Article

Evaluation of the Nutritional Value of *Prunus dulcis* Blossoms and the Antioxidant Compounds of Their Extracted Oil Using Green Extraction Method

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Abstract: Edible blossoms and extracted oils from various parts of plants have gained the interest of researchers in recent years due to their strong antioxidant activity and their high content of vitamins. In addition, they contain a plethora of polyphenols, and they do not have high caloric content. The blossoms of *Prunus dulcis* (i.e., almond tree) are edible; however, they have not been examined in terms of nutritional value. The present study aimed to examine the nutritional value of almond blossoms, as well as their extracted oil. The fat content of the blossoms was 1.75 g/100 g dry weight (dw), while the defatted blossoms were found to contain 1.34 g/100 g dw of crude protein and 29.97 g/100 g dw of carbohydrates. In addition, the blossom oil was tested for its composition of fatty acids, polyphenols, and total carotenoids. According to the results, several important fatty acids for human health were identified, such as oleic (25.17%), linoleic (15.64%), and linolenic (10.15%). Simultaneously, a low oxidation index (COX), i.e., 4.05, and many monounsaturated (25.17%) and unsaturated (67.56%) fats were detected, while both polyphenols (51.86 mg GAE/kg) and carotenoids were in abundance. Finally, the combination of simple stirring with ultrasound (a green extraction method) was found to be the most appropriate method to ensure maximum amounts of various antioxidant compounds in the blossom extracts (i.e., polyphenols and L-ascorbic acid). After optimization, the total polyphenol content increased by 23.98% and L-ascorbic acid content by 6.96%. In addition, antioxidant activity was tested by different antioxidant assays and specifically FRAP, DPPH, and H_2O_2, which showed a corresponding increase (14.46, 17.23, and 8.79%, respectively). Therefore, it can be concluded that *Prunus dulcis* blossoms, besides being edible, are also highly nutritious, and their oil has nutritional value and deserves further exploration.

Keywords: almond tree; edible blossoms; proteins; fatty acids; ultrasound extraction; antioxidants; polyphenols; carotenoids; L-ascorbic acid

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1. Introduction

The almond tree (*Prunus dulcis*), a member of the Rosaceae family, has garnered significant attention due to its remarkable features [1,2]. Almonds belong to the ancient cultivates as their cultivation in Europe dates back to 3000 BC [3,4]. In more recent data, global almond production exhibits an annual increase, with the 2017–2018 period recording the production of 1.76 million tons, while in 2018–2019 the production escalated to 3.2 million tons. The United States stands as the country with the highest annual production, with the state of California leading, contributing 84% of global production [5]. Spain, Iran, and Morocco follow, each accounting for approximately 18% of global production on an annual basis. Subsequent countries in the ranking include Iran and Turkey, with annual productions amounting to 139,000 and 100,000 tons, respectively [6,7]. This wide distribution is indicative of the almond tree's adaptability to varying climatic conditions. Almond trees

are cultivated mainly for their nut, which contains 4% water [8], 22% carbohydrates [9], 21% protein [10], and 50% fat, with a high monounsaturated (39%) and low saturated (3.7%) fatty acid content [8]. Furthermore, it is a valuable source of minerals such as calcium, copper, iron, magnesium, manganese, phosphorus, and zinc [11]. Owing to its rich nutritional value, the almond nut can be consumed raw but is also used for the production of almond milk.

Beyond their widespread recognition for their nuts, almond trees have also drawn attention due to their lesser-known but also valuable parts, such as the furry outer shuck and their edible flowers. Almond flowers have piqued the interest of researchers in recent years due to their beneficial properties [12]. In particular, they contain numerous compounds that exhibit antioxidant activity and free radical scavenging effects [13]. In addition, they are a rich source of vitamins, such as vitamins A and C [14], while they also possess an abundance of polyphenols [15] and flavonoids [16]. It is noteworthy that almond flowers have minimal caloric content [17], even though they are a source of such important substances. Finally, it is worth noting that the flowers are already used in many restaurants and homemade dishes [18] since they offer different colors and flavors, greatly impacting the sensory and nutritional value of food [15].

