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EDITORIAL

It is my proud privilege to welcome you all to the The IRES International Conference at Mexico City, Mexico. I am happy to see the papers from all part of the world and some of the best paper published in this proceedings. This proceeding brings out the various Research papers from diverse areas of Science, Engineering, Technology and Management. This platform is intended to provide a platform for researchers, educators and professionals to present their discoveries and innovative practice and to explore future trends and applications in the field Science and Engineering. However, this conference will also provide a forum for dissemination of knowledge on both theoretical and applied research on the above said area with an ultimate aim to bridge the gap between these coherent disciplines of knowledge. Thus the forum accelerates the trend of development of technology for next generation. Our goal is to make the Conference proceedings useful and interesting to audiences involved in research in these areas, as well as to those involved in design, implementation and operation, to achieve the goal.

I once again give thanks to the Institute of Research and Journals, The IIER & The IRES for organizing this event in Mexico City, Mexico. I am sure the contributions by the authors shall add value to the research community. I also thank all the International Advisory members and Reviewers for making this event a Successful one.

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POLYPHENOLS RECOVERY FROM SAMBUCUS EBULUS L., BY USING NADES SOLVENT

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Abstract - NADES solvents are the latest generation of "green" solvents. Prepared from exclusively natural components, such as: amino acids, sugars, water, etc. they are completely biodegradable, edible and absolutely harmless to the environment and humans. This is one of the main reason why their application in the extraction of bioactive molecules is extremely interesting. Natural extracts obtained with NADES solvents can be incorporated directly, without any purification, into a number of functional products, e.g. in food, cosmetic or pharmaceutical products. In the frame of this study, Sambucus ebulis L. plant was extracted using several NADES solvents. NADES solvents were obtained by mixing different components in different ratios. The obtained extracts were characterized in terms of the content of the main bioactive compounds – polyphenols and flavonoids, as well as the ability to neutralize free DPPH radicals. The obtained data clearly show the potential of natural and harmless solvents to be used in the extraction of medicinal plants and the preparation of natural preparations. The price of obtaining them also justifies their use at the industrial level.

I. INTRODUCTION

During the last 20 years an increasingly important role is played by natural medicine in the prevention and in the treatment of different diseases. Interest to include traditional approach into modern way of healing became popular and widespread in both industrially developed countries as well as in less developed countries, primarily because of the negative effects that may be caused by a prolonged application of strong synthetic drugs, or even due to the insufficient therapeutic outcomes of the conventional drugs[1,2].In addition to natural remedies, a big turn has been made in terms of many other products. For example, people are increasingly resorting to natural foods, i.e. foods that have not been processed and that do not contain synthetic additives[3]. In this sense, foods enriched with natural bioactive molecules that increase its functionality are gaining in popularity. The increase in interest in natural preparations has led to an increase in the number of scientific studies on the chemistry and biology of bioactive molecules that are carriers of biological activities of plants. In this way, the mechanism of action of many molecules was discovered and many of them have found application in the pharmaceutical, cosmetic or food industries. A of compounds special important group are polyphenolic compounds of plants. These biologically important molecules are actually one of the most common dietary antioxidants[4], as well as the most important nutritional food ingredients. Their role in the plant itself is multiple, and their intake in the body has irreplaceable benefits for human health and the strengthening of the immune system. Also, numerous in vitro and in vivo analyses have proven other properties of polyphenols such as ability to

