



JERUSALEM ARTICHOKE DRY EXTRACT AND ITS NATURAL PRODUCTS. (HELIANTHUS TUBEROSUS)

Ismailov Shokhrukh Farrukh ugli

Master's student of Pharmaceutical Biotechnology and Bioengineering at Tashkent Pharmaceutical Institute.

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ABSTRACT

This article discusses the Jerusalem artichoke plant, its useful properties, where it grows, and ways to obtain a useful dry elixir from it. Also, identifying the nutritional and health properties of Helianthus tuberosus, and learning more about this valuable species. It is believed that increased consumption of Jerusalem artichoke (JA) products is related to low blood pressure. One of many questions to answer is whether supplementation of inulin and inulin derivatives obtained from Helianthus tuberosus tubers and aerial parts can be used as antidiabetic, anti-carcinogenic, anti-fungistatic, anti-constipation, body mass-reducing, metabolism-improving agents.

Among biologically protective polyphenols, phenolic acids are widely distributed in plants, and

have antioxidant capacity by neutralizing radicals by donating or accepting electrons. Major classes of phenolic acids comprise carbon chains of C₆+C₁ (hydroxybenzoic acids, HBA) and of C₆+C₃ (hydroxycinnamic acids, HCA), where C₆ is the phenyl ring and C₃ the propanoid moiety of various trans-cinnamic acids (caffeic, p-coumaric, and ferulic acid). Jerusalem artichoke (*Helianthus tuberosus* L.) is a perennial tuberous plant for which the content of phenolic substances has been described by several authors. Tchone and colleagues identified 22 phenolic compounds in methanolic extracts of tubers; salicylic acid ranked highest of the HBAs, and highest of the HCAs was chlorogenic acid (CQA), an ester of caffeic acid and quinic acid. Within the family of caffeoyl quinic acids, the hydroxyl groups of quinic acid can be esterified with caffeic acid in multiple positions (1, 3, 4, 5) to produce mono-, di-, and even tricaffeoyl isomers. The leaves, in particular, contain a broad spectrum of mono- and dicaffeoyl esters of CQA; more than 30 variants were identified by LC-MS. In the tubers of Jerusalem artichoke, three isomers of CQA and four isomers of dicaffeoylquinic acid have been identified.

Due to the diversity of the CQA isomers, their abundance in solvent extracts and corresponding

antioxidant activity depend on the strength and polarity of the solvent system used. Exploratory



extractions of polyphenols can be lengthy, stretching from several hours up to overnight, followed

by classical sequential extractions and separations into more non-polar solvents. The most abundant phenolic acid obtained from leaves extracted by ethyl acetate was 1,5-diCQA (104 mg/g dry substance (DS), or 39%), while 3-CQA (74 mg/g DS or 55%) dominated in the n-butanol fraction extracts. Ethyl acetate extracts also showed the highest radical scavenging activity (SC

50). Polar solvent systems, e.g., ethanol or methanol, are frequently used for the initial extraction of polyphenols from leaf and tuber material. Moreover, to improve yields, extractions are sometimes intensified with ultrasound heat or pressure. For example, Petrova and colleagues made extracts of Jerusalem artichoke flowers in 95%, 80%, and 70% ethanol applying an ultrasound bath, which produced the highest total phenolic content (TPC; 17.4 mg gallic acid equivalents (GAE) per gram fresh weight) in 70% ethanol. In a high-yielding preparative method, the 3-CQA, an isomer of considerable commercial value, was purified from leaves. The protocol contained repeated extractions in methanol (60% at 60 °C and 3×30 min), followed by column adsorption on a polar resin eluted with sequential flushes of 60–90% ethanol, resulting in a more than 5-fold enrichment and 89% yield of 3-CQA. The potential commercial value of polyphenolic substances in Jerusalem artichoke, including the CQAs, is connected to the many reported beneficial bioactivities, besides antioxidants, found in tuber and aerial organs (leaf, flower, and stems), reviewed by Lim and Yang and colleagues.

The phenolic content of ethanol extracts of tubers and leaves elicited the effects observed in anti-inflammatory and cytotoxic as well as antifungal bioassays. Although tubers of Jerusalem artichoke are considered both a valuable food and energy (inulin) crop, its cultivation in northern countries remains limited. From a bio-economy perspective, finding better uses also for the top residues or aerial parts other than as animal feed may increase the overall value of the crop.

Considering the usefulness and biological potential of phenolic acids, the objectives of the study were to assess the distribution and antioxidant activity of the major phenolic acids throughout the whole plant (tuber, stem, leaf, and flower). We also wanted to chart the boundaries of critical variables (holding time, solvent strength, and temperature) for ethanol extraction of phenolic acids from leaf material, starting out investigating whether thermal intensification could improve yields.

Results.

Phenolic Acids Extracted under Different Conditions.

