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Comparative Study of Natural Antioxidants from *Glycine max*, *Anethum graveolensand Pimpinella anisum* Seed and Sprout Extracts Obtained by Ultrasound-Assisted Extraction

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Abstract: The study aimed to evaluate the antioxidant potential of sprout and seed extracts from three species of plants, namely *Glycine max* (GMsp-sprouts, GMsd-seeds), *Anethum graveolens* (AGsp-sprouts, AGsd-seeds) and *Pimpinella anisum* (PAsp-sprouts, PAsd-seeds), which are widely accepted by consumers and have various applications in food flavoring, and also in natural medical treatments in the pharmaceutical industries. These plants are rich in valuable compounds that show a remarkable antioxidant power and are associated with many health benefits. Ethanol extracts were obtained by ultrasound-assisted extraction and they were comparatively evaluated for their in vitro antioxidant properties. The extracts were characterized by HPTLC, HPLC-DAD, total phenol content (TPC), total flavonoid content (TFC) analysis and antioxidant activities with different assays, such as total antioxidant capacity (TAC), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assay (ABTS), 1,1-diphenyl 1-2-picryl-hydrazyl (DPPH) and iron binding ability of chelators. Our results showed that the sprout and seed extracts of the studied plants exhibited a high content of phytochemicals and promising antioxidant properties. The highest polyphenols content was detected for AGsd (53.02 ± 0.57 mg/g DW), PAsd (48.75 ± 0.34 mg/g DW) and the highest flavonoids content for PAsp (26.84 ± 0.57 mg/g DW). Moreover, the presence of valuable compounds was demonstrated by using HPTLC, FT-IR and HPLC-DAD techniques. In order to have a better understanding of the relationship between the biological properties and the electronic structure, a molecular modelling study of genistein was also conducted. Our approach to the comparative assessment of these three plant species was based on a priori knowledge from literature data; however, this study demonstrated that these plant extracts of seeds and also sprouts are excellent sources of natural antioxidants. Significant additional differences that were found in the phytochemical composition could be exploited in future research for pharmaceutical purposes.

Keywords: plant extracts; antioxidants; seeds; sprouts; natural products

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1. Introduction

Many plant species have received attention as functional foods due to their important nutritional values [1–3]. For this purpose, the rich composition of certain plant seeds and sprouts in fiber, phenolic acids, flavonoids, amino acids, vitamins and trace elements has been demonstrated and the consumption of seeds and sprouts has become increasingly

popular among those interested in improving and maintaining their health by adopting an improved diet [4,5]. Recently, many researchers have investigated how germination can influence the chemical composition of seeds during germination. Predominantly, the germination process would increase total phenolic and flavonoid contents or individual phenolics, however, some seed extracts showed contrasting behavior, exhibiting a decrease in phenolics, in some cases [4]. This shows that the mechanism of phytochemical development during germination varies between plant species. Phenolic compounds are considered secondary metabolites that are synthesized by plants during normal development, or in response to stress conditions, and their antioxidant properties may draw more attention to their potential as functional foods [4,6,7]. These valuable natural sources help to reduce the risk of developing various diseases and/or exerting health-promoting effects, in addition to their nutritional values.

Glycine max (L.) (Soybean) is an important source of high-quality protein and vegetable oils and is also one of the most economically valuable crops. *G. max* is a subtropical plant native to South-East Asia, the seeds of which are known to contain important chemical components, such as proteins, isoflavonoids (genistein, daidzein and glycitein), saponins, cumestrol, lecithin, phytosterols, vitamin E and dietary fibers with multiple beneficial properties for health [6]. Over time, studies have shown that there is a major difference between the chemical composition of plant sprouts and seeds [7]. Large amounts of soybeans are consumed worldwide due to their nutritional value and health benefits. Many soybean products, such as milk, raw sprouts, processed pasta and sauces, are used as sources of protein in the human diet and a new direction has also emerged in the increasing application of soybeans in the pharmaceutical and cosmetic industries [8]. Soy isoflavones have a strong estrogenic effect in tissues by binding to estrogen receptors and, therefore, they become beneficial to women with polycystic ovary syndrome by lowering testosterone, cholesterol, insulin values, weight gain, inflammatory markers and oxidative stress [9]. The estrogenic effects on women of soy isoflavones have been reported in many studies by demonstrating a low incidence of hot flashes due to menopausal symptoms [10]. Thus, in the case of moderate to severe hot flashes, soy isoflavones could lead to a significant improvement in the quality of life of premenopausal and postmenopausal women [11]. Many organic compounds from the phytochemical composition of soybean extracts have demonstrated antidepressant properties, the mechanism of action of which is based on noradrenergic transmission influence on the serotonergic system, stimulating the noradrenergic system [12].

Anethum graveolens is a common aromatic medicinal plant that belongs to the family *Umbelliferae* (*Apiaceae*). Various compounds were identified in the chemical composition of seeds, leaves and inflorescence of this plant [13]. *A. graveolens* contains many beneficial compounds for human health, such as anethine, felandrene and D-limonene, and its leaves are rich in tannins, steroids, terpenoids and flavonoids. Dill seeds are rich in volatile compounds, flavonoids, coumarins, xanthones and triterpenes [14]. Dill is a medicinal plant with a pleasant spicy aroma, and its essential oil, and extracts from dill seeds and leaves, are widely used for flavoring food and beverages. Studies have demonstrated that they have antimicrobial and antioxidant activities, being used as natural and safe preservatives in the food and pharmaceutical industries [15]. In vivo studies have demonstrated that extracts of *A. graveolens* show a positive influence on cerebral infarction and neuronal loss in rats with ischemic-induced cerebral ischemia by reducing the oxidative state and the inflammation, along with improving cerebral blood supply [16]. Moreover, a high dose of dill extract administered to rats acted as an agent that changed the duration of the luteal phase, based on increased progesterone, without pathological changes in ovarian tissue, thus being effective in regulating menstrual irregularities, and also as a natural contraceptive [17]. Extracts of dill introduced into a suppository had a similar efficacy to clotrimazole in reducing the rate of a positive culture for *Candidiadis* and relieving the severity of clinical symptoms, this action being of great importance in the current

context in which the resistance of microorganisms to synthetic drugs has significantly increased [18,19].

Anise (*Pimpinella anisum* L.) is a delicate annual herbaceous plant with white flowers from the *Apiaceae* family. The essential oil obtained from its seeds is used in food processing, perfumes, and toothpaste, as well as in pharmaceutical products [20]. Studies on the chemical composition of *P. anisum* have focused on lipophilic compounds, especially volatile compounds, for example, *trans*-anethole. On the other hand, aqueous extracts of *P. anisum* presented biological effects, such as antioxidant, antimicrobial, and anti-HSV effects, and acted as selective modulators of estrogen receptors [21]. These extracts are found in traditional medicine in many cultures to treat various ailments and are largely recommended as antioxidants, estrogens, galactagogues [22], antimicrobials, digestives, antispasmodics, expectorants and anti-inflammatories [23,24]. *P. anisum* showed therapeutic potential for the management of depressive disorders, showing an effect comparable to fluoxetine, which is a well-known drug for this disorder [25]. In addition, anise seeds are also a good source of many essential B-complex vitamins, such as niacin, riboflavin and thiamine. Seeds are also an important source of minerals, such as calcium, copper, potassium, iron, manganese, magnesium and zinc [26].

The aim of our study was to evaluate the antioxidant potential of ultrasonic ethanolic extracts from seeds and sprouts of dill, soybean and anise, and also to compare the chemical composition of these extracts by spectroscopic, chromatographic and molecular modelling studies. This study demonstrated that the analyzed plants represent an excellent source of natural organic compounds with various biological activities that could be exploited in future studies for pharmaceutical applications.

2. Materials and Methods

2.1. General

All the reagents and organic solvents used for analysis were purchased from Sigma Aldrich and Merck (Darmstadt, Germany). Standard compounds daidzin, daidzein, genistin and genistein ($\geq$95%, HPLC grade) were purchased from Merck (Darmstadt, Germany). Absorbance measurements for antioxidant activities were done by using a multiplate reader (Tecan Pro 200, Tecan Trading AG, Männedorf, Switzerland). The seeds were collected in March, from local suppliers. The seed specimens were deposited at the Botanical Garden in Galati, Romania, until processing.

2.2. Germination

The seeds of *Glycine max* (GMsd), *Anethum graveolens* (AGsd) and *Pimpinella anisum* (PAsd), were germinated in germination boxes with 24 compartments, on sterile filter papers, cut according to the dimensions of each compartment (2 cm $\times$ 4 cm). Prior to germination, healthy and mature soybean, dill and anise seeds were sterilized, to prevent fungal growth, by washing the seeds with sodium hypochlorite (0.1%) twice for 5 min, then they were rinsed six times with sterile ultrapure water and transferred to the germinator. The grains were incubated for germination, in optimal conditions of 80% humidity and under temperature control (23 $\pm$ 2 °C), in the absence of light [27]. Germination time was varied for each species, from 4 to 7 days. The sprouts were grown in the laboratory and used for further processing in fresh condition.

2.3. Sample Preparation

The sprouts of *Glycine max* (GMsp), *Anethum graveolens* (AGsp) and *Pimpinella anisum* (PAsp) and their seeds were dehydrated (60 °C, 24 h) and ground.

Both fresh and dried samples are used in herbal studies. In most cases, the dry sample is preferred given the time required for the experimental design. Comparison between fresh and dried plant materials showed no significant effect on the chemical composition of the extracts in the total phenolic compounds, but a higher flavonoid content was noted in the dry sample. Freeze-drying is a good method of drying samples. Stove drying is another

method of preparing plant samples that uses thermal energy to remove moisture from the samples. This sample preparation is considered one of the easiest and fastest thermal processes that can preserve phytochemicals. This method reduced the extraction time. The samples used in powder form have smaller homogenized particles and this aspect leads to better contact on the surface with the extraction solvents. This special pre-preparation is important because, in order for an efficient extraction to take place, the solvent must come into contact with the target analytes and having a particle size as small as possible is ideal for efficient extraction [28–30].