cardiovascular, protect organisms from neurodegenerative, metabolic and carcinogenic diseases. In recent years, their ability to regulate the activity of some biologically important enzymes has been intensively studied[5]. Due to the great benefits which they have for human health, as well as due to the demands of the market and consumers, an increasing number of industries are trying to include polyphenols in the formulations of their products. Therefore, one of the most important issues is their isolation from the plant matrix. Various techniques for their extraction have been developed and optimized. Although conventional techniques have been most commonly used up until recently, the industry is intensively turning to modern extraction techniques in the desire to achieve higher yields, better utilization of plant resources, and reduce waste generation[6]. Reducing extraction time, solvent volume, production costs, and energy consumption are the demands of modern industry that dictate the development of new technologies in the production of plant extracts. Also, the goal of newly developed techniques is to obtain extracts with increased biological activity compared to those obtained by conventional techniques[7]. In each extraction technique, selection of solvent is of utmost importance and it depends primarily on the type of compound we want to obtain from the selected plant material. With the desire to meet all the requirements of industry, but also "green" chemistry and environmental protection, many alternative solvents are widely used. One of these are Natural Deep Eutectic Solvents (NADES) which represent the latest generation of solvents[8]. Taking into account that they are biodegradable, non-toxic, non-volatile, composed of exclusively natural components, they are extremely suitable medium for the separation of bio-molecules.

Sambucus ebulus L. is one of the many self-seeding plant species that has been slightly forgotten over the recent years, but absolutely deserves its rehabilitation. Although it grows all around as a weed of an unpleasant smell, this plant also has healing properties. The use of S.ebulus in medicine and diet dates back to the antiquity and numerous preparations made of this plant have been used for centuries on the territory of whole Europe[9]. The traditionally made healing preparations include all parts of this plant. The root is used in the case of rheumatism, arthritis, sciatica and neuralgias. Its use against psoriasis is particularly important and well-spread because of its ability to connect and excrete uric acid from the body.

The leaf is recommended for the detoxification of liver and for regulating the function of kidneys[9]. Also, the healing preparations made of the danewort leaf in the traditional manner are used for treating common cold and sore throat, as well as for the lowering of the body temperature or for the wound healing. The fruit has multiple uses and it is its role as a purgative and immunological stimulator that is particularly important[9]. The plant has often been used in the case of a bee sting or a snake bite, and also for oedema, eczema or inflammatory processes. There is a large number of preparations that are based on S.ebulus which are traditionally used in the case of some types of cancer.

Also, there is a well-known traditional medicine against Helicobacter Pilory[10].The purpose of this work was to prepare extracts of Sambucus ebulus L. with NADES solvent and to find extraction conditions for maximal recovery of polyphenols as one of the most important bioactive ingredients of the plant.

II MATERIALS AND METHODS

A. Preparation of Plant Material

Sambucus ebulusL. was collected in the southeastregion of Serbia. Plant material was stacked in a crate with perforated bottom, in order toensure air flow. Drying was performed naturally on draft in the darkuntil a moisture content of 10%. Dry plant material was packedin glass jars and stored in the dark until use.

B. Preparation of NADES Solvents

All NADES used in the present study are composed of organic acids as hydrogen-bond donors and betaine or sugars as hydrogen-bond acceptors with different molar ratios. In total, 5 different NADES were selected: N1-lactic acid: fructose (5:1), N2-Lproline:Glicerol:water (1:2:1), N3-malic acid:betaine:water (2:1:5), N4-holin chloride:citric acid (1:1), N5-1.2propandiol: cholinchloride:water (1:1:1). NADES were prepared in a water bath at 80 $^{\circ}$ C placed on a magnetic stirrer hot plate. Mixing lasted approx. 10 min until the stable, transparent liquid was formed. All created NADES were stable at room temperature (~15 $^{\circ}$ C) for more than seven days.

C.Extraction of Plant Material

Extractions were performed in a water bath at 50 ± 1 °C placed on a magnetic stirrer hot plate with thermocouple for temperature regulation. Solvent (NADES: N1–N5) and plant matrix (0.05 g) were placed together with a small magnet into a glass extraction vial with a 1:20 (m/m) sample to solvent ratio. The vial was tightly closed with a cap and immersed in the water bath for 60 min. Water (4 mL) was added after the extraction to ease separation of solid and liquid phases, and samples were centrifuged for 15 min at 4000 rpm. The supernatant was separated from solid plant residue and stored in a fridge at 4 °C until the analysis of total phenol content (TP) and antioxidant activity toward DPPH radicals.