The study of the nutritional value of edible flowers dates back many years ago [19]. Typically, the examination of the chemical compounds within these flowers involves organic solvent extraction [20]. However, conventional extraction with organic solvents is time-consuming and involves hazardous chemicals [21]. Amor and Allaf [22], in order to optimize the process and enhance conventional extraction for the study of the edible flower of *Hibiscus sabdariffa*, proposed thermomechanical transient control pressure drop processing methods. The results showed that the thermomechanical transient control method improved the diffusion rate of anthocyanin extraction and increased the yield of anthocyanins [22]. Nonetheless, as mentioned by Zhao et al. [20], new and green extraction technologies, such as ultrasound-assisted extraction, should be implemented in edible flower extraction.

As reported by Banji et al. [23], great interest has arisen in the extraction of edible oils from various parts of plants such as peels, seeds, leaves, flowers, and fruits. Thus, it has become apparent that edible flowers may also be used for oil production. Edible plant oils are an essential component of human nutrition [24], exhibiting health benefits, since they have a wide variety of fatty acids [25]. Although oil can be extracted from various parts of the plant, studies have mainly been focused on the extraction of oil from the kernel of the plant [26,27], while no studies have previously been conducted regarding the extraction of oil from flowers, except for essential oil [28].

Despite the significant commercial value of *Prunus dulcis* [29], the nutrient composition of *Prunus dulcis* blossoms, including their fat/fatty acid content, total polyphenols, total carotenoids, crude protein, and carbohydrates, has not been thoroughly studied. The antioxidant capacity was determined through the measurement of antioxidant activity, using different assays. To study the above parameters, extraction steps were conducted. The extraction techniques used were the conventional stirring-assisted extraction (ST), ultrasound-assisted extraction (US), and a hybrid approach involving both methods (ST and US). This study aimed to obtain a comprehensive composition of almond blossoms and their oil, as well as contribute to the optimization of extraction strategies for antioxidant compounds.

2. Materials and Methods

2.1. Chemicals and Reagents

All information regarding the solvents and reagents used is presented in detail in the Supplementary Materials.

2.2. Sample Collection and Preparation

The blossoms were collected during their full bloom in early May 2023 from an almond farm in Sykourio, Larisa, Greece by expert agriculturalists, Ms. Kotsou and Mr. Makrygiannis. Sykourio is located in Thessaly where the largest almond production (~40%) in Greece takes place. After collection, the blossoms were placed in a freezer at −40 °C, and then the water was removed by freeze-drying (Biobase BK-FD10P freeze dryer, Jinan, China). The water content of the blossoms was found to be 73%. After the water was removed, the dried blossoms were ground up and separated according to size using a series of sieves (Analysette 3, Fritsch GmbH, Oberstein, Germany). Particles of an average diameter of 133 μm were used for further study since a smaller particle size facilitates the extraction process and gives a better overview of the contained compounds [30,31].

2.3. Extraction of Oil and Determination of Fat Content

A total of 5 g of blossom powder was mixed with 50 mL of *n*-hexane in an amber glass bottle. The combination was stirred for two hours at room temperature with 500 rotations per minute. Following this, it was centrifuged for 10 min at 3600× *g* using a NEYA 16R centrifuge from Remi Elektro-technik Ltd. (Mumbai, India). The liquid portion (supernatant) was separated, while the solid residue underwent the same extraction process. The collected supernatants were merged, and a rotary evaporator (Laborota 4000 efficient, Heidolph Instruments GmbH and Co. KG, Schwabach, Germany) was utilized to eliminate the solvent. The fat content was then determined through gravimetric analysis. The oil was then used for further analyses. The solid residue after the centrifugation steps (defatted blossom powder) was dried and used for further extractions and analyses.

2.4. Analyses of the Oil

2.4.1. Fatty Acid Composition

The method employed for the qualitative and quantitative determination of fatty acids was based on a previous study [32]. Details are given in Supplementary Materials.

2.4.2. Determination of Total Carotenoid Content (TCC)

The determination of TCC was based on a previously reported procedure [33]. Details are given in Supplementary Materials.

