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THE WORD OF WELCOME

Dear colleagues,

We warmly welcome you to the **3rd International UNIFood Conference – UNIFood2024** organized by the Faculty of Agriculture, University of Belgrade and University of Belgrade. This event engages not only academics, but also stakeholders from all relevant industries and business sectors, and will serve as a meeting point and platform for the dissemination of new ideas and the development of new partnerships. Food scientists, technologists, researchers, nutritionists, engineers and entrepreneurs will share their knowledge on the latest advances in all aspects of food production, processing, sustainability, safety and quality, nutrition and health, and knowledge transfer supporting environment.

The first UNIFood conference, organized as a national, was launched in 2018. as one of the events in honor of the **210th anniversary** of the **University of Belgrade** which was ranked 35th on the 2017 Shanghai list in Food Science and Technology. More than 250 proceedings from thirteen countries including 83 oral presentations (including three plenary lectures, eight invited lecturers and three section lecturers) and a round table dedicated to better cooperation between academia and industry highlighted the importance of food research in various fields of science and technology that require multidisciplinary and multistakeholder approaches. The second UNIFood conference was organized as an international in 2021. and gathered 273 participants from 23 countries, with 52 oral presentations (including four plenary lectures, five key note speakers, seven invited lectures and three section lecturers) as well as round table and workshops.

We are delighted that you have choosen to participate in this collaborative conversation, where authors from 19 countries will present their recent work in 99 poster presentations and 60 oral communications (including four plenary lectures, four keynote lectures, nine invited lectures and seven section lecturers). You will have the opportunity to participate in three educational workshops, round table and discuss current EU project results.

Belgrade, one of the oldest cities in Europe, always young, at the confluence of the Sava and Danube rivers, will be your host. At the confluence of new ideas and experiences we welcome you again and wish you a fruitful discussion and the establishment of new collaborations.

Sincerely,

Prof. Dr Mirjana Pešić
President of the Scientific Committee
of UNIFood2024

OPTIMIZING PARAMETERS RELATED TO THE PHENOLIC AND FLAVONOID CONTENTS OF STEEPE PEONY LEAVES

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The current study had two objectives: (1) the determination of the total phenolic content (TPC) and total flavonoid content (TFC) of steppe peony leaf extracts prepared in two different extraction methods, maceration using conventional orbital shaker (M_{COS}) and ultrasound-assisted extraction (UAE) and (2) optimization of the extraction process parameters *via* varying solvent type (aqueous, and ethanolic (50% and 70%, *v/v*), time of extraction (5, 15, 30, and 60 min), and solid-to-solvent ratio (1:10, 1:20, 1:30, 1:40, and 1:50). The total phenolics were extracted most effectively by UAE (411,3 mg gallic acid equivalents (GAE)/mL of raw extract). The M_{COS} gave lower values, approximately 112,9 mg GAE/mL of raw extract. Total flavonoids content were ranged from 3,0 to 20,0 mg catechin equivalents (CE)/mL of raw extract for UAE, and from 2,98 to 22,87 mg CE/mL of raw extract for M_{COS}. For all tested analytes, it was observed that a concentration of ethanol of 50% (*v/v*), a time of extraction of 15 min, and the solid-to-solvent ratio of 1:20 were the most effective for extracting bioactive substances (phenolics and flavonoids) from Steppe peony.

Keywords: *phenolics, colorimetric determination, gallic acid equivalents, conventional and „green" extraction*

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Vaccinium myrtillus LEAF EXTRACT-LOADED LIPOSOMES: THE INFLUENCE OF UV IRRADIATION

