROS scavenging action, giving rise to less DMPDQ production. By this method, the extent of ROS generation (oxidative status) can be estimated directly from the color formed on the membrane, while the absorbance decrease on the sensor was linearly dependent on antioxidant concentration over a reasonable concentration range, enabling the simultaneous determination of OS and AA-against ROS. The proposed antioxidant sensing method was tested in synthetic antioxidant mixtures, and validated against standard antioxidant capacity assays (*i.e*. ABTS and CUPRAC) for a variety of polyphenolic and antioxidant compounds.

PP-33

COMPARISON OF ANTIOXIDANT AND ANTIRADICAL ACTIVITIES OF LYOPHILIZED WATER EXTRACTS OF TWO DIFFERENT TYPES OF GARLIC (*ALLİUM SATİVUM* L. AND *ALLİUM TUNCELİANUM* L.)

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In this study, we determined and compared the antioxidant and antiradical capacities of lyophilized water extracts of two garlic species (*Allium Sativum* L. and *Allium Tuncelianum* L.) belong to the family of *Liliaceae.* In order to evaluate the antioxidant and antiradical capacities of *Allium Sativum* L. and *Allium Tuncelianum* L.; N,N-dimethyl-p-phenylenediamine radical ($DMPD^{\bullet+}$) scavenging activity, superoxide anion radical ($O_2^{\bullet-}$) scavenging activity, ferric thiocyanate method for total antioxidant activity, ferric ions (Fe^{3+}) reducing capacity by FRAP assay, cupric ions (Cu^{2+}) reducing capacity by Cuprac assay and ferrous ions (Fe^{2+}) chelating activity were studied. The amounts of total flavonoid was determined as quercetin equivalents. BHA, BHT, α-tocopherol and trolox were used as reference antioxidants to compare the antioxidant activity of the extracts. 30 µg/mL of water extracts of *Allium Sativum* L. and *Allium Tuncelianum* L. inhibited linoleic acid peroxidation 63.8 and 72.2%, respectively.

In addition to protection of metabolism against the harmful effects of free radicals, natural antioxidants prevent the oxidative deterioration of lipids in foods and retard the process of chronic disorders due to the chemical structures of their phenolic contents [1, 2]. Because of polyphenol compounds in garlic, Tunceli garlic (*Allium Tuncelianum* L.) an endemic species was thought to have a strong antioxidant and antiradical activity than culture garlic (Allium sativum L.). According to the results compared with reference antioxidants, *Allium Tuncelianum* L. was found to have better activity than *Allium Sativum* L. in all assays. The results obtained by comparing the antioxidant and radical scavenging activities of these two different species of garlic are expected to make a major contribution to design of medicaments and their pharmacological applications.

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PP-34

ANTIOXIDANT ACTIVITY OF AMINOADAMANTANE ANALOGUES WITH AMINO ACID CYSTEIN

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Due to the ability of thiols to undergo redox reactions, cysteine has antioxidant properties. Cysteine's antioxidant properties are typically expressed in the tripeptide glutathione, which occurs in humans as well as other organisms. The synthesis and the antioxidant activities of new analogues of amantadine and rimantadine with amino acid cystein are reported. The new aminoadamantanes have been synthesized from amantadine and rimantadine with the Cys(Acm)-OH. The antioxidant properties of the newly synthesized amides have been studied for then antioxidative activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH)* test.

PP-35

DETERMINATION OF ANTIOXIDANTS CAPACITY OF NIGELLA SATIVA VIA CUPRAC, FRAP, DPPH AND ABTS/TEAC METHODS

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Antioxidants which reduced the hazardous effect of free radicals in food and human body, plays an important role in converting hazardous compounds into non-toxic products. A diet including antioxidant nutrition's may inhibit the oxidative damage of free radicals and active oxygen. For this reason, food, beverages and plant's antioxidant activity become a very important issue. Several antioxidant capacity measurement methods were developed in order to determine the antioxidant activity of these nutrition's. *Nigella sativa* L. was commonly used for thousands years as spice and medicine. The plant itself and its essential oil exhibit a potential drug property in alternative medicine. Hundreds of study were done to investigate the effect of that plant on many illnesses. In this study, ethanol extract of *Nigella sativa* L. were analyzed with different antioxidant activity methods as CUPRAC, FRAP, DPPH and ABTS/TEAC which are the most common and cited antioxidant capacity measurement method.

In procedure, *Nigella sativa* L. ethanol extract were diluted in between 1 to 1/32 (v/v) and then the samples were analyzed with FRAP, CUPRAC, DPPH and ABTS/TEAC methods. In order to compare and calculate the equivalant antioxidant capacity of each sample, different concentration of trolox reference samples were prepared in between 1-30 ug/mL. All measurements were performed in Biotek Elisa Reader and results were evaluated with Microsoft Excel 2010 software.

According to the results, *Nigella sativa* L. ethanol extract's ½ diluted concentration have equivalant trolox concentration as 48.05, 47.64, 43.17, 1.74 for FRAP, ABTS/TEAC, DPPH and CUPRAC respectively. It is claimed that ethanol extract of Nigella sativa has showed antioxidant property.

PP-36

DETERMINATION OF ANTIOXIDANT CAPACITY OF COMMERCIAL PEACH BEVERAGES VIA CUPRAC, FRAP, DPPH AND ABTS/TEAC METHODS

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Antioxidants which reduced the hazardous effect of free radicals in food and human body, plays an important role in converting hazardous compounds into non-toxic products. A diet including nutrition that have antioxidant property may inhibit the oxidative damage of free radicals and active oxygen. For this reason, food, beverages and plant's antioxidant activity become a very important issue. Several antioxidant capacity measurement methods were developed in order to determine the antioxidant activity of these nutrition. Peach which is known as summer fruit is very rich in terms of A, B_3, vitamin C, folic acid, β-karoten, potassium, calcium, ferrous, and natrium. In addition to this, peach contains many phenolic compounds which makes this fruit a very antioxidant nutrition. In this study, different trademark (a,b,c) peach beverages were analyzed with different antioxidant activity methods as CUPRAC, FRAP, DPPH and ABTS/TEAC which are the most common and cited antioxidant capacity measurement method.

Peach beverages were diluted in between 1 to 1/32 (v/v) and then the samples were analyzed with FRAP, CUPRAC, DPPH and ABTS/TEAC methods. In order to compare and calculate the equivalant antioxidant capacity of each sample, different concentration of trolox reference samples were prepared in between 1-30 ug/mL. All measurements were performed in Biotek Elisa Reader and results were evaluated with Microsoft Excel 2010 software.

According to the results, peach beverages ½ diluted concentration have equivalant trolox concentration as (a:9.88, b: 9, c:10.02), (a:49.58, b:41.43, c:36.32), (a:36.42, b:28.17, c: 37.82), (a:80.73, b:65.07, c:83.17) for FRAP, ABTS/TEAC, DPPH and CUPRAC, respectively. It is claimed that ethanol extract of peach beverages has showed antioxidant property.

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