2.4.3. Determination of Total Polyphenol Content (TPC)

For the determination of the TPC of the oil samples, a previously described method was followed [34].

2.5. Analyses of the Defatted Solid Residue

2.5.1. Extraction and Determination of Crude Protein Content

Determination of crude protein content was carried out with the Bradford assay, according to a previous study [35]. Details are given in Supplementary Materials.

2.5.2. Extraction and Determination of Carbohydrate Content

The carbohydrate levels in the samples were assessed utilizing the phenol/sulfuric acid technique [35]. Details are given in Supplementary Materials.

2.5.3. Extraction and Determination of L-ascorbic Acid Content

The amount of L-ascorbic acid was measured using a previous method [36]. Details are given in Supplementary Materials.

2.5.4. Determination of TCC

For the estimation of the total carotenoid content, a previously used technique described by Chatzimitakos et al. was followed [37]. Quantification was carried out as described in Section 2.4.2.

2.5.5. Extraction and Determination of TPC

Details about the extraction of polyphenols are given in Supplementary Materials. The TPC of the extracts was examined according to a previous study [38]. Details are given in Supplementary Materials.

2.5.6. Antioxidant Activity by Ferric-Reducing Antioxidant Power (FRAP) Assay, DPPH Radical Scavenging Activity, and Hydrogen Peroxide (H_2O_2) Scavenging Activity

All experimental methods that were applied for the exploration of the antioxidant activity of defatted solid residue were carried out according to previous experimental surveys [38–40] and are described in Supplementary Materials.

2.6. Statistical Analysis

All extractions and assays were conducted in triplicate to ensure accuracy and reliability. Statistical analysis was performed to assess the significance of differences between mean values, with a significance level set at $p < 0.05$. The analysis was carried out using the ANOVA test, utilizing IBM SPSS Statistics 29.0 software developed by SPSS Inc. in Chicago, IL, USA.

3. Results and Discussion

3.1. Nutritional Composition of Prunus dulcis Blossoms (PDBs) and Prunus dulcis Blossom Oil (PDBO)

Protein, a fundamental dietary element important for daily consumption, was the first nutrient examined. According to the results and Table 1, PDBs exhibit a notably low protein content (1.34 ± 0.02%), in stark contrast to the nut, which contains a substantial protein content of 21.26% [41]. Comparative studies on protein content across edible flowers yielded insightful findings. Notably, *Brassica oleracea* var. *italica* (broccoli) contained 3% crude protein, hibiscus exhibited 2.7%, and *Brassica oleracea* var. *botrytis* (cauliflower) contained only 1.9% [42]. Similarly, an analysis by Sheng et al. [43] on banana flowers of two cultivars (cvs. *Baxijiao* and *Paradisical*) revealed a protein content in the range of 1.62–2.07 g/100 g. These values are comparable with our findings. Furthermore, an exploration of wild edible flowers in Mexico demonstrated higher protein content, ranging from 11.3 g/100 g in *Arbutus xalapensis* to 27.4 g/100 g in *Erythrina caribaea* [44]. This substantiates the notion that edible flowers generally lack a high protein content, specifically blossoms.

Table 1. Nutritional composition of *Prunus dulcis* blossoms.

Crude Protein (g/100 g dw)	Carbohydrates (g/100 g dw)	Fat (g/100 g dw)
1.34 ± 0.02	29.97 ± 1.04	1.75 ± 0.05

Carbohydrates, as pivotal sources of energy for the human body, are rich in dietary fiber and vital nutrients. Our study showed that PDBs contain a quantity of carbohydrates significantly lower than that found in the kernel of *Prunus dulcis*, which ranges from 13.34 ± 1.54 to 18.59 ± 2.22 g/100 g [45]. Comparatively, conventional foods like chicken breast contain far less carbohydrates, whereas vegetables such as broccoli and cauliflower contain a carbohydrate content of up to 5% [42]. However, compared with other well-known edible flower species like banana, primula, and chive, our findings vary. PDBs can be considered relatively rich in carbohydrates compared to banana blossoms, which contain a carbohydrate content of only 1.2% [41], while compared with primula and chive blossoms that contain carbohydrate contents of 76.8% [46] and 50.0% [47], respectively, it can be considered poorer in carbohydrates.