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Due to the present phytochemicals (phenolic acids, flavonoids, and tannins), the extracts of *Vaccinium myrtillus* leaf were shown to scavenge free radicals *in vitro*, to enhance glutamate decarboxylase gene expression in dermal fibroblasts, resulting in the stimulation of cell growth and synthesis of hyaluronic acid and glutathione, to inhibit collagenase and elastase, enzymes responsible for the ragging and wrinkled nature of skin, to decrease the melanin content in B16 melanoma cells, and to suppress the release of histamine from mast cells. With the aim to improve the stability and bioavailability of the extract compounds, *V. myrtillus* leaf extract was encapsulated in the liposomes, and the influence of the UV irradiation on the particle size, polydispersity index (PDI), zeta potential, and mobility of obtained liposomes was investigated. The liposomal particles were prepared using the proliposome method and 4 g of phospholipids, 50 mL of *V. myrtillus* ethanol extract, and 20 mL of ultrapure water. Vesicle size, PDI, zeta potential, and mobility were measured by photon correlation spectroscopy before and after UV irradiation. The size and PDI did not change after UV irradiation and amounted to 5508.67±56.58 nm and 0.249±0.047, respectively. On the other hand, the zeta potential (as a parameter of the stability of the system) and mobility possessed low values (absolute values) at the beginning and additionally significantly changed in the UV-treated sample (-3.93±0.10 mV and -0.315±0.016 μmcm/Vs, respectively). The beneficial effects of biologically active *V. myrtillus* leaf phytochemicals on human and animal health, as well as their sensitivity, particularly under UV irradiation, encourage their encapsulation in liposomes. However, future experiments should be focused on the improvement of the stability of *V. myrtillus* extract-loaded liposomes, *i.e.*, increasing the zeta potential value.

Keywords: encapsulation, liposomal particles, Vaccinium myrtillus, UV irradiation

Acknowledgments: The authors acknowledge their gratitude to the Ministry of Education, Science and Technological Development of Serbia, contract numbers 451-03-66/2024-03/200003, and 451-03-66/2024-03/200019.

ANTIOXIDANT CAPACITY OF SILIBININ-LOADED LIPOSOMES

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Silibinin is the major active constituent of silymarin, a standardized extract of milk thistle seeds. Silibinin has been used traditionally as a chemopreventive and therapeutic agent in human lung cancer. It also possesses hepatoprotective, antioxidant, hypocholesterolemic, antitumor, cardioprotective, neuroprotective, and antiviral activities. However, its application is limited due to poor water solubility, intestinal resorption, and low bioavailability. Liposomes, as non-toxic, biodegradable, and biocompatible lipid carriers, can provide controlled delivery of bioactive components and their protection from degradation caused by light, oxygen, UV irradiation, different pH values, and enzymes. Additionally, phospholipids from the liposomes do not provoke a reaction with taste receptors, and, therefore, the liposomal bilayer is an appropriate carrier for covering the unpleasant taste of numerous polyphenols. With the aim of investigating the antioxidant potential of silibinin-loaded liposomes after different technological procedures (UV irradiation and lyophilization), two antioxidant assays were employed (ABTS and DPPH tests). Liposomes with silibinin were prepared using the proliposome method and phospholipids. According to the results of the ABTS test, the antioxidant activity of pure silibinin was 0.769 μmol Trolox equivalent (TE)/mL, while the antioxidant potential was lower after the encapsulation in liposomes; 0.548 μmol TE/mL after preparation, 0.549 μmol TE/mL after UV irradiation, and 0.436 μmol TE/mL after lyophilization. Furthermore, the DPPH radical scavenging activity was 20.97% for pure silibinin, 22.48% immediately after the liposomal preparation, 22.24% after UV irradiation, and 18.73% after lyophilization. As can be seen, UV irradiation did not cause significant changes in the overall antioxidant potential of silibinin-loaded liposomes. Nevertheless, lyophilization significantly decreased the radical scavenging activity of the liposomes. Considering that the two used antioxidant assays are based on different principles and reactions, the obtained results provide good insight into the overall antioxidant activity of silibinin-loaded liposomes.

Keywords: antioxidant capacity, encapsulation, liposomes, proliposome method, silibinin

Acknowledgments: The authors thank the Ministry of Education, Science and Technological Development of Serbia, contract numbers 451-03-66/2024-03/200019, 451-03-66/2024-03/200003, 451-03-66/2024-03/200026, and 451-03-66/2024-03/200135.