Ultrasound-assisted extraction has proven to be among the most efficient methods in the extraction of plant materials, based on high efficiency, less extraction time, reduced solvent consumption and high selectivity [31,32]. This method is increasingly used in the extraction of thermolabile compounds to reduce the extraction time, the costs of the procedure and to avoid exposure to high temperatures. Ultrasonic extraction involves the use of ultrasound from 20 kHz to 2000 kHz, and can be used in both small and large scale extraction of phytochemicals, but at high frequencies the effect on active phytochemicals, through the formation of free radicals, is taken into account [31]. Ultrasound-assisted extraction is based on the mechanical effect of the acoustic cavity from ultrasound which leads to an increase in surface contact between solvents and the permeability of cell walls of plant samples. Therefore, under the action of ultrasound the physical and chemical properties of cell walls are altered, facilitating release of compounds from plant cells into the solvent [32,33]. The procedure is simple, involving relatively low-cost technology, and having high efficiency, and, therefore, it was chosen as the extraction method in this study. Solvent selection is one of the most important stages of preparation for extraction given that the selectivity of the solvent to extract the target compound from a plant material is related to the polarity compatibility of the two and the uneven distribution of phytochemicals in the plant matrix. The recovery of antioxidant compounds from plant materials is usually done by various extraction techniques, considering their chemical structure. Ethanol is a solvent that is safe for human consumption as an extraction solvent for natural substances for both food and natural medicines purposes. Absolute ethanol and aqueous ethanol have been successfully used to extract phenolic compounds and their antioxidant derivatives from natural ingredients, with remarkable results. Solvent extraction for soluble plant components is one of the safest forms of extraction, providing similar results to other commonly used methods, and is one of the least expensive methods available [34]. Thus, this solvent has proven to be easy and effective to use to produce extracts rich in antioxidants, both as a raw material for functional foods and for natural medicine. Alcoholic extraction with absolute methanol or ethanol, or aqueous solutions, has been successfully used in recent years on a large scale to extract antioxidant compounds from various plant materials (fruits, vegetables, seeds, stems, leaves, roots) [34–38]. Many studies have shown that aqueous alcohol extraction is one of the most effective extraction methods, and, due to the additional purpose of this work, which was to obtain a plant product safe for human consumption, 70% ethanol was chosen as the extraction solvent for this study. All the extraction conditions were chosen after a careful selection of data from the literature, but also to minimize the costs of extraction, solvent consumption and to facilitate the transition of these methods to the industrial scale. The powders (10 g) were extracted in 70% ethanol (100 mL) for 2 h in a temperature-controlled ultrasonic bath (40–50 °C, Bandelin Sonorex Ultrasonic, Bandelin, Berlin, Germany, operating frequency 35 kHz, with digital timer and temperature control). The resulting extracts were filtered and concentrated on a rotary evaporator.

2.4. HPTLC Analysis

Pure standard compounds (1 mg/mL) and extracts (2 mg/mL) were dissolved in ethanol. The standard compounds were: daidzin, daidzein, genistin and genistein. The system used was HPTLC CAMAG AG (Linomat 5, Dehumidifier, ADC2 Development Camera, TLC Scanner, and TLC Visualizer). The stationary phase: HPTLC Si 60 F254. A

quantity of 2 µL of each sample was sprayed in the form of bands by using Linomat 5 with compressed air, and by using a 100 µL syringe. The first optimized mobile phase used was ethyl acetate:methanol:water (6:1.1:1, *v*/*v*/*v*). The second optimized mobile phase used was toluene:ethyl acetate:formic acid:acetic acid (0.8:6:0.8:0.4, *v*/*v*/*v*/*v*). The development was carried out with Automatic Developing Chamber ADC 2, in a steam-saturated chamber, at a distance of 80 mm from the baseline. The visualization of the plate was performed with TLC Visualizer at UV 254 nm and 366 nm. The Planar Chromatography Manager winCATS was used in all steps of thin-layer chromatography [39,40].

The TLC profile of seed and sprout extracts showed that they were mixtures of spots at various R_f. These spots were separated using PTLC (Preparative thin layer glass plates, silica gel 60 matrix, binder, Polymeric, fluorescent indicator, Merck, Darmstadt, Germany) and four bands, containing the specified compounds, were obtained for each sample. These compounds were further checked for their chemical structure by FT-IR analysis.

2.5. FT-IR Analysis

The infrared spectra were collected using a Nicolet iS50 FT-IR spectrometer (Thermo Scientific, Waltham, MA, USA) equipped with a built-in ATR accessory, DTGS detector and KBr beam splitter.

A total of 32 scans were co-added over the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}. Air was taken as the reference for the background spectrum before each sample. After each spectrum, the ATR plate was cleaned with ethanol solution. In order to verify that no residue from the previous sample remained, a background spectrum was collected each time and compared to the previous background spectrum. The FT-IR spectrometer was placed in a room that was air conditioned with controlled temperature (21 ± 2 °C).

2.6. Identification of Bioactive Compounds by HPLC-DAD

HPLC-DAD (High Performance Liquid Chromatography with Photodiode Detector) analysis was performed using an L-3000 High Performance Liquid Chromatography System (Rigol Technologies Inc., Beijing, China). The Kinetex EVO C18 column (1504.6 mm, 5 m particle size), with an injection volume of 10 µL, was used in the chromatographic analysis. The solvents used were (A) trifluoroacetic acid (TFA) 0.1% in water and (B) trifluoroacetic acid (TFA) 0.1% in acetonitrile. The degree of elution was 2% to 100% B at 30 °C for 60 min, and the rate of elution was set at 10 µL/min. Wavelengths of 230, 250 and 280 nm, respectively, were used to detect the organic compounds.

2.7. Total Polyphenol Content (TPC)

The determination of the total polyphenol content was performed according to a previously published method, adapted to microplate reader with 96-well plates (Tecan Pro 200, Tecan Trading AG, Männedorf, Switzerland) using Folin-Ciocalteu reagent [41,42]. The Folin-Ciocalteu reagent is a mixture of phosphomolybdic acid ($H_3PMo_{12}O_{40}$) and phosphotungstic acid ($H_3PW_{12}O_{40}$) that reacts with phenols and non-phenolic reducing substances to form chromogens which can be detected spectrophotometrically, since in alkaline conditions the oxotungstate and oxomolybdate formed in this redox reaction show a blue coloration proportional to the concentration of polyphenols [43,44]. Briefly, in this method 10 µL of sample were incubated with 25 µL of Folin-Ciocalteu reagent for 5 min, then 25 µL of 20% Na_2CO_3 and 140 µL of ultrapure water were added and incubated for 30 min at room temperature. A blank sample was prepared by replacing Folin-Ciocalteu reagent with ultrapure water. All experiments were performed in triplicate. Absorbance was recorded at 760 nm. For the standard curve, freshly prepared gallic acid solutions were used, with known concentrations (0.9–500 µg/mL), and the results were expressed in mg of gallic acid equivalents (GAE)/g of dry weight.

2.8. Total Flavonoids Content (TFC)

Total flavonoid content was performed according to a previously published method [41,45]. Briefly, 100 µL of sample were incubated with 100 µL of 2% methanolic solution of aluminum chloride at room temperature for 15 min, after which absorbance at 415 nm was recorded with a microplate reader with 96-well plates (Tecan Pro 200, Tecan Trading AG, and Männedorf, Switzerland). Quercetin was used as a reference standard (0.078–40 µg/mL) to quantify the total flavonoid content. All experiments were performed in triplicate and the results were expressed as µg quercetin equivalent (QE)/mg dry weight.

2.9. Total Antioxidant Capacity (TAC)

Total antioxidant capacity (TAC) of plant extracts and sprouts were evaluated by the Prieto et al. phosphomolybdenum microspectrophotometric modified method [46]. This technique is based on the formation of a blue/Mo (V) phosphate complex at acidic pH by reducing molybdenum Mo (VI) to Mo (V). An aliquot of 0.5 mL of samples solution was combined with 0.5 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). In the case of the blank, 0.5 mL of PBS was used in place of sample. The tubes were incubated in a boiling water bath at 95 °C for 90 min. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each sample was measured at 695 nm against the blank in a UV-2450 spectrophotometer (Tecan Pro 200, Tecan Trading AG and Männedorf, Switzerland). The total antioxidant activity was expressed as the absorbance of the sample at 695 nm. The higher absorbance values revealed higher antioxidant activity of the extracts.

2.10. Free Radical Scavenging Activity DPPH

The antioxidant effects of plant extracts and sprouts were analyzed by the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging test [47]. This method is based on a color change from purple to yellow, resulting in a reduced form of DPPH radical. A quantity of 100 µL of the sample were mixed with 100 µL of a methanolic solution of DPPH in a 96-well plate and the absorbance values were recorded at 517 nm after specific periods of time with a microplate reader (Tecan Pro 200, Tecan Trading AG and Männedorf, Switzerland). Gallic acid and ascorbic acid were used as references at different concentrations. All experiments were performed in triplicate. The percentage of DPPH inhibition was calculated using the following formula:

$$\% \text{ Inhibition of DPPH radical} = (1 - A_s/A_c) \times 100 \quad (1)$$

where A_c is the absorbance of the control and A_s is the absorbance of the sample.

2.11. ABTS Radical Cation Decolorization Assay

This assay was determined as previously described in the literature with slight modification [48].The $ABTS^{\bullet+}$ solution was prepared and then stored for 16 h in dark conditions at room temperature, then diluted with absolute ethanol until the absorbance at 734 nm was 0.700 ± 0.02, then used as $ABTS^{\bullet+}$ reagent. The absorbance of the final reaction solution was measured at 734 nm in a 96-well plate, by using a microplate reader (Tecan Pro 200, Tecan Trading AG and Männedorf, Switzerland). A volume of 100 µL of each extract was mixed with 100 µL of $ABTS^{\bullet+}$ reagent and the absorbance was measured after 30, 60 and 90 min. The results were expressed using a Trolox calibration curve (calibration curve at different concentrations in the range of 50–0.25 µM). All experiments were performed in triplicate.

2.12. Determination of Iron Binding Ability of Chelators

As previously described in the literature, ferrous ion was monitored by measuring the formation of a red ferrozine-Fe^{2+} complex at 562 nm, as previously described in other studies [41,49–51], adapted to the microplate analysis. If the ferrous-ferrozine ion complex

was formed, the absorbance at 562 nm would be increasing. A sample volume of 225 µL was mixed with 10 µL of ferrous sulfate (2 mM), then stored at room temperature for 5 min at room temperature and 15 µL (0.2 mM) of ferrozine was added to start the reaction. The resulting mixture was left for 10 min at room temperature and then the absorbance was measured at 562 nm. Na_2EDTA was used as a positive control. For the blank sample, distilled water was used. All experiments were performed in triplicate. Iron chelating ability was calculated using the formula given below:

$$\text{Iron binding ability (\%)} = [(A_0 - A_1)/A_0] \times 100 \quad (2)$$

where A_0 is the absorbance of the ferrozine-Fe^{2+}complex and A_1 is the absorbance of the tested compound.

2.13. Molecular Modelling Study of Genistein

The geometry optimization of genistein compound in the gas phase was carried out by using the Gaussian 09 program, Inc.: Wallingford, CT, USA) [52] at DFT/B3LYP/6-311G(d,p) level of theory. Also, by computing the IR frequencies we found that the optimized geometry of genistein was located at the minimum on the potential energy surface because it did not yield any IR imaginary frequency.

In order to verify the performance of the DFT method used for molecular modelling we compared the theoretical parameters, i.e., bonds length and angle, of the optimized molecular structure of genistein with the experimental XRD parameters [53] of the same chemical compound (CCDC number-677691).

The chemical potential of genistein compound was evaluated using a series of electronic parameters, such as the dipole moment (DM), the energy of the highest occupied molecular orbital (E_{HOMO}) and the energy of the lowest unoccupied molecular orbital (E_{LUMO}), which were extracted from the .log output file of the Gaussian 09 program, and the gap energy (Egap), the ionization potential (IP), the electron affinity (EA), the chemical hardness (η), the chemical softness (σ), the electronegativity (χ) and electrophilic index (ω), which were calculated using specific formulas [54–56]. The .chk output file of the Gaussian 09 program was used as the input file for Avogadro program (Compiling Avogadro on Linux, http://avogadro.openmolecules2012, accessed on 18 March 2022) to represent the frontier molecular orbitals i.e., HOMO and LUMO, and the molecular electrostatic potential (MEP) map of the genistein compound.