The *Prunus dulcis* nut is globally known for its oil content that can vary from 46 to 64% [48]. The fat content of PDBs was found to be low (1.75 ± 0.05%), but the fatty acids that were identified and quantified (Table 2) are of great interest. Particularly noteworthy are significant compounds like oleic acid (C18:1) at 25.17%, linoleic acid (C18:2, ω-6) at

15.64%, and linoleic acid (C18:3, ω-3) at 10.15%. The presence of α-Eleostearic acid (C18:3, ω-3 isomer) is of particular significance. α-Eleostearic acid functions as an antioxidant and has demonstrated lipid peroxidation reduction in plasma and erythrocyte membranes in patients with diabetes [49]. Monounsaturated fatty acids (MUFAs) are also important, accounting for 25.17% of fatty acids. MUFAs are known for their anti-inflammatory and cardiovascular benefits [50], and are commonly abundant in nuts, avocados, and olive oil [51]. Additionally, an examination of unsaturated fats (UFAs), which are important for improving blood cholesterol levels and combating inflammation, yielded noteworthy results [52]. Our study identified a 67.56% UFA content in PDBO. It is important to mention that a large amount of saturated fatty acids (SFAs) (32.44%) were also found, that is, fats that can be synthesized naturally by the organism but can be found in foods such as full-fat dairy products, red meat, and poultry [53]. Moreover, our findings highlight a substantial presence of polyunsaturated fatty acids (PUFAs) at 42.39%, whose daily consumption should be limited [54]. Moreover, the PUFA: SFA ratio is the most commonly used indicator for assessing the nutritional value of dietary foods; the higher the value, the higher the nutritional value of the food. PDBO has a value of 1.31, while the most consumed meats have a PUFA:SFA ratio between 0.13 and 0.48. The PUFA:SFA ratio in fish ranges from 0.8 to 1.6 [55]. In addition, the COX (calculated oxidizability) value is a high priority since the lower its value, the better the oxidative stability and therefore the longer the shelf life of the oil. The COX value of sunflower oil is close to 6, and it is one of the most used oils, both in domestic and industrial use. In the present study, the COX value is 4.05, ~32.5% less than sunflower oil [32]. Finally, a balanced ω-6:3 ratio has great benefits to human health, whereas regular intake of imbalanced dietary essential fatty acids has detrimental effects on health. Depending on its value, this ratio can cause various immune-mediated diseases such as cardiovascular disease (4/1), colon cancer (2.5/1), and rheumatoid arthritis (2–3/1) [56]. Hence, it becomes clear that PDBO is a nutritious and valuable edible oil. The problem, however, arises from the fact that it is difficult to isolate in a large quantity.

Table 2. Fatty acid (%) content in *Prunus dulcis* blossom oil determined using GC-FID.

Fatty Acid	*Prunus dulcis* Blossom Oil (%)
Lauric (C12:0)	1.36 ± 0.09
Myristic (C14:0)	1.82 ± 0.13
Palmitic (C16:0)	14.88 ± 1.09
Arachidic (C20:0)	14.38 ± 0.4
∑ *Saturated (SFA)*	32.44 ± 1.71
Oleic (C18:1)	25.17 ± 1.26
∑ *Monounsaturated (MUFA)*	25.17 ± 1.26
Linoleic (C18:2, ω-6)	15.64 ± 0.61
Linolenic (C18:3, ω-3)	10.15 ± 0.55
α-Eleostearic (C18:3, ω-3 isomer)	16.6 ± 0.68
∑ *Polyunsaturated (PUFA)*	42.39 ± 1.84
∑ *Unsaturated (UFA)* [1]	67.56 ± 3.1
PUFA:SFA ratio	1.31 ± 0.01
MUFA:PUFA ratio	0.59 ± 0
ω-6:3 ratio	0.58 ± 0
COX [2]	4.05 ± 0.19

[1] UFAs, sum of MUFAs and PUFAs. [2] COX, calculated oxidizability value.