THE INFLUENCE OF LYOPHILIZATION ON LIPOSOMAL PARTICLES WITH SILYMARIN

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Silymarin is the group of biologically active polyphenols from *Silybum marianum* (milk thistle) that contains silibinin, isosilybin, silydianin, and silychristand. The mentioned components show numerous pharmacological activities promoting human health and well-being, including antioxidant, antimicrobial, anti-inflammatory, antiviral, immunomodulatory, and antitumor effects. Nevertheless, silymarin is quite sensitive to temperature, light, and oxidation and has poor water solubility and low bioavailability. Therefore, their application in food, functional food, dietetic supplements, and pharmaceutics is limited. The encapsulation of silymarin in liposomes represents a technique that can be widely used to strengthen and supplement formulations by enhancing stability and bioavailability and controlling the delivery of the active compound. Lyophilization is a widely employed procedure for drying thermosensitive components to obtain freeze-dried products with active compounds that are stable over a long period, due to the prevention of hydrolytic and oxidative degradation which can occur in water surrounding. Hence, lyophilization can result in significant modifications of the liposomal vesicles, thus its effect should be examined. The liposomes were prepared using 0.5 g of silymarin, 5 g of phospholipids, 10 mL of ethanol, and 40 mL of water in the proliposome procedure. After the preparation, the liposomes were freeze-dried for 24 h. The characterization is performed using photon correlation spectroscopy. Vesicle size and polydispersity index (PDI) of lyophilized silymarin-loaded liposomes were changed from 4080.0±24.0 nm and 0.346±0.044 to 4628.1±45.2 nm 0.426±0.038, respectively. Zeta potential was -20.55±1.34 mV, mobility was -1.55±0.13 µmcm/Vs, and conductivity was 20.15±1.06 µS/cm. In comparison to non-treated liposomes, lyophilization caused an increase in vesicle size and the absolute value of zeta potential, and a decrease in the conductivity value. On the other hand, freeze-drying did not have a significant influence on PDI values and mobility of silymarin-loaded liposomes.

Keywords: lyophilization, silymarin, size, zeta potential

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ANTIOXIDANT CAPACITY OF APIUM GRAVEOLENS, APIUM GRAVEOLENS VAR. RAPACEUM, AND DAUCUS CAROTA EXTRACTS

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Celery (*Apium graveolens*) represents an edible plant used in traditional medicine due to its numerous health benefits: prevention of cardiovascular disease, lowering blood glucose and blood pressure, antifungal anti-inflammatory, anticoagulant, antioxidant, and antitumor properties. Celeriac (*Apium graveolens* var. *rapaceum*) is commonly used for its edible fleshy tap root and stalk. It contains flavonoids, violate oil, vitamins, and minerals, showing anticancer effects. Carrot (*Daucus carota*) is recognized for its nutraceutical and health benefits and due to the presence of phenolic compounds, carotenoids, and ascorbic acid possess antioxidant, anti-aging, anti-inflammatory, and anti-proliferative activities. Ultrasound-assisted extraction represents a modern technique for the extraction of various antioxidant compounds from plant material. Thus, in the present study, the extracts were prepared using celery root, celeriac stalk and leaves, or carrot root, and water or 30% ethanol in an ultrasound bath. The antioxidant potential was determined by analyzing total reducing (Folin-Ciocalteu assay) and DPPH radical scavenging activities. The total reducing capacity varied in a range of 0.27 to 2.10 mg gallic acid equivalents (GAE)/g of fresh plant material, achieving the highest values in the following samples: ethanol celeriac leaf extract > water celeriac leaf extract > water celery root and celeriac stalk extracts. On the other hand, the lowest total reducing capacity was obtained in the samples: water and ethanol carrot root extracts < ethanol celery root and celeriac stalk extracts. The DPPH radical scavenging potential, expressed as the concentration required for neutralization of 50% of radicals, follows the trend: ethanol celeriac leaf extract (0.10±0.01 g/mL) < water celeriac stalk extract (0.13±0.00 g/mL) < water celery and carrot extracts (0.18 g/mL) < ethanol celeriac stalk extract (0.26±0.03 g/mL) < water celeriac leaf extract (0.43±0.07 g/mL) < ethanol carrot root extract (0.62±0.05 g/mL) < ethanol celery root extract (1.32±0.06 g/mL) (the lowes IC_{50} = the highest antioxidant capacity). As can be seen, the highest antioxidant capacity was determined in ethanol celeriac leaf extract in both assays. Thus future experiments should be focused on the chemical characterization of the mentioned extract and individual compounds responsible for the antioxidant activity.