3. Results and Discussion

3.1. Germination

The seeds of the three analyzed species were considered sprouts after 10 days if they had radicles of at least 2 mm in length (Figure 1). These were taken over to the extraction step and subsequent analysis.

(**a**) (**b**) (**c**)

Figure 1. Sprouts of the analyzed plants (**a**) *Anethum graveolens* (AGsp), (**b**) *Glycine max* (GMsp), and (**c**) *Pimpinella anisum* (PAsp).

3.2. High Performance Thin Layer Chromatography Analysis (HPTLC)

Thin layer chromatography is known as a characterization method of the chemical composition of certain plant seeds, sprouts, fruits or leaves [57]. This method is useful, not only in identification, discrimination and quality control of certain species, but also because it provides primary information for identification, isolation, purification and characterization of various organic compounds [57,58]. The HPTLC fingerprint has proven to be a reliable and precise tool for identifying and demonstrating the presence of many compounds from plant extracts [59]. Thus, in the present study, HPTLC was used as separation and identification method for daidzin, daidzein, genistin and genistein in the analyzed samples. The mobile phase was selected after optimization steps for a good, clear and easily identifiable migration of the organic compounds. Genistin and daidzin were identified by using the first mobile phase, mentioned in materials and methods, but genistein and daidzein migrated too close to the finish developing line, so another mobile phase was used in order to identify their presence in the analyzed samples. In Figures 2 and 3 the HPTLC chromatograms at 254 nm of the extracts and the four standard pure compounds are represented. It was observed that in all the analyzed extracts from seeds and sprouts, the four isoflavone compounds of major interest could be identified.

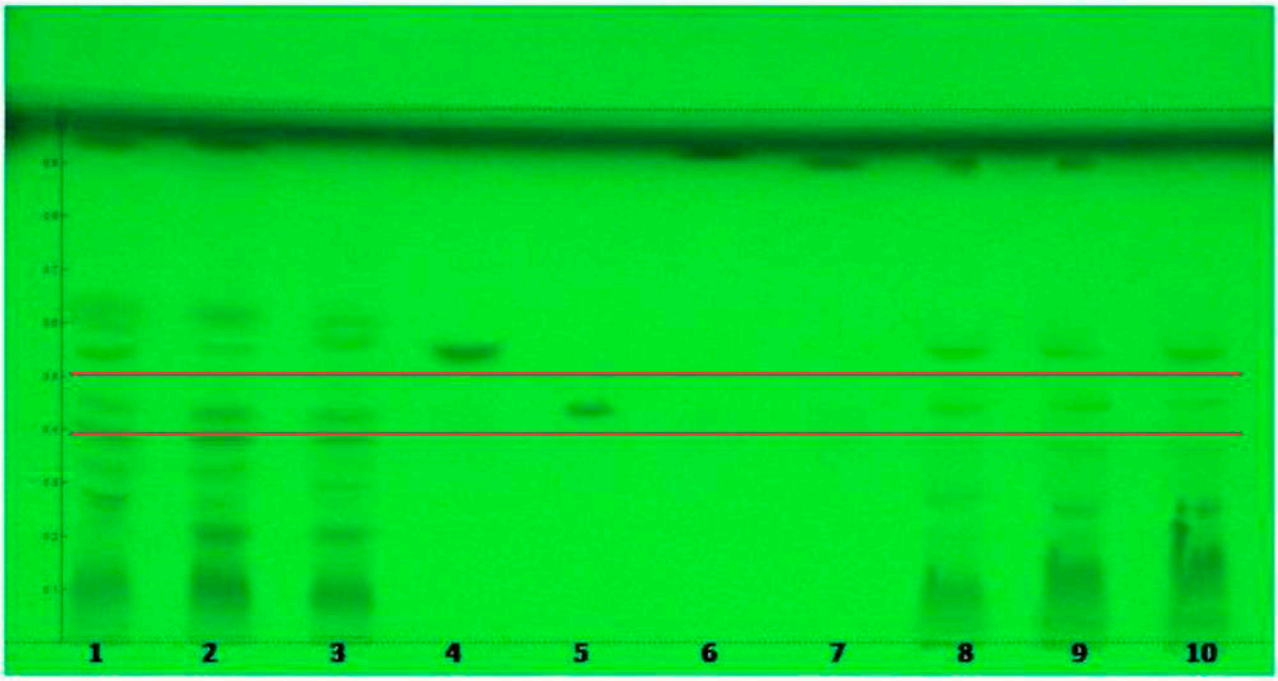


Figure 2. HPTLC chromatogram at 254 nm, where 1-GMsp, 2-AGsp, 3-PAsp, 4-genistin, 5-daidzin, 6-genistein, 7-daidzein, 8-GMsd, 9-AGsd, 10-PAsd.

The most intense spots corresponding to daidzin were identified especially in the sprout extracts, namely GMsp and PAsp, followed by PAsd. The spots corresponding to genistin could be identified in the sprout extracts of all the analyzed plant species.

In the second chromatogram daidzein and genistein were separated from the extracts and could be identified in greater concentration in anise seeds, soy seeds and dill sprout extracts. Therefore, the presence of these compounds in the analyzed samples was confirmed, and was in accordance with the results from the literature [60–62].

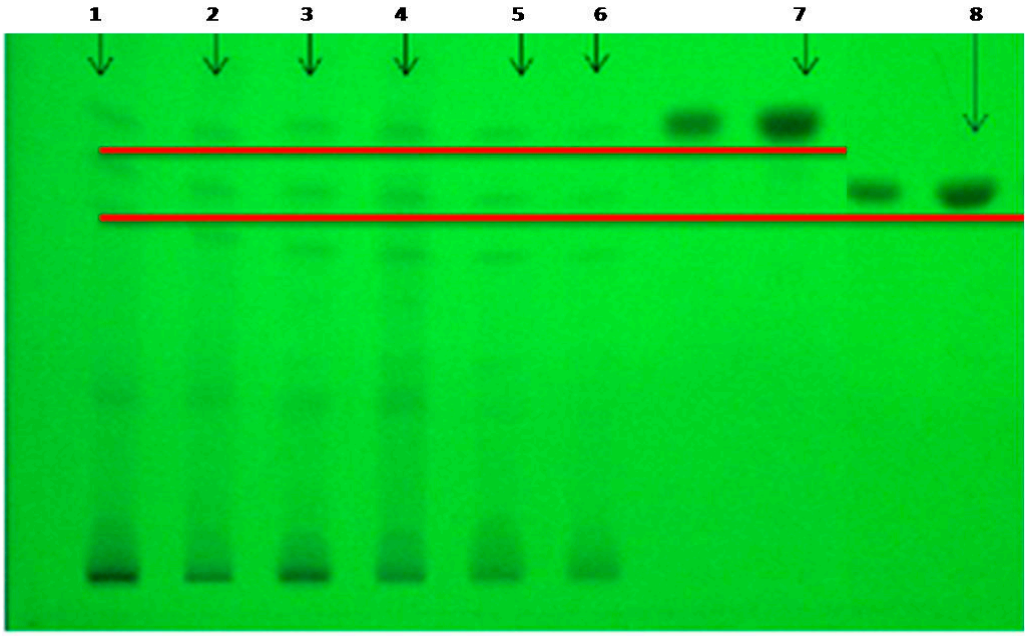


Figure 3. HPTLC chromatogram at 254 nm, where 1-GMsd, 2-GMsp, 3-AGsd, 4-AGsp, 5-PAsd, 6-PAsp, 7-daidzein, 8-genistein.

3.3. FT-IR Spectra

The characterization of active compounds, based on their functional groups, was possible by Fourier transform infrared spectroscopy (FT-IR) and, at the same time, valuable information about their chemical composition was obtained by comparing the extracts spectra with those of the standard compounds. The ATR-FTIR spectra, in a range from 4000 to 400 cm^{-1}, of pure daidzin and genistin, and also the samples, are shown in Figure S1a–e.

In all analyzed samples the IR absorption bands characteristic of the functional groups of daidzin and genistin could be identified. Bands at 3500–3300 cm^{-1}, related to the asymmetric and symmetrical stretching vibrations of the hydroxyl groups, were identified, and also bands related to the vibration of the C=O groups at 1700–1600 cm^{-1}. The bands at 2980–3100, and also 1400 cm^{-1}, could be due to the stretching vibrations of the aromatic bonds υ-C=C-H [61,63]. The bands appearing in the region of 1000–1100 cm^{-1} appeared due to O-C vibrations bond (Table 1).

Table 1. The experimental FTIR-ATR bands (cm^{-1}) and their functional groups assignments.

Genistin	Daidzin	PAsd	AGsd	GMsp	PAsd	AGsp	GMsp	Corresponding Functional Groups
IR (ATR, $cm^{We\ d}$) Bands								
3325.72 $_{s}$	3320.01 $_{s}$	3331.43	3320.10	3321.20	3336.78 $_{s}$	3286.57 $_{s}$	3288.10 $_{s}$	υ–O-H
2972.93 $_{m}$	2944.06 $_{m}$	2944.72	2944.06	2981.61	2916.32	2917.38 $_{m}$	2917.38	υC-H
1652.96 $_{w}$	1653.14	1653.00	1653.14	1640.02	1633.98	1743.61	1634.57	υC=O
1418.82 $_{w}$	1414.28	1410.66; 1448.87	1414.28; 1448.75	1417.78	1412.30	1457.03	1406.89	υ-C=C-H, aromatic
1045.28 $_{s}$	1020.93	1019.44	1020.93	1044.23; 1084.92	1070.48	1057.81	1067.67	υ –O-C

PA = *Pimpinella anisum*, AG = Anethum graveolens, GM = *Glycine max*, sd = seeds, sp = sprouts.

3.4. Identification of Bioactive Compounds by HPLC-DAD

Table 2 shows the analytical data obtained from HPLC indicating different polyphenols and flavonoids, but the presence of the isoflavonoids, daidzein and genistein, was noted in all samples.

Table 2. Chemical composition of seed and sprout extractsof *Glycine max*, *Anethum graveolens* and *Pimpinella anisum* (expressed as mg/kg ± standard deviation).

Compounds	RT	GM-sd	GM-sp	AG-sd	AG-sp	PA-sd	PA-sp
Hydroxybenzoic acids							
Gallic acid	4.40	n.d.	n.d.	n.d.	n.d.	579.65 ± 1.84	n.d.
Catechin	19.02	n.d.	30.45 ± 0.77	n.d.	n.d.	n.d.	n.d.
Epicatechin	23.29	n.d.	29.44 ± 0.79	n.d.	n.d.	370.99 ± 1.62	n.d.
Hydrocinnamic acids							
Chlorogenic acid	20.50	n.d.	n.d.	n.d.	n.d.	886.88 ± 3.31	632.38 ± 5.76
Acid tanic	2.36	763.18 ± 1.6	39.64 ± 1.17	n.d.	n.d.	96.68 ± 1.7	40.00 ± 3.78
p-Coumaric acid	28.90	n.d.	128.37 ± 1.32	n.d.	n.d.	n.d.	n.d.
Flavonols							
Rutin	44.71	86.19 ± 0.98	42.01 ± 0.44	n.d.	n.d.	661.58 ± 0.71	n.d.
Quercetin	38.03	n.d.	-	491.65 ± 2.23	n.d.	341.08 ± 2.34	243.07 ± 2.56
Quercetin-3-galactoside	29.13	12.76 ± 3.22	76.09 ± 1.08	-	n.d.	231.62 ± 1.55	76.62 ± 1.06
Flavanone							
Naringenin	38.95	505.86 ± 1.11	n.d.	380.53 ± 1.87	n.d.	n.d.	82.69 ± 3.9
Naringin	30.74	36.17 ± 0.76	12.51 ± 1.14	n.d.	n.d.	n.d.	803.07 ± 2.03
Isoflavone							
Daidzein	25.57	110.18 ± 0.98	303.86 ± 2.8	633.69 ± 0.83	750.76 ± 2.29	349.72 ± 5.6	537.37 ± 6.36
Genistein	38.79	39.02 ± 1.22	102.41 ± 2.5	573.74 ± 2.76	698.21 ± 3.46	101.39 ± 2.16	520.36 ± 2.16

RT = retention time, n.d. = not detected, PA = *Pimpinella anisum*, AG = *Anethum graveolens*, GM = *Glycine max*, sd = seeds, sp = sprouts.