D. Determination of Total Phenols Content

The total phenolics content (TPC) in the S.ebulus L. extracts was determined by Folin–Ciocalteu procedure [11,12] using gallic acid as a standard. Absorbance was measured at 750 nm. The content of phenolic compounds was expressed in mg of gallic acid equivalent (GAE) per g of plant material (mg GAE/g). All experiments were performed in triplicate.

E. Determination of Total Flavonoid Content

Flavonoids in obtained extracts were determined using colorimetric assay based on the procedure described by Markham [13]. NADES extracts of Sambucus ebulus L. (1 mL) were mixed with 5% NaNO₂ solution (0.3 mL). After 5 min aluminiumchoride hexahydrate (10%, 0.3 mL) was added and allowed to stand for 6 min. Sodiumhidroxide (1 mol/dm³, 1 mL) was added to the mixture. Immediately, distilled water was added to bring the final volume to 10 mL. The blank was prepared by replacing the extract with distilled water. Immediately after mixing, absorbance was measured at 510 nm. Total flavonoid content in obtained extracts was calculated by interpolating the measured sample absorbance into calibration curve defined with standard solutions of rutin, defined for the 0.02-0.1 mg/mL concentration range $(A = 1.88727C - 0.0096, R^2 = 0.997)$. The results were expressed as rutin equivalents (mg RE/g).

F. DPPH Radical Scavenging Assay

Antioxidant activity towards DPPH radicals was determined by a spectrophotometric method [14]. First, 100 μ L of examined extract solutions in series of different concentrations were prepared and then added to 2900 μ L of DPPH methanolic solution (26 mg/L). After 1 h, absorbances were recorded at a

wavelength of 517 nm. All experiments were performed in triplicate, and the mean values of the antioxidant potential were presented as mg of Trolox equivalents (TE) per g dry mass.

III. RESULTS AND DISSCUSION

Phenolic compounds are recognized as one of the most powerful bioactive molecules in plants. These compounds may have physiological effects on living organisms, or may have direct/indirect therapeutic effects that make them suitable for use in the different forms. They can be used in food industry, due to their strong antioxidant and antimicrobial effects or can be used as natural colorant and additives. Further, they are also suitable for preparing medicinal or cosmetic products taking into account their strong biological activity (antioxidant, anticancer, antiproliferative, antimutagenetic, antimicrobial, etc). Numerous benefits offered by phenolic compounds and need of industry to isolate them from nature, influence new trend in the extraction technology. In that sense, green solvent, acceptable in high-demanding industry, such as pharmaceutical or food industry, are getting on their popularity. In this research, 5 different NADES solvents were applied for the extraction of phenolic compounds from Sambucus ebulus L. Determination of total phenolic content in obtained extracts was done spectrophotometrically, and the obtained results are presented in the Figure 1.



Figure 1. Total Phenolic content in Sambucus ebulus L. extracts obtained with different NADES solvents. N1-lactic acid: fructose (5:1), N2-L-proline:Glicerol:water (1:2:1), N3malic acid:betaine:water (2:1:5), N4-holin chloride:citric acid (1:1), N5-1.2propandiol: cholinchloride:water (1:1:1)

As it can be seen from the Figure 1. All obtained extracts were rich in total phenols, but certain differences among the extracts were presented. These differences originate from the used solvent, actually from the composition of used NADES. The highest phenolic content was determined in the extracts obtained with solvent made with L-proline, glycerol and water (58.04 mg GAE/g). The second NADES system in terms of extraction efficiency was holinchloride:citric acid (1:1) (49 mg GAE/g), than lactic acid: fructose (5:1) (48.68 mg GAE/g). However, the lowest phenolic content was determined in the extracts made with malic acid,

betaine and water (43.19 mg GAE/g) and very similar value (43.44 mg GAE/g) was reached with N5 system (1,2-propandiol, choline chloride and water). Apart of total phenols obtained extracts were characterized in terms of their flavonoids content. Flavonoids are subclass of polyphenols and in the same time the largest group of phenolic compounds responsible for many bioactivities of plants. Their high biological activity in nature can be used after their isolation from plans as well. From that reason numerous attempt were performed in order to obtained plant extracts rich in these very potent bioactives and to use them in different kind of product. In the frame of this study, total flavonoid content was determined spectrophotometrically and obtained results are presented in the Figure 2.