Keywords: antioxidant activity, carrot, celery, celeriac, extraction

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ENCAPSULATION OF *FUMARIA OFFICINALIS* EXTRACT IN THE LIPOSOMAL VESICLES

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Fumaria officinalis (fumitory) extracts have shown antimicrobial, antioxidative, antispasmodic, laxative, anthelmintic, cholagogue, cytotoxic, and sedative effects. The mentioned health benefits can be attributed to the high content of polyphenol and alkaloid compounds. However, the mentioned compounds possess low solubility, stability, and bioavailability. Therefore, their encapsulation in different carriers is necessary. Liposomes are widely used as carriers for the encapsulation, preservation, and controlled release of numerous hydrophilic and lipophilic bioactive principles from different plant sources. Thus, the aim of the presented study is the development and physicochemical characterization of *F. officinalis* extract-loaded liposomes in terms of encapsulation efficiency, vesicle size, polydispersity index (PDI), zeta potential, conductivity, and mobility. The extract-loaded liposomes were obtained in the proliposome method using 1 g of phospholipids, 1 mL of fumitory ethanol extract, and 10 mL of ultrapure water. Encapsulation efficiency was indirectly calculated by the polyphenol concentration determined in the supernatant. Particle size, PDI, zeta potential, conductivity, and mobility were measured by photon correlation spectroscopy. The encapsulation efficiency of polyphenols was >73%. The vesicle size and PDI were 274.0±0.7 nm and 0.307±0.020, respectively. The zeta potential, conductivity, and mobility were -6.34±0.16 mV, 0.465 mS/cm, and -0.491±0.011 μmcm/Vs, respectively. The beneficial effects of biologically active compounds from *F. officinalis* herba on human health, as well as their sensitivity, highlight the application of small, uniform, and stable phospholipid liposomal vesicles as their carrier.

Keywords: encapsulation, fumitory, liposomes, polyphenols

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TOTAL POLYPHENOL AND PROTEIN CONTENT IN DIFFERENT *FUMARIA OFFICINALIS* EXTRACTS

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Fumaria officinalis L. (Fumariaceae) is a component of various phytotherapeutic formulations in the European ethnobotany used in hepatobiliary dysfunction, illnesses of gastrointestinal and urogenital tracts, cancer, rheumatism, high blood pressure, and skin disorders. Various extraction techniques can isolate bioactive compounds from plant material; however, they differ in terms of extraction speed and efficiency, the yield of target molecules, solvent and energy consumption. Therefore, in the present study, microwave- and ultrasound-assisted extractions (MAE and UAE, respectively) and two different solid-to-solvent ratios (1:20 and 1:30) were used for polyphenol and protein extractions from *F. officinalis* herba. MAE process was performed in a microwave reactor for 2 min, while UAE was done in an ultrasound bath for 15 min. The total polyphenol content (TPC) was determined in the Folin-Ciocalteu assay, while the total protein yield was measured in the Bradford protein assay. The TPC varied in a range of 15.2 to 23.7 mg gallic acid equivalents (GAE)/g of dried plant material (dw), achieving the highest value in the extract prepared using MAE and a 1:30 ratio, followed by MAE and a 1:20 ratio and UAE and a 1:30 ratio, while the lowest polyphenol yield was obtained using UAE and a 1:20 ratio. The concentration of proteins in *F. officinalis* extracts follows the trend: MAE and a 1:30 ratio (68.3 mg/g of dw) > MAE at a 1:20 ratio (67.2 mg/g of dw) and UAE at a 1:30 ratio (67.5 mg/g of dw) > UAE and a 1:20 ratio (64.3 mg/g of dw). Due to significantly higher polyphenol and protein yields, *F. officinalis* extract prepared using MAE (as a more rapid technique) and a higher employed level of solid-to-solvent ratio (1:30) can be potentially implemented in different food, functional food, dietetic supplement, or pharmaceutical formulations.