Other studies have already identified different bioactive compounds in the seed and sprout extracts of these three plants [19,25,63,64]. However, our study investigated, and comparatively highlighted, the fact that extracts obtained from seeds or sprouts significantly influenced their chemical composition. Daidzein was found in the largest quantity in sprouts of all the analysed plants, of which sprouts of *Pimpinella anisum* contained the largest quantity (537.3778 ± 6.36 mg/kg), followed by *Anethum graveolens* sprouts (75.0768 ± 2.29). The biggest quantity of genistein was found in *Anethum graveolens* seeds (573.7410 ± 2.76 mg/kg). To our knowledge, there has thus far been no such study for the three studied species. This study confirmed the chemical composition of soy, dill and anise rich in daidzein, genistein, rutin, naringenin, quercetin-3-galactoside and tannic acid [64–69]. *G.max* seed extracts showed a similar chemical composition with those from sprouts. In the case of *Anethum graveolens* and *Pimpinella anisum* the chemical composition showed significant differences between seed and sprout extracts. The extracts were further evaluated by antioxidant assays.

Flavonoids are among the most explored classes of natural compounds of phenylpropanoid-derived plant specialized metabolites, structurally consisting of two main groups, 2-phenylchromans and 3-phenylchromans, with approximately 10,000 different members [70]. Isoflavonoids have become a class of interest due to numerous researches into their positive effects on human and animal health, such as antioxidant, antimutagenic, estrogenic [71], antiproliferative and anticancer effects [65,72]. Usually they are known as dietary antioxidants that play an important role in human nutrition as natural chemicals that promote health [73].

Isoflavones are considered the most estrogenic compounds [74] and the most representative among this class are daidzein, genistein, biochanin A, formononetin and glycitein [75]. Findings have shown that traditional herbal formulations have positive effects in ameliorating menopausal symptoms and improving the quality of sleep and also the quality of life [76]. The phytoestrogens daidzein and genistein, having a structural resemblance to

17-β-estradiol, can protect against a lot of illnesses, including various forms of breast, prostate or bowel cancer, cardiovascular diseases, and osteoporosis and can relieve menopausal symptoms, as illustrated in Figure 4 [11,77–79].

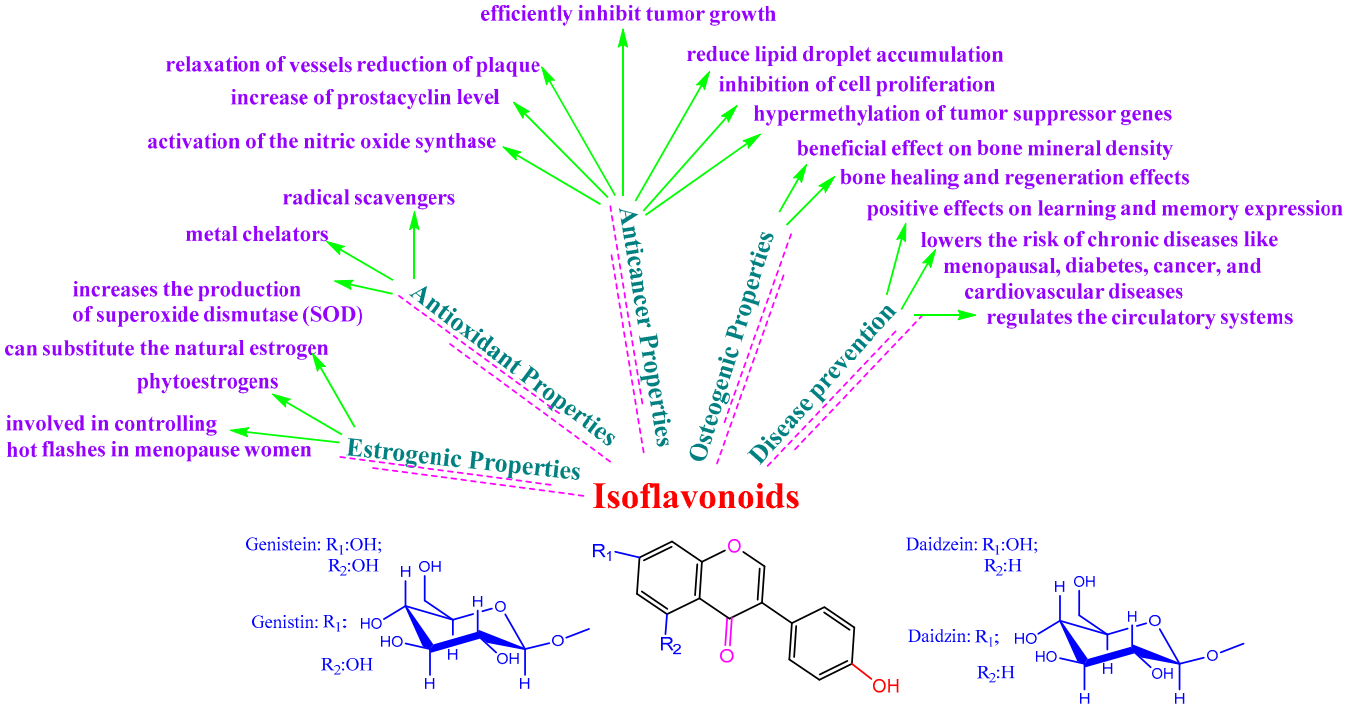
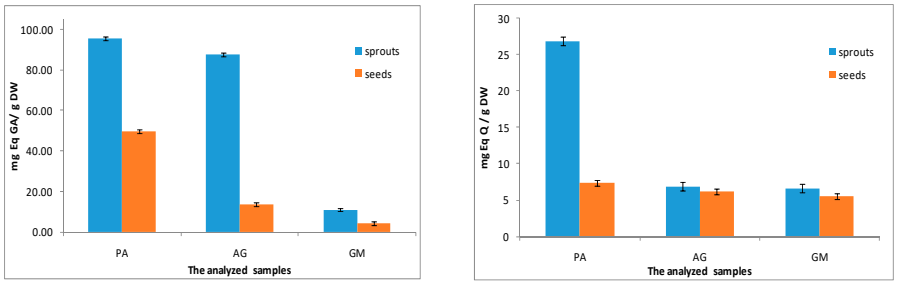


Figure 4. The chemical structures of some phytoconstituents of *Glycine max*, *Anethum graveolens* and *Pimpinella anisum* and their role in human health, according to literature data [11,77–79].

3.5. Total Polyphenol Content

In Figure 5a the total phenolics content of the tested samples, determined by Folin–Ciocalteu colorimetric method, is represented. The good concentration found in PAsd and AGsd samples, expressed as 49.74 ± 0.61 mg and 13.02 ± 0.79 mg GAE/100 mg in the extract samples, accorded well with literature studies, and the amount of polyphenols in dill seeds coincided with the data obtained [80]. Studies on different types of soybean seeds have shown the presence of polyphenols in quantities between 2.68 ± 0.47 and 6.22 ± 0.68 mg of GAE/g of dry material [81]. The amounts of polyphenols in the studied soybeans were similar to previous studies reported in the literature. Our data correlated with the results obtained from chromatographic results.



(**a**) (**b**)

Figure 5. (**a**) Total polyphenol content of seed (sd) and sprout (sp) extracts (PA = *P. anisum*, AG = *A. graveolens*, GM = *G. max*). The results were expressed as mg gallic acid equivalents/g of dry weight (Eq GA/g DW); (**b**)Total flavonoids content of tested samples. The results were expressed as mg quercetin equivalents /g of dry weight (mg Eq Q/g DW). Error bars represent ± standard deviation of 3 replicates.

3.6. Total Flavonoids Content (TFC)

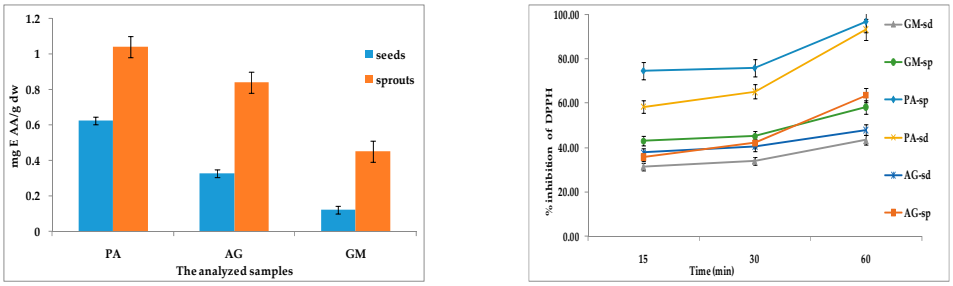
The flavonoid contents ranged from higher contents, such as 26.83 ± 0.92 mg EqQ/g DW in sprouts extract of dill (PAsp), to lower ones 5.55 ± 0.42 mg EqQ/g DW in *Glycine max* (GMsd) seeds extract (Figure 5b). Other studies also showed the presence of small amounts of flavonoids in soybeans [81]. Our results were in agreement with Aly et al. research where anise seed extracts showed a great flavonoid content, ranging between 65.9 ± 0.9–46.8 ± 0.64 mg CAT Eq/g DW [82]. In Figure 5b the flavonoids content values in all analyzed samples are presented, which confirm the HPLC results, in which in all samples great amounts of isoflavonids, namely daidzein, genistein, daidzin and genistin, were identified.

Previous studies of dill seed extracts have shown flavonoids content of 5.07 ± 0.38 mg EqQ/g dw [83], which were lower than those from the present study (6.13 ± 0.69 mg EqQ/g DW for seeds (AGsd) and 6.90 ± 0.75 mg EqQ/g DW for sprouts (AGsp)).

3.7. Antioxidant Activity

3.7.1. Total Antioxidant Capacity (TAC)

The antioxidant capacity of plants has attracted considerable interest, since antioxidants exert protective properties against numerous pathologies. Therefore, this assay revealed the antioxidants capacity of seed and sprout extracts of the studied species. Our results demonstrated that all the extracts had good antioxidant activities, based on the reduction of the molybdenum cation. The soybean extract (GMsp) registered a slightly lower activity 0.45 ± 0.31 mg Eq AA/gD W (Figure 6a). Results were expressed as equivalents of ascorbic acid.



