processes

MDPI

Article

Phenolic Compounds, Fatty Acid Composition, and Antioxidant Activities of Some Flaxseed (*Linum usitatissimum* L.) Varieties: A Comprehensive Analysis

Mehmet Zeki Koçak

Department of Field Crops, Faculty of Agriculture, Igdir University, 76000 Igdir, Turkey; mzeki.kocak@igdir.edu.tr

Abstract: Flaxseed, also known as flax or linseed (*Linum usitatissimum* L.), is one of the oldest crops. It is used for oil and fiber production. The species displays a broad range of biological activities due to its chemical compounds. It has a widespread geographical distribution, and a large number of its varieties have been evaluated for their biological efficacy and nutritional value. This study investigates fifteen varieties of the species, some of which are examined for the first time. In this regard, a series of chemical composition analyses and antioxidant assays were carried out. Accordingly, total phenolic content ranged between 613.6 (Michael) and 3164.6 (Atalanta) mg GAE/g, whilst total flavonoid content varied from 176.25 (BonnyDoon) to 689.20 (Mcduff) mg QE/g. Regarding the radical scavenging assays, the values obtained were significantly higher than those of the standard antioxidant (ascorbic acid). Furthermore, the extracts exhibited chelating activity for ferrous ions and a cupric reducing capacity that was comparable to that of the standard. The oil content values of the varieties ranged from 0.82 g/100 g (Michael) to 2.14 g/100 g (McGregor). The percentage of α-linolenic acid varied between 39.21% (McGregor) and 54.13% (Nareum).

Keywords: *Linum usitatissimum*; linseed; α-linolenic acid; antioxidant activity; phenolic ingredient

Citation: Koçak, M.Z. Phenolic Compounds, Fatty Acid Composition, and Antioxidant Activities of Some Flaxseed (*Linum usitatissimum* L.) Varieties: A Comprehensive Analysis. *Processes* **2024**, *12*, 689. https://doi.org/10.3390/pr12040689

Academic Editors: Jung-Feng Hsieh, Chun-Chi Chen, Ken-Lin Chang and Dariusz Dziki

Received: 16 February 2024
Revised: 24 March 2024
Accepted: 27 March 2024
Published: 29 March 2024

1. Introduction

Flax (*Linum usitatissimum*) is an herbaceous, annual plant that is primarily self-pollinated. It is one of the main domesticated crops. The genus *Linum*, belonging to the family *Linaceae*, consists of several diploid species with a chromosome number of 2n = 30. Also, one of the species belonging to this family is *L. usitatissimum*, commonly known as linseed or common flax. Flax is a valuable crop grown for its fiber and oil-rich seeds, and it is cultivated with 22 genera around 300 species worldwide [1,2]. In archaeology, evidence in Tell Abu Hureyra in Northern Syria shows it originated in the Middle East and was farmed by the ancient "Egyptians and Somaris" around 10,000 years ago. According to research, the primary center of flax was Ancient Egypt and Mesopotamia; India, Ethiopia, and Central Asia are thought to be secondary centers of the plant. The species is distributed in other parts of the world, including North and South-West America and the Mediterranean Basin [3]. Although flax is grown at a lower rate in many other parts of the world, the major producers of flax are Canada, China, India, and Russia [4]. The industrial uses of flaxseed are abundant, including the preparation of soaps, pastes, paints, varnishes, and polymers due to its fast-drying properties. Flaxseed is also used in biodiesel production [5], as well as the food industry and the cosmetic industry [6].

Flaxseed oil is one of the richest sources of polyunsaturated omega-3 fatty acids from plant sources and is known for its significant health benefits including cardiovascular diseases [7], cancer [8], and diabetes [9]. The importance of the flaxseed for their oil-related attributes can be demonstrated with the high number of documents, according to SCOPUS database. Of 26,890 documents, 8344 included oil-related keywords. Of the documents identified, approximately 10% are related to "biological activities" of "oil". According to the literature available, flaxseed oil is one of the richest plant sources of α-linolenic