For initial screening of conditions, a two-step extraction from leaves and tubers were compared for the overall yield of total phenolics. In the second step, the residue from the first step was re-extracted with a new volume of fresh solvent. Leaf extracts contained double to threefold more phenolics than the tuber extracts. For the same extractions, the effect of incubation temperature (20 °C and 60 °C) and microwaving (MW) was also tested. Temperature had a significant effect on extraction from room temperature up to 60 °C; only a marginal effect was obtained with microwave-assisted extraction. First-step extraction from leaves gave 70% of the combined yield at room temperature, and increased further to 78% at 60 °C and 82% at microwave conditions. In the continued study, all three temperature



conditions were used, but applying only single-step extraction due to the significantly higher yield obtained. *Helianthus tuberosus* L., commonly known as Jerusalem artichoke (abbreviated as JA), is an annual herb that belongs to the sunflower family, Asteraceae. This plant is well known for its resistance to environmental stress and thrives in diverse soil (arid-saline) and temperature (cold-hot) conditions. It is cultivated in most parts of the world due to its diverse habitat and resistance to both biotic and abiotic stresses. In addition to its ability to thrive, the plant is also well known for its significance in feedstock, functional food, biomass, biomedical, and chemical industries. A variety of phytochemicals such as phenols, flavonoids, coumarins, unsaturated fatty acids, polyacetylene derivatives, sesquiterpenes, protein, amino acid, reducing sugars, organic acids, lactones, cardiac glycoside, terpenoids, amino acids such as linoleic, α -linoleic acid, and inulin are produced in various parts (stems, leaves, flowers, and tubers) of the plant. The plant species grown in Japan has been presented as a reference to understand its various growth stages from sprouting to tuber formation and harvesting.

Due to this diversity in functional-molecular components, the plant plays an important role in the health sector. More specifically, the areal parts of the plant are useful in pharmaceutical applications such as antioxidant, anticancer, antifungal, antidiabetic, antimicrobial, immune stimulation, etc.,. Although the tubers also provide such benefits through direct consumption (as food or supplements), they are more useful in promoting gut bacteria and treating chronic diseases such as diabetes. Type 2 diabetes mellitus (DM2) is a common term for increased blood sugar level. It is a metabolic disorder characterized by hyperglycemic (lack of insulin) condition in the body. The condition is also associated with excessive loss of proteins, damage of several metabolic pathways and other disorders such as chronic kidney disease (CKD) and non-alcoholic fatty liver (NAFL). Most of these conditions are a result of unbalanced dietary habits that include an excessive intake of high-calorie and high-fat foods. The use of plant extracts to decrease blood sugar level has been promoted since ancient times. However, it is only recently that the constituents of the plants and their role in human disease treatment are more keenly understood through advanced omics approaches such as genomics, transcriptomics, proteomics, and metabolomics. Several metabolomics and bio-chemical studies have been performed to unravel the chemical composition of the JA plant and its tubers. However, very less is known about the proteome of the tubers. Plant proteomics provide significant insights into the biochemical pathways of essential metabolites, markers for disease treatment, as well as plant phenotype identification. Most recent proteomic studies focused on understanding the salt stress responses and molecular basis of carbohydrate metabolism in JA. The latter provides more information on the biosynthesis mechanism of inulin (a linear polymer of D-fructose) in JA. It is one of the major bioactive components produced by JA and highly abundant in its tubers. Inulin is known for promoting gut bacteria, improves glucose tolerance and liver lipid profile. Apart from Inulin, other phytochemicals such as phenolics (catechins, flavonoids, etc.) are also known to have significant roles in treating DM2 and related diseases such as chronic kidney disease (CKD) and Nonalcoholic Fatty Liver (NAFL) disease through their antioxidant properties. Therefore, investigating the JA tuber proteome might provide new insights into the protein components and their healing properties. In particular, essential pathways related to bioactive components that are useful in promoting health including fighting life-style diseases such as DM2. Importantly, the proteome



of a commercial or processed edible tuber source could help us to understand the significance of JA tubers as a functional food source.

With this background, and a first step in our research project, we decided to investigate the proteins present in the commercial edible samples (processed from tubers of the same plant species) that are available in Japan. The commonly available ready to eat (or use as additive in foods) products are first, the dried powder (as it can easily added into rice, or other food preparations) and second, dried chips that can be eaten as a healthy snack. It was also reasoned that the availability of their proteome profiles would give insight into not only common but unique proteins, which could be further used to explain the value of these products. Two different dried tuber powders (labeled as samples 1 and 2; see prepared using slightly different drying techniques were used as a comparison. The powdered extracts of all three samples (1, 2, and 3) were used to extract the total proteins that were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS), and the highly significant proteins and pathways were identified through bioinformatics analyses.

Conclusions.

Jerusalem artichoke tuber proteomes from two different processing techniques, dry powder (tuber 1 and 2), and dry chips (tuber 3), were unraveled using a high-throughput label-free LC-MS/MS-based omics technology. Out of the 3065 proteins detected, only 2967 were identified with high confidence. Among the many different proteins identified relating to health and disease, our data particularly revealed the presence of 1-SST, which is involved in inulin biosynthesis. This was a main reason that our group chose to study this plant. The proteins identified, and their classifications, suggest functions which support data (as yet unpublished) on kiku-imo: data which suggest a reduction effect on blood sugar levels, and a relation to glycated hemoglobin (Hb-A1c) in humans (Genboku Takahashi et al., n.d., unpublished data). These protein data constitute a novel experimental dataset for the tuber sample.

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