(**a**) (**b**)

Figure 6. (**a**) TAC assay of seed (sd) and sprout (sp) extracts (PA = *P. anisum*, AG = *A. graveolens*, GM = *G. max*). The results were expressed as mg ascorbic acid equivalents/g of dry weight (E AA/g DW); (**b**) DPPH percent inhibition of 20 µg/mL seeds (sd) and sprouts (sp) extracts (PA = *P. anisum*, AG = *A. graveolens*, GM = *G. max*). The error bars represent ± standard deviation of 3 replicates.

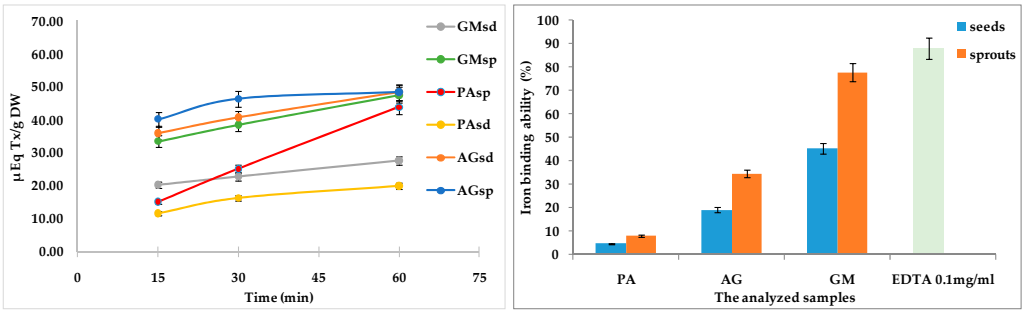
3.7.2. Free Radical Scavenging Activity DPPH

The radical DPPH is sensitive enough to detect active compounds at low concentrations. Thus, the study of ethanolic extracts has demonstrated a growing ability to scavenge the DPPH radical in time, especially seed and sprout extracts of *P. anisum* (PAsp). Seed extracts of *G. max* (GMsd) showed the lowest scavenging activity of the DPPH radical (Figure 6b).

The results of a study analyzing antioxidant activity of the aqueous and alcoholic extracts of anise and dill [84] showed that the given extracts have strong free radical scavenging effects, this was also highlighted in our study, especially for the anise extracts (PAsd, PAsp). The antioxidant activities of the Indian soybean varieties and the Bulgarian soybean varieties registered a great antioxidant activity at the same concentration by reducing the DPPH radical in [52] by 63–89.40% ± 0.31 [64]. Similarly, results were obtained in the present study with 31.36–43.36% in the case of seeds (GMsd) and better activity of 42.9–58.16% ± 0.49 for sprouts (GMsp).

3.7.3. ABTS Radical Cation Decolorization Assay

The $ABTS^{\bullet+}$ test is a useful tool for determining the antioxidant activity of hydrophilic and lipophilic compounds. The ABTS radical cation reacts rapidly with antioxidant compounds, due to its solubility in both organic and aqueous solvents. All extracts showed over time a good percentage of ABTS inhibition at a concentration of 20 µg/mL. Seed and sprout extracts of *Pimpinela anisum* (Figure 7a) showed high antioxidant capacity, inhibiting the $ABTS^{\bullet+}$ with 48.43 ± 0.33 µEqTx/gDW and 48.41 ± 0.46 µEqTx/gDW.



(**a**) (**b**)

Figure 7. (**a**) ABTS radical scavenging activity of 20 µg/mL seed (sd) and sprout (sp) extracts. The results were expressed as µM trolox equivalents/g of dry weight (µEq Tx/g DW); (**b**) Iron binding ability of seeds (sd) and sprouts (sp) extracts. PA = *P. anisum*, AG = *A. graveolens*, GM = *G. max*. The error bars represent ± standard deviation of 3 replicates.

It has been shown that at the beginning of maturity, soybean seeds have a stronger antioxidant potential than in the period of growth from the beginning of complete seeds to full maturity [85]. Thus, soybeans have a promising antioxidant potential compared to mature seeds. All extracts showed over time a good percentage of $ABTS^{\bullet+}$ inhibition at a concentration of 20 µg/mL.

3.7.4. Iron Binding Ability of Chelators

In our study, the reducing iron chelating power of seed and sprout extracts were compared to that of a known strong reducing iron chelator, Na_2EDTA (Figure 7b).

Seed and sprout extracts of *G. max* exhibited high iron binding abilities of 77.64% ± 0.53 and 45.02% ± 0.64, respectively, as compared to the standard Na_2EDTA, which had a chelating activity of 99.89% ± 0.35. Data from other studies have shown that anise extracts present a high capacity for iron binding, suggesting that their action as oxidation protectors may be related to their ability to bind iron [86]. Seed and sprout extracts of *P. anisum* (PAsd, PAsp) had relatively low iron binding abilities of 7.97% ± 0.44 and 4.75% ± 0.72, respectively.

3.8. Molecular Modelling Study of Genistein

Regarding the 3D orientation of the optimized molecular structure of genistein (Figure 8), we may specify that the basic chemical skeleton, i.e., the benzopyran, is plane and the phenolic group bound to the benzopyran structure in C3 position is rotated with a torsion angle τ(C8-C9-C11-C12) of 139.31°. Also, there is an intramolecular hydrogen bond (1.68 Å length), which is formed between the hydrogen H25 from the hydroxyl group (O20-H25) at C5 position and the O18oxygen from the carbonyl group (C10=O18).

The theoretical and experimental values of geometrical parameters, i.e., bond lengths and bond angles of the genistein compound, are presented in the Table 3. For a better visualization of the differences between the theoretical and experimental values of geometrical parameters, we plotted the data as graphs, namely, Figure 9a for bonds length and Figure 9b for bonds angle.

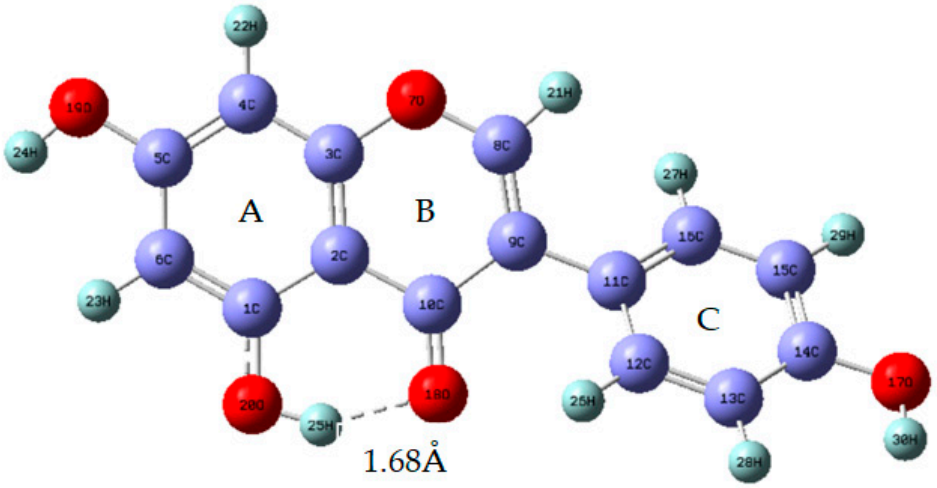
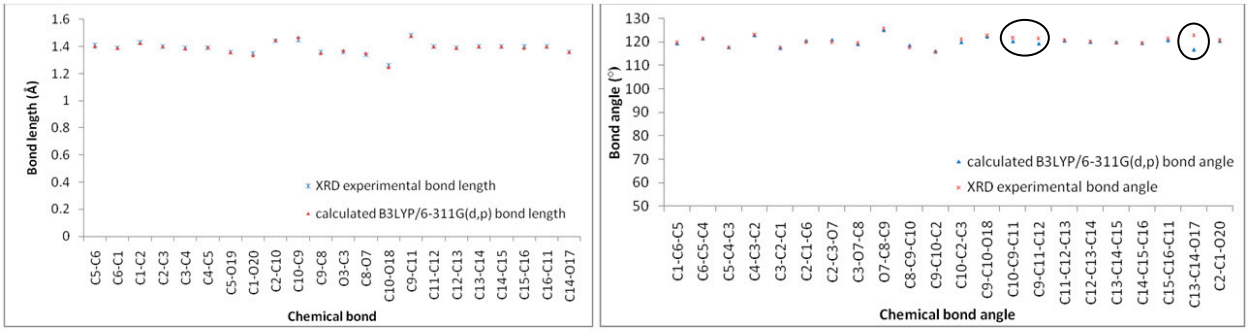


Figure 8. The optimized molecular structure of genistein compound.

Table 3. Theoretical and experimental values of geometrical parameters of the genistein compound.

Chemical Bond	XRD Bond Length [64] (Å)	Theoretical Bond Length (B3LYP/6-311G(d,p)) (Å)	Chemical Bond Angle	XRD Bond Angle [64] (°)	Theoretical Bond Angle (B3LYP/6-311G(d,p)) (°)
C5-C6	1.41	1.4	C1-C6-C5	119.5	120.02
C6-C1	1.39	1.39	C6-C5-C4	121.5	121.59
C1-C2	1.43	1.425	C5-C4-C3	117.9	117.67
C2-C3	1.4	1.4	C4-C3-C2	122.8	123.11
C3-C4	1.39	1.385	C3-C2-C1	117.6	117.71
C4-C5	1.39	1.395	C2-C1-C6	120.6	119.89
C5-O19	1.36	1.357	C2-C3-O7	120.9	119.9
C1-O20	1.35	1.335	C3-O7-C8	119.2	119.5
C2-C10	1.44	1.45	O7-C8-C9	125	125.92
C10-C9	1.45	1.47	C8-C9-C10	118.6	117.78
C9-C8	1.36	1.35	C9-C10-C2	116.2	115.64
O3-C3	1.36	1.37	C10-C2-C3	120	121.19
C8-O7	1.34	1.35	C9-C10-O18	122.3	122.75
C10-O18	1.26	1.245	C10-C9-C11	120.4	121.9
C9-C11	1.48	1.48	C9-C11-C12	119.4	121.49
C11-C12	1.4	1.4	C11-C12-C13	120.6	120.94
C12-C13	1.39	1.39	C12-C13-C14	120.1	120.34
C13-C14	1.4	1.4	C13-C14-C15	120	119.54
C14-C15	1.4	1.4	C14-C15-C16	119.6	119.71
C15-C16	1.4	1.39	C15-C16-C11	120.8	121.62
C16-C11	1.4	1.4	C13-C14-O17	116.9	122.85
C14-O17	1.36	1.36	C2-C1-O20	120.5	120.74

Analyzing these graphs, we noticed a very good agreement between the theoretical and experimental values of geometrical parameters with the specification that this agreement was better for the bonds length (root mean square error, i.e., RMSE = 0.00835) than for the bonds angle (RMSE = 1.4868).The slight differences between the theoretical and experimental bonds angles were due to the fact that the XRD data was obtained for solid phase (single crystal), while the theoretical data was computed for the molecule in gaseous phase. Therefore, we may conclude that the B3LYP/6–311G (d,p) method used for the molecular modelling of genistein was very suitable.



a) b)

Figure 9. Theoretical and XRD [53] experimental values of the bonds length (**a**) and the bonds angle (**b**) of the genistein compound. The circles show the bonds angles which have low differences between their theoretical and experimental values.