3.2. Total Polyphenol Content (TPC) and Total Carotenoid Content (TCC) of Prunus dulcis Blossom Oil (PDBO)

Incorporating polyphenols into diets contributes significantly to reducing blood pressure, insulin resistance, and systemic inflammation. Furthermore, polyphenols exert a discernible influence on the gut microbiome composition, fostering improved human health [57]. Unlike almond oil, PDBO has not been studied before. According to the results, it was found that PDBO contains 51.86 ± 3.97 mg GAE/kg TPC. The oil extracted from the

almond nut of *Prunus dulcis* contains 85.33–141.66 mg/kg TPC [58], while almond as a nut contains 392.00–11,030.50 mg/kg TPC [59]. Noteworthy is that almond polyphenols are mostly found in the skin, with values ranging from 9100.00 to 32,100.00 mg/kg TPC [60]. Although blossom oil does not contain such high TPC, it does contains a high amount. In fact, it could be compared with the TPC of olive oil which, depending on the variety and the area of cultivation and production, can vary from 50 to 800 mg/kg [61]. This outcome is quite promising as it seems that the oil extracted from PDBO not only has a high fatty acid content and low oxidation rate but also a high TPC.

Carotenoids are well known for their antioxidant properties since they have beneficial effects on human health by reducing the risk of certain types of cancers and eye conditions [62]. Commonly, vegetable oils contain low levels of carotenoids [63]; however, in the present study, PDBO appears to have significant amounts of TCC (i.e., 62.19 mg/kg). Since PDBO appears to be a promising edible oil, a comparison with other common edible oils was deemed interesting. For instance, rapeseed oil is one of the oldest known vegetable oils and contains 10.47 mg/kg of total carotenoids [64], almost 500% (493.98) lower than that found in PDBO. Comparing it to even more well-known and widely consumed oils such as soy and olive oil, TCC was found to be almost the same. Specifically, soy oil contains 64.43 mg/kg, olive oil contains 62.98 mg/kg [65], and PDBO contains 62.19 mg/kg TCC. This substantiates the conclusion that PDBO is a highly nutritious oil, rivaling the well-known olive oil in terms of TCC.

3.3. Antioxidant Properties of *Prunus dulcis* Blossom (PDB) Extracts after Three Different Extraction Methods

As can be seen in Table 3, employing US either alone or coupled with ST results in an increase in the TPC. More specifically, an increase of 15.39 and 23.99% can be observed, demonstrating the pivotal role of ultrasonic extraction in maximizing TPC. In a previous study, 13 edible plants (such as *Petunia hybrida*, *Pentas lanceolate*, and *Cosmos sulphureus*) were evaluated for their polyphenol concentration, with values ranging from 1.47 to 13.08 mg GAE/g fresh weight (fw) [46]. This fact indicates that PDBs, irrespective of the extraction method, are a rich source of polyphenols compared to other known edible flowers.

Table 3. Total polyphenols, antioxidant activities (FRAP, DPPH, and H_2O_2), and L-ascorbic acid content of *Prunus dulcis* blossoms, using conventional and green extraction methods.

Technique	TPC (mg GAE/g)	FRAP (μmol AAE/g)	DPPH (μmol AAE/g)	H_2O_2 (μmol AAE/g)	L-ascorbic Acid (mg/100 g)
ST [1]	10.13 ± 0.06 ^{c}	54.22 ± 1.02 ^{b}	14.16 ± 0.48 ^{b}	129.74 ± 4.62 ^{a}	978.86 ± 2.03 ^{c}
US [2]	11.69 ± 0.13 ^{b}	59.61 ± 0.2 ^{a}	15.56 ± 0.72 ^{a,b}	130.57 ± 6.84 ^{a}	1013.15 ± 1.16 ^{b}
US + ST [3]	12.56 ± 0.17 ^{a}	62.06 ± 1.89 ^{a}	16.6 ± 0.51 ^{a}	141.15 ± 1.36 ^{a}	1047.03 ± 0.51 ^{a}

[1] ST, stirring-assisted extraction. [2] US, ultrasound-assisted extraction. [3] US + ST, combined extraction method. Values are expressed as the mean values (±SD) of triplicate determinations, and means within each column with different superscript letters (a–c) are significantly ($p < 0.05$) different.