Figure 2. Total Phenolic flavonoid content in Sambucus ebulus L. extracts obtained with different NADES solvents. N1-lactic acid: fructose (5:1), N2-L-proline:Glicerol:water (1:2:1), N3malic acid:betaine:water (2:1:5), N4-holin chloride:citric acid (1:1), N5-1.2propandiol: cholinchloride:water (1:1:1)

As in the case of total phenols, the highest flavonoid content (35.02 mg RE/g) was determined in the case of the extract obtained with N2 solvent. However, no further correlation with TPC was in regard to all other extracts were present. The lowest flavonoid contentwas recorded in the N4 extract (13.6 mg RE/g). It was noticed that in all extract approximately 2/3 part of total phenols were flavonoids, which suggested that flavonoids made the largest contribution to the total phenol content. This indicates very high bioactivity and high biopotential of all extracts.

Flavonoids are a group of secondary biomolecules with the most different roles in plant growth, development and survival. The role they play in the plant can be, among others, protective towards to pathogenic microorganisms or harmful UV radiation due to the high antimicrobial and antioxidant potential. The role they play in the plant can be preserved even when we isolate them from matrix. Thus, a high degree of antioxidant protection as well as antimicrobial potential can be exploited in the formulation of many products. The extracts obtained in this study with NADES solvents are characterized by a green character, which increases the degree of their potential application. The next step in the study was to measure their ability to neutralize free radicals, which was conducted using the DPPH test. The obtained results are presented in the Figure 3.



Figure 3. Antioxidant activity of Sambucus ebulus L. Extracts obtained with different NADES solvents. N1-lactic acid: fructose (5:1), N2-L-proline:Glicerol:water (1:2:1), N3-malic acid:betaine:water (2:1:5), N4-holin chloride:citric acid (1:1), N5-1.2propandiol: cholinchloride:water (1:1:1)

In a DPPH test (Figure 3), the values of antiradical activity towards DPPH radicals for Sambucus ebulus extracts varied from 11.53 to 15,49 mg TE/g, which indicates that all investigated extracts showed good scavenging activity against DPPH radicals. The highest scavenging capacity was expressed byN2 extract and determined result revealed very good antiradical activity. This was expected taking into account that this sample was with the highest phenols and flavonoids content. Extracts prepared with N3 solvent exhibited the lowest free radical scavenging capacity, which was also correlated with the content of total phenols. The obtained values shows that all extracts express very high potential to neutralize free DPPH radicals, indicating that all used NADES solvent are suitable for the isolation of bioactive molecules from plant matrices. Incorporating the obtained extracts into products for various purposes can result in increased stability of the obtained products. Their green character allows their direct incorporation, without purification, offering increased process economy.

IV. CONCLUSION

Within this work, the wild plant Sambucus ebulus L was extracted with NADES solvent. This plant has long been known for its biological potential. So far, the isolation of polyphenolic compounds from this plant has been mainly basedon the use of organic solvents. The needs of environmental protection, as well as human health, require the replacement of organic solvents with a suitable "green" alternative. In this paper, by using exclusively biodegradable components, 5 different NADES solvents were composed, which further was used for extraction of polyphenols from Sambucus ebulus L. In all obtained extracts, the content of total phenols, flavonoids as

well as their potential to neutralize DPPH radicals was examined within this paper. The results showed that the polyphenol content in the obtained extracts varied in the range of 43.19-58.04 mg GAE/g, while the content of flavonoids was 13.6-35.02 mg ER/g, indicating the high efficiency of the used NADES solvents. Using an in vitro test, the ability of the obtained extracts to neutralize free DPPH radicals was measured. The obtained values were in the range of 11.53-15.49 mg TE/g. The results show the potential of these green solvents for the isolation of plant bioactive principles. Taking into account their acceptability from the environmental point of view. economic value for the process and high efficiency, these solvents represent a good alternative for modern industry.

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