In order to characterize the genistein compound in terms of chemical stability/reactivity, we evaluated the values of the computed electronic parameters, i.e., the energy gap, the chemical hardness, the chemical softness, the electronegativity and the electrophilicity index, which are shown in the Table 4. The Egap, energy gap, helped us to characterize the chemical reactivity, optical polarizability and chemical hardness–softness of a compound. Thus, if the energy gap decreased, the reactivity of the molecule increased, due to the fact that the electron donating efficiency also increased. Therefore, a lower energy gap leads to an enhancement of the antioxidant properties of a compound [55,56]. In the case of genistein, the value of the E_{gap} parameter, of 4.18 eV, indicated the fact that the genistein was a compound with a medium chemical reactivity and an important antioxidant character.

Table 4. Quantum chemical parameters of genistein compound.

Chemical Compound	Dipole Moment (D)	E_{HOMO} (eV)	E_{LUMO} (eV)	E_{gap} (eV)	IE (eV)	EA (eV)	η (eV)	σ $(eV)^{-1}$	χ (eV)	ω (eV)
genistein	1.29	−5.95	−1.77	4.18	5.95	1.77	2.09	0.24	3.86	3.56

The electrophilicity index supplies us information about the electrophilic or nucleophilic character of a molecule. More exactly, the electrophilicity index gives a measure of the energy stabilization of a molecule when it acquires an additional amount of electron density from the environment. Strong electrophiles are the molecules which present an electrophilicity index higher than 2.0 eV [56]. In our case, we remarked that the genistein had a strong electrophilic character, the value of the electrophilicity index being 3.56 eV (Table 4).

The dipole moment is a quantum parameter related to the charge distribution (charge separation) in a molecule. The magnitude of the dipole electric moment influences the ability of a molecule to pass through a cellular membrane [55]. A recent research paper [68], revealed that there is a relationship between the dipole moment and biological activity (antibacterial, antifungal, anticancer etc.) of a molecule, but whether it is a positive or negative dependence has still not been clear elucidated. Our studied molecule presented a small dipole moment namely, 1.29 D.

It is known that the degree of conjugation and the planarity/non-planarity of a molecule have a great influence on the electric charge distribution of the frontier molecular orbitals [55]. In the case of genistein, the electric charge distribution of the HOMO orbital is along the entire molecular structure (Figure 10) due to the presence of the C2=C3 double bond, which allows a more pronounced delocalization of the electrical charge when switching from the HOMO molecular orbital to the LUMO molecular orbital. In the case of the LUMO molecular frontier orbital, the electric charge distribution is located only on the benzopyran structure (Figure 10).

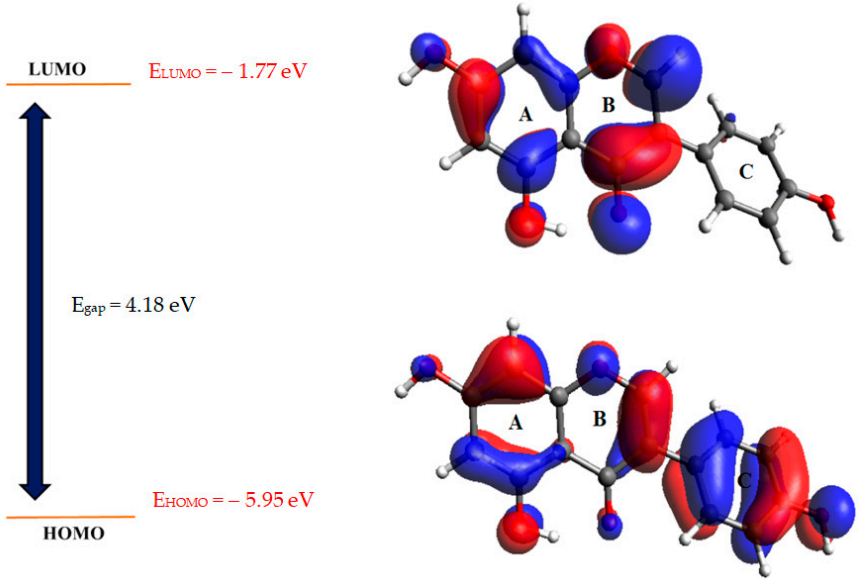


Figure 10. Charge distribution of HOMO and LUMO molecular orbitals in the optimized genistein structure.

The molecular electrostatic potential (MEP) diagram of the studied compound (Figure 11) presents the regions with negative electrostatic potential in red and positive electrostatic potential in blue. In the case of the genistein compound, we found five negative regions located around the oxygen atoms which belong to the carbonyl group, hydroxyl groups and pyranic ring, these regions being active sites for possible electrophilic attacks. The negative regions were localized around all the hydrogen atoms and were active sites for possible nucleophilic attacks.

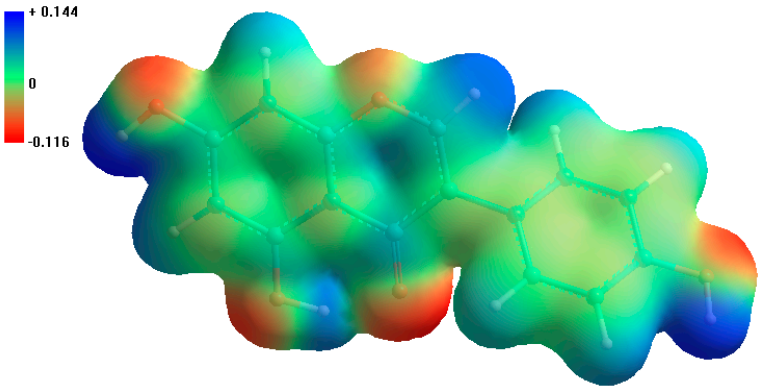


Figure 11. Molecular electrostatic potential 3D—diagram of genistein.

This study is part of our ongoing research. Therefore, these results will be used to study the interaction of genistein with enzymes involved in various biological activities.

4. Conclusions

In conclusion, our results provide evidence that ethanol extracts obtained from the three plant species are rich in antioxidant compounds and could draw more attention to their potential as functional foods or herbal medicines. In all the analyzed extracts, daidzin, daidzein, genistin and genistein could be identified, but in the sprout extracts it was found that these compounds appeared in higher concentrations than in seeds. The germination process can influence the chemical composition of the seeds during germination, leading to a variation in the total content of phenols and flavonoids or individual phenolic compounds, the mechanisms of phytochemical development during germination being varied between

plant species. Isoflavones are valuable plant secondary metabolites that play multiple essential roles in plants, as well as for human health. It has also been shown that all extracts of the analyzed species had a high antioxidant potential, due to their chemical composition being rich in polyphenols and flavonoids. Our preliminary experimental data show that these extracts have pronounced antixanthine-oxidase and antidiabetic activity (ongoing research, unpublished). Therefore, we are in the process of molecular docking of these isoflavonoids with xanthine-oxidase and amylase. Given the great need to develop alternative treatments against current emergent diseases, such as cancer, COVID-19, diabetes or hormonal dysfunctions, traditional medicine and nutrition should play a major contribution, either by improving diet or by introducing effective pharmaceutical herbal formulations. Future work will focus on shaping this attractive avenue by demonstrating the synergic or antagonistic properties of the analyzed extracts when used in combination, but also when used with other foods and in drug interactions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations9060152/s1, Figure S1: Overlaid FTIR spectra for pure daidzein (D) and genistein (G) and the analyzed samples: (a) seeds and sprouts of *G. max* (GMsd, GMsp); (b) seeds and sprouts of *P. anisum* (PAsd, PAsp); (c) seeds and sprouts of *A. graveolens* (AGsd, AGsp); (d) genistin and (e) daidzin FTIR analysis for the pure compounds (Sigma Aldrich) and for the compounds isolated through PTLC.

Author Contributions: Conceptualization, R.M.D. and F.B.; Data curation, S.M.A., R.M.D. and B.F.; Funding acquisition, R.M.D. and A.C.B.; Investigation, A.C.B., F.B. and A.V.D.B.; Methodology, A.V.D.B., A.C.B., S.G. and S.M.A.; Project administration, R.M.D. and A.C.B.; Resources, R.M.D., S.M.A. and B.F.; Software S.G., A.V.D.B. and A.C.B.; Supervision, R.M.D. and B.F.; Visualization, A.V.D.B., A.C.B. and F.B.; Writing—original draft, A.C.B., A.V.D.B. and R.M.D.; Writing—review & editing, R.M.D., F.B., A.V.D.B., A.C.B., S.G. and B.F. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kaur, N.; Chugh, V.; Gupta, A.K. Essential fatty acids as functional components of foods—A review. *J. Food Sci. Technol.* **2014**, *51*, 2289–2303. [CrossRef] [PubMed]
2. Wang, Z.; Wu, Z.; Zuo, G.; Lim, S.S.; Yan, H. Defatted seeds of oenotherabiennis as a potential functional food ingredient for diabetes. *Foods* **2021**, *10*, 538. [CrossRef] [PubMed]
3. Hidalgo, M.; Sánchez-Moreno, C.; de Pascual-Teresa, S. Flavonoid-flavonoid interaction and its effect on their antioxidant activity. *Food Chem.* **2010**, *121*, 691–696. [CrossRef]
4. Pająk, P.; Socha, R.; Broniek, J.; Królikowska, K.; Fortuna, T. Antioxidant properties, phenolic and mineral composition of germinated chia, golden flax, evening primrose, phacelia and fenugreek. *Food Chem.* **2019**, *275*, 69–76. [CrossRef]
5. BrglezMojzer, E.; KnezHrnčič, M.; Škerget, M.; Knez, Ž.; Bren, U. Polyphenols: Extraction Methods, Antioxidative Action, Bioavailability and Anticarcinogenic Effects. *Molecules* **2016**, *21*, 901. [CrossRef]
6. Sridhar, A.; Ponnuchamy, M.; Kumar, P.S.; Kapoor, A.; Vo, D.V.N.; Prabhakar, S. *Techniques and Modeling of Polyphenol Extraction from Food: A Review*; Springer International Publishing: Berlin/Heidelberg, Germany, 2021; Volume 19, ISBN 0123456789.
7. Wang, Z.; Zhang, Y.; Yan, H. In situ net fishing of α-glucosidase inhibitors from evening primrose (Oenothera biennis) defatted seeds by combination of LC-MS/MS{,} molecular networking{,} affinity-based ultrafiltration{,} and molecular docking. *Food Funct.* **2022**, *13*, 2545–2558. [CrossRef]
8. Badole, S.L.; Bodhankar, S.L. *Glycine Max (Soybean) Treatment for Diabetes*; Elsevier: Amsterdam, The Netherlands, 2013; ISBN 9780123971531.