The antioxidant activity of PDBs was assessed using three distinct assays. These included the ferric-reducing antioxidant power (FRAP) assay, the DPPH radical scavenging assay, and the hydrogen peroxide (H_2O_2) scavenging assay. In all three cases, the combination of US and ST seemed to be the most suitable method for ensuring maximum antioxidant activity (at $p < 0.05$). The blossoms of *Prunus dulcis* have not been studied extensively. On the other hand, the antioxidant activity of the leaves and the nut has been examined. Several edible flowers have been investigated. For instance, the edible flowers of *Brassica oleracea* and *Brassica oleracea* var. *italica* show an antioxidant activity, measured by DPPH assay, of 2.71 μmol/g and 3.85 μmol/g, respectively [66], which is significantly lower than that of PDBs. Meanwhile, the antioxidant activity of the edible flower of Musa ABB was also investigated, using the FRAP method, registering a value of 20.60 μmol/g [67], 201.26% lower than that of PDBs. As far as the method of inhibiting peroxide action is

concerned, the antioxidant capacity increased (at $p > 0.05$) between the ST and US methods by 0.64% and 8.79% compared to the combination of ST and US.

Last but not least, L-ascorbic acid, also known as vitamin C, is among the best-known antioxidants and is of high importance for human health, as the body requires it for normal physiological functions [68]. Moreover, since humans are unable to synthesize L-ascorbic acid on their own [69], daily consumption of fresh fruits, vegetables, and other plant sources rich in L-ascorbic acid is recommended to achieve the required intake of it. In the present survey, L-ascorbic acid was recorded in large quantities irrespective of the extraction method. The amount recorded with ST extraction was 3.50% lower than with US extraction and 6.96% lower than with the combined ST and US extraction ($p < 0.05$). The vitamin C content of edible flowers has been extensively studied in previous research with varying results. For instance, Garzón and Wrolstad [70] analyzed the flowers of *Tropaeolum majus* and recorded an L-ascorbic acid content of 71.5 mg/100 g, while years later Khattak [71], studying the flowers of *Tagetes erecta*, noted L-ascorbic acid values of 57.3–91.0 mg/100 g. As for the highest vitamin C content, it was recorded in yellow pepper *Capsicum annuum* flowers where it reached 159.61 mg/100 g fw, a quantity exceedingly low compared to that contained in PDBs. Overall, it is obvious that PDBs are remarkably rich in antioxidants and can be enhanced even more by using the right extraction method.

4. Conclusions

Within the context of a continuously expanding global population, where the scientific community actively explores novel avenues for alternative, nutritionally rich, and economically sustainable food sources, *Prunus dulcis* blossoms offer a viable alternative. Despite their low protein content (1.34 g/100 g), these blossoms have proven to be nutritious, as even a simple extract reveals a substantial content of polyphenols (10.13 mg GAE/g) and L-ascorbic acid (978.86 mg/100 g). The oil extracted from *Prunus dulcis* blossoms is also exceptionally nutritious and suitable for consumption or general use in the food industry, pharmaceutical products, and cosmetics. In addition to the aforementioned nutrients, it contains all essential fatty acids in significant quantities. Consequently, it is a highly nutritious floral component of the plant that remains underutilized today, and more attention should be given to the blossoms since the fruit is already one of the most widely consumed nuts worldwide. Furthermore, as expected, by utilizing a green extraction method and, even more effectively, by combining the green method with conventional extraction, bioactive compounds in the blossoms can easily be isolated in higher percentages, achieving an increase of up to 19.35% in TPC and 6.50% in vitamin C content. This promotes naturally enriched extracts in essential nutrients without the addition of chemical substances.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app14052001/s1. Supplementary Material File—Materials and Methods.

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