Keywords: Fumaria officinalis, extraction, polyphenols, proteins

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THE IMPACT OF DIFFERENT SOLID-TO-SOLVENT RATIOS ON *SATUREJA MONTANA* L. POLYPHENOL AND FLAVONOID CONTENT AND ANTIOXIDANT POTENTIAL

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The presented study aimed to optimize the extraction of polyphenolic compounds from *Satureja montana* L. cultivated at the experimental site of the Institute for Medicinal Plants Research "Dr Josif Pančić", Serbia, by varying on of the most important parameter for maceration, solid-to-solvent ratio. The obtained extracts were characterized on the basis of the total polyphenol content (TPC), and total flavonoid content (TFC). The TPC varied from 35.01 ± 10.41 mg GAE (gallic acid equivalent)/g to 87.96 ± 4.73 mg GAE/g. On the other hand, the TFC values were in the range from 1.8 ± 0.09 to 5.6 ± 0.01 mg CE (catechin equivalent)/g. The highest TFC was obtained at a solid-to-solvent ratio of 1:50, whereas in the case of TFC the optimal ratio was 1:40. The highest DPPH neutralization level (86.12 %) as well as the highest anti-ABTS antioxidant activity (90.35 %) were obtained for the extracts prepared using a solid-to-solvent ratio of 1 g:50 cm^3. Our study shows the initial steps in obtaining polyphenol-rich extract of *S. montana* with good satisfactory antioxidant potential, which could be used in the pharmaceutical, food, or cosmetic industry.

Keywords: polyphenols, winter savory, maceration, flavonoids

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ULTRASOUND AND NATURAL DEEP EUTECTIC SOLVENTS AS TOOLS FOR IMPROVING EXTRACTION YIELD AND ANTIOXIDANT POTENTIAL OF WILD THYME EXTRACTS

***Ana Plećić*[1], Sandra Rakin[1], Lidija Mlađenović[1], Natalija Čutović[2], Predrag Petrović[3], Marija Gnjatović[1], Aleksandra Jovanović[1]**

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The aerial part of wild thyme has widespread use in traditional medicine for treating gastrointestinal and respiratory disorders, rheumatism, menstrual pain, wounds, and eczema. Its extracts possess antioxidative, anti-inflammatory, antihypertensive, antibacterial, and antifungal properties. Ultrasound extraction is a novel procedure used to isolate various plant bioactive compounds, due to the increased extraction yield, fast kinetics, and simple operation. Furthermore, natural deep eutectic solvents can increase phenolic recovery from plant matrix. In the present research, the extracts were prepared using dried wild thyme herb and water, 35% ethanol, or two types of natural deep eutectic solvents with 50 % water - betaine+citric acid and citric acid+saccharose in an ultrasound bath. The obtained extracts were characterized *via* analyzing the total phenolic and flavonoid contents (TPC and TFC, respectively) and antioxidant capacity ($ABTS^{\bullet+}$ and $DPPH^{\bullet}$ assays). The TPC of water, ethanol, betaine+citric acid, and citric acid+saccharose extracts were 25.08±2.3, 27.5±1.2, 30.2±2.9, and 28.1±3.3 mg gallic acid equivalents (GAE)/g of dried plant material, respectively. At the same time, the TFC values amounted to 10.5±0.8, 13.1±1.3, 1.0±0.5, and 2.8±0.1 mg catechin equivalents (CE)/g of dried plant material, respectively. In the $ABTS^{\bullet+}$ assay, antioxidant activity was 7.7±1.0 µmol Trolox equivalent (TE)/g of dried plant material (water extract), 8.5±0.9 µmol TE/g (ethanol extract), 10.8±0.11 µmol TE/g (betaine+citric acid extract), and 17.0±0.7 µmol TE/g (citric acid+saccharose extract). DPPH radical scavenging capacity was expressed as IC_{50} (concentration of the extract required to neutralize 50% of free DPPH radicals); IC_{50} was 4.3±0.2 mg/mL for water extract, 4.0±0.5 mg/mL for ethanol extract, 3.5±0.2 mg/mL for betaine+citric acid extract, and 2.9±0.1 mg/mL for citric acid+saccharose extract. pH values for ethanol, betaine+citric acid, and citric acid+saccharose extracts were 6.97, 6.18, 2.47, and 1.60, respectively. Due to higher $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging activity, wild thyme extract obtained using citric acid and saccharose with 50% water compared to water, ethanol, and betaine+citric acid extracts was favored as an ingredient in food and pharmaceutical products.

Keywords: antioxidants, natural deep eutectic solvents, phenolics, ultrasound, wild thyme

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