9. Lee, K.S.; Woo, S.Y.; Lee, M.J.; Kim, H.Y.; Ham, H.; Lee, D.J.; Choi, S.W.; Seo, W.D. Isoflavones and soyasaponins in the germ of Korean soybean [*Glycine max* (L.) Merr.] cultivars and their compound-enhanced BMP-2-induced bone formation. *Appl. Biol. Chem.* **2020**, *63*, 1–8. [CrossRef]
10. Choi, Y.M.; Yoon, H.; Lee, S.; Ko, H.C.; Shin, M.J.; Lee, M.C.; Oh, S.; Desta, K.T. Comparison of Isoflavone Composition and Content in Seeds of Soybean (*Glycine max* (L.) Merrill) Germplasms with Different Seed Coat Colors and Days to Maturity. *Korean J. Plant Resour.* **2020**, *33*, 558–577.
11. Al-Maharik, N. Isolation of naturally occurring novel isoflavonoids: An update. *Nat. Prod. Rep.* **2019**, *36*, 1156–1195. [CrossRef]
12. Messina, M.; Hughes, C. Efficacy of soyfoods and soybean isoflavone supplements for alleviating menopausal symptoms is positively related to initial hot flush frequency. *J. Med. Food* **2003**, *6*, 1–11. [CrossRef]
13. Taku, K.; Melby, M.K.; Kronenberg, F.; Kurzer, M.S.; Messina, M. Extracted or synthesized soybean isoflavones reduce menopausal hot flash frequency and severity: Systematic review and meta-analysis of randomized controlled trials. *Menopause* **2012**, *19*, 776–790. [CrossRef] [PubMed]
14. Nina Estrella, R.E.; Landa, A.I.; Lafuente, J.V.; Gargiulo, P.A. Effects of antidepressants and soybean association in depressive menopausal women. *Acta Pol. Pharm. Drug Res.* **2014**, *71*, 323–327.
15. Jana, S.; Shekhawat, G. Anethum graveolens: An Indian traditional medicinal herb and spice. *Pharmacogn. Rev.* **2010**, *4*, 179–184. [CrossRef]
16. Memariani, Z.; Gorji, N.; Moeini, R.; Farzaei, M.H. *Traditional Uses*; Elsevier: Amsterdam, The Netherlands, 2019; ISBN 9780128153543.
17. Najaran, Z.T.; Hassanzadeh, M.K.; Nasery, M.; Emami, S.A. *Dill (Anethum graveolens L.) Oils*; Elsevier Inc.: Amsterdam, The Netherlands, 2016; ISBN 9780124166448.
18. Wattanathorn, J.; Ohnon, W.; Thukhammee, W.; Muchmapura, S.; Wannanon, P.; Tong-Un, T. Cerebroprotective Effect against Cerebral Ischemia of the Combined Extract of Oryza sativa and Anethum graveolens in Metabolic Syndrome Rats. *Oxid. Med. Cell. Longev.* **2019**, *2019*, 1–19. [CrossRef]
19. Moon, H.J.; Xiang, L.P.; Jong, M.K.; Sung, W.K.; Jeong, H.P. Inhibition of cholinesterase and amyloid-β aggregation by resveratrol oligomers from Vitisamurensis. *Phyther. Res.* **2008**, *22*, 544–549. [CrossRef]
20. Saghafi, N.; Karjalian, M.; Ghazanfarpour, M.; Khorsand, I.; Rakhshandeh, H.; Mirteimouri, M.; Babakhanian, M.; Khadivzadeh, T.; Najafzadeh, M.J.; Ghorbani, A.; et al. The effect of a vaginal suppository formulation of dill (Anethum graveolens) in comparison to clotrimazole vaginal tablet on the treatment of vulvovaginal candidiasis. *J. Obstet. Gynaecol.* **2018**, *38*, 985–988. [CrossRef] [PubMed]
21. Afonso, A.C.; Oliveira, D.; Saavedra, M.J.; Borges, A.; Simões, M. Biofilms in diabetic foot ulcers: Impact, risk factors and control strategies. *Int. J. Mol. Sci.* **2021**, *22*, 8278. [CrossRef] [PubMed]
22. Orav, A.; Raal, A.; Arak, E. Essential oil composition of *Pimpinella anisum* L. fruits from various European countries. *Nat. Prod. Res.* **2008**, *22*, 227–232. [CrossRef]
23. Lee, J.B.; Yamagishi, C.; Hayashi, K.; Hayashi, T. Antiviral and immunostimulating effects of lignin-carbohydrate-protein complexes from *Pimpinella anisum*. *Biosci. Biotechnol. Biochem.* **2011**, *75*, 459–465. [CrossRef]
24. Hosseinzadeh, H.; Tafaghodi, M.; Abedzadeh, S.; Taghiabadi, E. Effect of aqueous and ethanolic extracts of *Pimpinella anisum* L. seeds on milk production in rats. *JAMS J. Acupunct. Meridian Stud.* **2014**, *7*, 211–216. [CrossRef]
25. BettaiebRebey, I.; AidiWannes, W.; Kaab, S.B.; Bourgou, S.; Tounsi, M.S.; Ksouri, R.; Fauconnier, M.L. Bioactive compounds and antioxidant activity of *Pimpinella anisum* L. accessions at different ripening stages. *Sci. Hortic.* **2019**, *246*, 453–461. [CrossRef]
26. Huda, J.A.; Hameed, I.H.; Hamza, L.F. Anethum graveolens: Physicochemical properties, medicinal uses, antimicrobial effects, antioxidant effect, anti-inflammatory and analgesic effects: A review. *Int. J. Pharm. Qual. Assur.* **2017**, *8*, 88–91. [CrossRef]
27. Shahamat, Z.; Abbasi-Maleki, S.; MohammadiMotamed, S. Evaluation of antidepressant-like effects of aqueous and ethanolic extracts of *Pimpinella anisum* fruit in mice. *Avicenna J. Phytomedicine* **2016**, *6*, 322–328. [CrossRef]
28. Sun, W.; Shahrajabian, M.H.; Cheng, Q. Anise (*Pimpinella anisum* L.), a dominant spice and traditional medicinal herb for both food and medicinal purposes. *Cogent Biol.* **2019**, *5*, 1673688. [CrossRef]
29. Tabacaru, A.; Botezatu, A.V.D.; Horincar, G.; Furdui, B.; Dinica, R.M. Green accelerated synthesis, antimicrobial activity and seed germination test of quaternary ammonium salts of 1,2-bis(4-pyridyl)ethane. *Molecules* **2019**, *24*, 2424. [CrossRef] [PubMed]
30. Fotsing Yannick Stéphane, F.; Kezetas Jean Jules, B.; El-Saber Batiha, G.; Ali, I.; Ndjakou Bruno, L. Extraction of Bioactive Compounds from Medicinal Plants and Herbs. In *Natural Medicinal Plants*; El-Shemy, H., Ed.; IntechOpen: London, UK, 2022; Chapter 9; pp. 1–39. [CrossRef]
31. Maksoud, S.; Abdel-Massih, R.M.; Rajha, H.N.; Louka, N.; Chemat, F.; Barba, F.J.; Debs, E. Citrus aurantium l. Active constituents, biological effects and extraction methods. An updated review. *Molecules* **2021**, *26*, 5832. [CrossRef]
32. Dai, J.; Mumper, R.J. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules* **2010**, *15*, 7313–7352. [CrossRef]
33. Nn, A. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. *Med. Aromat. Plants* **2015**, *4*, 3–8. [CrossRef]
34. Chaves, J.O.; de Souza, M.C.; Da Silva, L.C.; Lachos-Perez, D.; Torres-Mayanga, P.C.; Machado, A.P.D.F.; Forster-Carneiro, T.; Vázquez-Espinosa, M.; González-De-Peredo, A.V.; Barbero, G.F.; et al. Extraction of Flavonoids from Natural Sources Using Modern Techniques. *Front. Chem.* **2020**, *8*, 507887. [CrossRef]

35. Sultana, B.; Anwar, F.; Ashraf, M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules* **2009**, *14*, 2167–2180. [CrossRef]
36. Ameer, K.; Shahbaz, H.M.; Kwon, J.H. Green Extraction Methods for Polyphenols from Plant Matrices and Their Byproducts: A Review. *Compr. Rev. Food Sci. Food Saf.* **2017**, *16*, 295–315. [CrossRef]
37. Heimler, D.; Romani, A.; Ieri, F. Plant polyphenol content, soil fertilization and agricultural management: A review. *Eur. Food Res. Technol.* **2017**, *243*, 1107–1115. [CrossRef]
38. Adebooye, O.C. Phyto-constituents and anti-oxidant activity of the pulp of snake tomato (*Trichosanthes cucumerina* L.). *African J. Tradit. Complement. Altern. Med.* **2008**, *5*, 173–179. [CrossRef] [PubMed]
39. Ajila, C.M.; Brar, S.K.; Verma, M.; Tyagi, R.D.; Godbout, S.; Valéro, J.R. Extraction and Analysis of Polyphenols: Recent trends. *Crit. Rev. Biotechnol.* **2011**, *31*, 227–249. [CrossRef] [PubMed]
40. Medi, M.; Jasprica, I.; Mornar, A.; Males, Z. Application of TLC in the Isolation and Analysis of Flavonoid. In *Thin Layer Chromatography in Phytochemistry*; CRC Press: Boca Raton, FL, USA; Taylor & Francis Group: London, UK, 2008; pp. 405–424.
41. Zhang, Y.; Liu, C.; Zhang, Z.; Qi, Y.; Wu, G.; Li, S. Solvent gradient elution for comprehensive separation of constituents with wide range of polarity in Apocynumvenetum leaves by high-speed counter-current chromatography. *J. Sep. Sci.* **2010**, *33*, 2743–2748. [CrossRef] [PubMed]
42. Busuioc, A.C.; Botezatu, A.V.D.; Furdui, B.; Vinatoru, C.; Maggi, F.; Caprioli, G.; Dinica, R.M. Comparative study of the chemical compositions and antioxidant activities of fresh juices from romaniancucurbitaceae varieties. *Molecules* **2020**, *25*, 5468. [CrossRef]
43. Yeo, D.; Dinica, R.; Yapi, H.F.; Furdui, B.; Praisler, M.; Djaman, A.J.; N'Guessan, J.D. Évaluation de l'activité anti-inflammatoire et screening phytochimique des feuilles de Annona senegalensis. *Therapie* **2011**, *66*, 73–80. [CrossRef]
44. Balanescu, F.; Mihaila, M.D.I.; Cârâc, G.; Furdui, B.; Vînătoru, C.; Avramescu, S.M.; Lisa, E.L.; Cudalbeanu, M.; Dinica, R.M. Flavonoid profiles of two new approved romanianocimum hybrids. *Molecules* **2020**, *25*, 4573. [CrossRef]
45. Robu, S.; Chesaru, B.I.; Diaconu, C.; Dumitriubuzia, O.; Tutunaru, D.; Stanescu, U.; Lisa, E.L. Lavandula hybrida: Microscopic characterization and the evaluation of the essential oil. *Farmacia* **2016**, *64*, 914–917.
46. El Moujahed, S.; Errachidi, F.; AbouOualid, H.; Botezatu-Dediu, A.V.; OuazzaniChahdi, F.; KandriRodi, Y.; Dinica, R.M. Extraction of insoluble fibrous collagen for characterization and crosslinking with phenolic compounds from pomegranate byproducts for leather tanning applications. *RSC Adv.* **2022**, *12*, 4175–4186. [CrossRef]
47. Mao, S.; Wang, K.; Lei, Y.; Yao, S.; Lu, B.; Huang, W. Antioxidant synergistic effects of Osmanthus fragrans flowers with green tea and their major contributed antioxidant compounds. *Sci. Rep.* **2017**, *7*, 1–10. [CrossRef]
48. Cudalbeanu, M.; Ghinea, I.O.; Furdui, B.; Dah-Nouvlessounon, D.; Raclea, R.; Costache, T.; Cucolea, I.E.; Urlan, F.; Dinica, R.M. Exploring New Antioxidant and Mineral Compounds from Nymphaea alba Wild-Grown in Danube Delta Biosphere. *Molecules* **2018**, *23*, 1247. [CrossRef] [PubMed]
49. Hsieh, S.-L.; Shih, Y.-W.; Chiu, Y.-M.; Tseng, S.-F.; Li, C.-C.; Wu, C.-C. By-Products of the Black Soybean Sauce Manufacturing Process as Potential Antioxidant and Anti-Inflammatory Materials for Use as Functional Foods. *Plants* **2021**, *10*, 2579. [CrossRef] [PubMed]
50. Govindarajan, R.; Rastogi, S.; Vijayakumar, M.; Shirwaikar, A.; Rawat, A.K.S.; Mehrotra, S.; Pushpangadan, P. Studies on the antioxidant activities of Desmodiumgangeticum. *Biol. Pharm. Bull.* **2003**, *26*, 1424–1427. [CrossRef] [PubMed]
51. Sudan, R.; Bhagat, M.; Gupta, S.; Singh, J.; Koul, A. Iron (FeII) chelation, ferric reducing antioxidant power, and immune modulating potential of arisaemajacquemontii (himalayan cobra lily). *Biomed Res. Int.* **2014**, *2014*, 1–7. [CrossRef] [PubMed]
52. Adjimani, J.P.; Asare, P. Antioxidant and free radical scavenging activity of iron chelators. *Toxicol. Rep.* **2015**, *2*, 721–728. [CrossRef]
53. Frisch, M.J.; Trucks, G.; Schlegel, H.; Scuseria, G.; Robb, M.; Cheeseman, J.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G.; et al. *Gaussian 09, Revis. B.01*; Gaussian, Inc.: Wallingford, CT, USA, 2009.
54. Yearley, E.J.; Zhurova, E.A.; Zhurov, V.V.; Pinkerton, A.A. Binding of genistein to the estrogen receptor based on an experimental electron density study. *J. Am. Chem. Soc.* **2007**, *129*, 15013–15021. [CrossRef]
55. Gosav, S.; Ion, A.; Praisler, M. DFT characterization of MDMA methylene homologue, a chemical compound with psychoactive properties. *AIP Conf. Proc.* **2019**, *2075*, 170027. [CrossRef]
56. Gosav, S.; Paduraru, N.; Maftei, D.; Birsa, M.L.; Praisler, M. Quantum chemical study of a derivative of 3-substituted dithiocarbamic flavanone. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2017**, *172*, 115–125. [CrossRef]
57. Domingo, L.R.; Ríos-Gutiérrez, M.; Pérez, P. Applications of the conceptual density functional theory indices to organic chemistry reactivity. *Molecules* **2016**, *21*, 748. [CrossRef]
58. Reich, E. *HPTLC of Medicinal Plants Identification and Quantification*; Camag, Sonnenmattstrasse: Muttenz, Switzerland, 2013; pp. 1–58.
59. Sajeeth, C.I.; Manna, P.K.; Manavalan, R.; Jolly, C.I. Quantitative estimation of Gallic Acid, Rutinand Quercetin in certain herbal plants by hptlc method. *Der Chem. Sin.* **2010**, *1*, 80–85.
60. Alam, P.; Kamal, Y.T.; Alqasoumi, S.I.; Foudah, A.I.; Alqarni, M.H.; Yusufoglu, H.S. HPTLC method for simultaneous determination of ascorbic acid and gallic acid biomarker from freeze dry pomegranate juice and herbal formulation. *Saudi Pharm. J.* **2019**, *27*, 975–980. [CrossRef] [PubMed]
61. Puri, A.; Panda, B.P. Simultaneous estimation of glycosidic isoflavones in fermented and unfermented soybeans by TLC-densitometric method. *J. Chromatogr. Sci.* **2015**, *53*, 338–344. [CrossRef] [PubMed]

62. Pandit, N.T.; Patravale, V.B. Design and optimization of a novel method for extraction of genistein. *Indian J. Pharm. Sci.* **2011**, *73*, 184–192. [CrossRef]
63. Shawky, E.; Sallam, S.M. Simultaneous Determination of Soyasaponins and Isoflavones in Soy (*Glycine max* L.) Products by HPTLC-densitometry-Multiple Detection. *J. Chromatogr. Sci.* **2017**, *55*, 1059–1065. [CrossRef]
64. Liggins, J.; Bluck, L.J.C.; Runswick, S.; Atkinson, C.; Coward, W.A.; Bingham, S.A. Daidzein and genistein contents of vegetables. *Br. J. Nutr.* **2000**, *84*, 717–725. [CrossRef]
65. Bhalla, Y.; Chadha, K.; Chadha, R.; Karan, M. Daidzein cocrystals: An opportunity to improve its biopharmaceutical parameters. *Heliyon* **2019**, *5*, e02669. [CrossRef]
66. Rimbach, G.; Boesch-Saadatmandi, C.; Frank, J.; Fuchs, D.; Wenzel, U.; Daniel, H.; Hall, W.L.; Weinberg, P.D. Dietary isoflavones in the prevention of cardiovascular disease—A molecular perspective. *Food Chem. Toxicol.* **2008**, *46*, 1308–1319. [CrossRef]
67. Sakthivelu, G.; Akitha Devi, M.K.; Giridhar, P.; Rajasekaran, T.; Ravishankar, G.A.; Nikolova, M.T.; Angelov, G.B.; Todorova, R.M.; Kosturkova, G.P. Isoflavone composition, phenol content, and antioxidant activity of soybean seeds from India and Bulgaria. *J. Agric. Food Chem.* **2008**, *56*, 2090–2095. [CrossRef]
68. Sulistyowati, E.; Martono, S.; Riyanto, S.; Lukitaningsih, E.; Rohman, A. Rapid quantitative analysis of daidzein and genistein in soybeans (*Glycine max* (L). Merr.) using FTIR spectroscopy and multivariate calibration. *J. Appl. Pharm. Sci.* **2020**, *10*, 117–123. [CrossRef]
69. Luan, F.; Tang, L.L.; Chen, X.X.; Liu, H.T. Simultaneous determination of daidzein, genistein and formononetin in coffee by capillary zone electrophoresis. *Separations* **2017**, *4*, 1. [CrossRef]
70. Puri, A.; Panda, B.P.; Singh, H.; Singh, S.; Srivastava, A.; Tandon, P.; Bharti, P.; Kumar, S.; Maurya, R. Conformational analysis and vibrational study of daidzein by using FT-IR and FT-Raman spectroscopies and DFT calculations. *J. Chromatogr. Sci.* **2014**, *53*, 338–344. [CrossRef] [PubMed]
71. Dixon, R.A.; Pasinetti, G.M. Flavonoids and isoflavonoids: From plant biology to agriculture and neuroscience. *Plant Physiol.* **2010**, *154*, 453–457. [CrossRef] [PubMed]
72. Thrane, M.; Paulsen, P.V.; Orcutt, M.W.; Krieger, T.M. Soy Protein: Impacts, Production, and Applications. In *Sustainable Protein Sources*; Elsevier: Amsterdam, The Netherlands, 2017; pp. 23–45. ISBN 9780128027769.
73. Park, S.Y.; Kim, J.K.; Kim, E.H.; Kim, S.H.; Prabakaran, M.; Chung, I.M. Comparison of 12 Isoflavone Profiles of Soybean (*Glycine max* (L.) Merrill) Seed Sprouts from Three Different Countries. *Korean J. Crop Sci.* **2018**, *63*, 360–377. [CrossRef]
74. Miadoková, E. Isoflavonoids—An overview of their biological activities and potential health benefits. *Interdiscip. Toxicol.* **2009**, *2*, 211–218. [CrossRef]
75. Vitale, D.C.; Piazza, C.; Melilli, B.; Drago, F.; Salomone, S. Isoflavones: Estrogenic activity, biological effect and bioavailability. *Eur. J. Drug Metab. Pharmacokinet.* **2013**, *38*, 15–25. [CrossRef]
76. Sajid, M.; Stone, S.R.; Kaur, P. Recent Advances in Heterologous Synthesis Paving Way for Future Green-Modular Bioindustries: A Review with Special Reference to Isoflavonoids. *Front. Bioeng. Biotechnol.* **2021**, *9*, 1–18. [CrossRef]
77. Nikolić, I.; Savić-Gajić, I.; Tačić, A.; Savić, I. Classification and biological activity of phytoestrogens: A review. *Adv. Technol.* **2017**, *6*, 96–106. [CrossRef]
78. De Franciscis, P.; Colacurci, N.; Riemma, G.; Conte, A.; Pittana, E.; Guida, M.; Schiattarella, A. A nutraceutical approach to menopausal complaints. *Medicina* **2019**, *55*, 544. [CrossRef]
79. Lee, S.G.; Brownmiller, C.R.; Lee, S.; Kang, H.W. Anti-Inflammatory and Antioxidant Effects of Anthocyanins of Trifolium pratense (Red Clover) in Lipopolysaccharide-Stimulated RAW-267.4 Macrophages. *Nutrients* **2020**, *12*, 1089. [CrossRef]
80. Siriyong, T.; Subhadhirasakul, S.; Chanwanitsakul, S.; Phungtammasan, S.; Wichayaworanan, S.; Boonchu, K.; Phaenoi, N.; Siangchin, P.; Klaingkaew, K. Therapeutic effects of traditional Thai herbal blood and wind tonic formulations for treatment of menopausal symptoms. *Explore* **2021**, *17*, 469–474. [CrossRef] [PubMed]
81. Taralkar, S.V.; Chattopadhyay, S. A HPLC Method for Determination of Ursolic Acid and Betulinic Acids from their Methanolic Extracts of Vitex Negundo Linn. *J. Anal. Bioanal. Tech.* **2012**, *3*, 1–6. [CrossRef]
82. Malenčić, D.; Cvejić, J.; Miladinović, J. Polyphenol content and antioxidant properties of colored soybean seeds from central Europe. *J. Med. Food* **2012**, *15*, 89–95. [CrossRef] [PubMed]
83. Aly, S.E.; Sabry, B.A.; Shaheen, M.S.; Hathout, A.S. Assessment of antimycotoxigenic and antioxidant activity of star anise (*Illicium verum*) in vitro. *J. Saudi Soc. Agric. Sci.* **2016**, *15*, 20–27. [CrossRef]
84. Kaur, N.; Chahal, K.K.; Kumar, A.; Singh, R.; Bhardwaj, U. Antioxidant activity of *Anethum graveolens* L. essential oil constituents and their chemical analogues. *J. Food Biochem.* **2019**, *43*, 1–11. [CrossRef] [PubMed]
85. Al-Ismail, K.M.; Aburjai, T. Antioxidant activity of water and alcohol extracts of chamomile flowers, anise seeds and dill seeds. *J. Sci. Food Agric.* **2004**, *84*, 173–178. [CrossRef]
86. Peiretti, P.G.; Karamać, M.; Janiak, M.; Longato, E.; Meineri, G.; Amarowicz, R.; Gai, F. Phenolic composition and antioxidant activities of soybean (*Glycine max* (L.) Merr.) plant during growth cycle. *Agronomy* **2019**, *9*, 153. [CrossRef]