acid (omega-3) and linoleic acid (omega-6) polyunsaturated fatty acids (PUFA), which are necessary for humans to obtain from food, since they cannot be synthesized in organisms. Flaxseed has many important uses, including a high oil content of approximately 35–65%. Regarding composition, flaxseed contains valuable amounts of fatty acids such as oleic acid, stearic acid, α-linolenic acid, linoleic acid, and palmitic acid. It also contains high amounts of protein, dietary fiber, lignans, vitamins, secoisolariciresinol diglucoside (SDG), and micronutrients [10]. Phenolic compounds in flaxseeds have recently gained attention. Furthermore, flaxseed has been reported to contain high levels of phenolics and exhibit radical scavenging activity [11]. In the same case, it was reported that oily flaxseeds contain significantly higher amounts of free phenolic compounds and exhibit the highest antioxidant capacity [12]. In addition to phenolic acids, such as *p*-coumaric, vanillic, sinapic, and ferulic, which are found in flaxseed as ester and ether bonds and glycosides, it also contains lignans in significant amounts. These lignans have biological activity, including antiradical, antioxidant, antimicrobial, anticancer, and anti-cardiovascular effects [11,13].

The aim of this study was to compare the antioxidant capacity, phenolic profile, and fatty acids of domestic and foreign registered flaxseed varieties for use in functional food applications, and to identify the phenolic compounds responsible for this bioactivity. Furthermore, there is limited information available regarding the comparison of various flaxseed cultivars from different countries across the globe. Numerous documents report that various factors affect the chemical composition and, consequently, the biological activities of plant species [14,15]. Environmental factors, in addition to genetic makeup, are critical in determining the characteristics of plants. The chemical composition of the same species may vary significantly depending on its spatial origin. It is important to investigate and monitor the chemical composition of plants from various geographical locations. The present study aimed to compare the antioxidant capacity, phenolic profile, and fatty acids of local and foreign registered flaxseed varieties for use in functional food applications. The study also aimed to identify the phenolic compounds responsible for this bioactivity.

2. Materials and Methods

2.1. Flaxseed Material and Chemicals

The flaxseed variants, including "Dillman, Karakız, Norman, BonnyDoon, Nareum, Barbara, McGregor, Atalanta, Michael, Florinda, Mcduff, Konya Beyazı, Milas, Lirina, and Zoltan", were provided by governmental and non-governmental agricultural organizations/institutions of Türkiye. The details of the varieties considered for analysis are clearly presented in Table 1. For the analysis, the chemicals were procured from Merck Co. (Darmstadt, Germany) and Sigma-Aldrich Co. (St. Louis, MO, USA). The research was conducted at Igdir University Agricultural Research and Application Laboratory; Igdir, Türkiye. In this regard, a series of phytochemical composition analyses and antioxidant assays were performed using fifteen varieties of flaxseeds. The schematic presentation of the workflow of the study is given in Figure 1.

Table 1. Origin and growing properties of flaxseed varieties.

Sample	Variety	Origin Country	Place of Supply	Breeding Season	Flower Color	Seed Color
M.Z.K.7601	Karakız	Türkiye	Agricultural institute of Türkiye	Summer	White	Brown
M.Z.K.7602	BonnyDoon	Avustralya	Agricultural institute of Türkiye	Summer	White	Brown
M.Z.K.7603	Norman	Canada	Agricultural institute of Türkiye	Spring	Blue	Brown
M.Z.K.7604	McGregor	United States of North Dakota	Agricultural institute of Türkiye	Summer	Blue	Brown
M.Z.K.7605	Dillman	United States of North Dakota	Agricultural institute of Türkiye	Summer	Blue	Brown

Table 1. *Cont.*

Sample	Variety	Origin Country	Place of Supply	Breeding Season	Flower Color	Seed Color
M.Z.K.7606	Michael	USA	Agricultural institute of Türkiye	Spring	Blue	Brown
M.Z.K.7607	Konya Beyazı	Türkiye	Agricultural institute of Türkiye	Summer	Blue	Brown
M.Z.K.7608	Florinda	Romania	Agricultural institute of Türkiye	Spring	Blue	Brown
M.Z.K.7609	Lirina	Russian	Agricultural institute of Türkiye	Spring	White	Brown
M.Z.K.76010	Nareum	USA	Agricultural institute of Türkiye	Spring	Blue	Brown
M.Z.K.76011	Barbara	Romania	Agricultural institute of Türkiye	Spring	Blue	Brown
M.Z.K.76012	Atalanta	Türkiye	Agricultural institute of Türkiye	Summer	Blue	Brown
M.Z.K.76013	Milas	Türkiye	Agricultural institute of Türkiye	Spring	White	Brown
M.Z.K.76014	Zoltan	Hungary	Agricultural institute of Türkiye	Summer	Blue	Brown
M.Z.K.76015	Mcduff	Canada	Agricultural institute of Türkiye	Summer	Blue	Brown

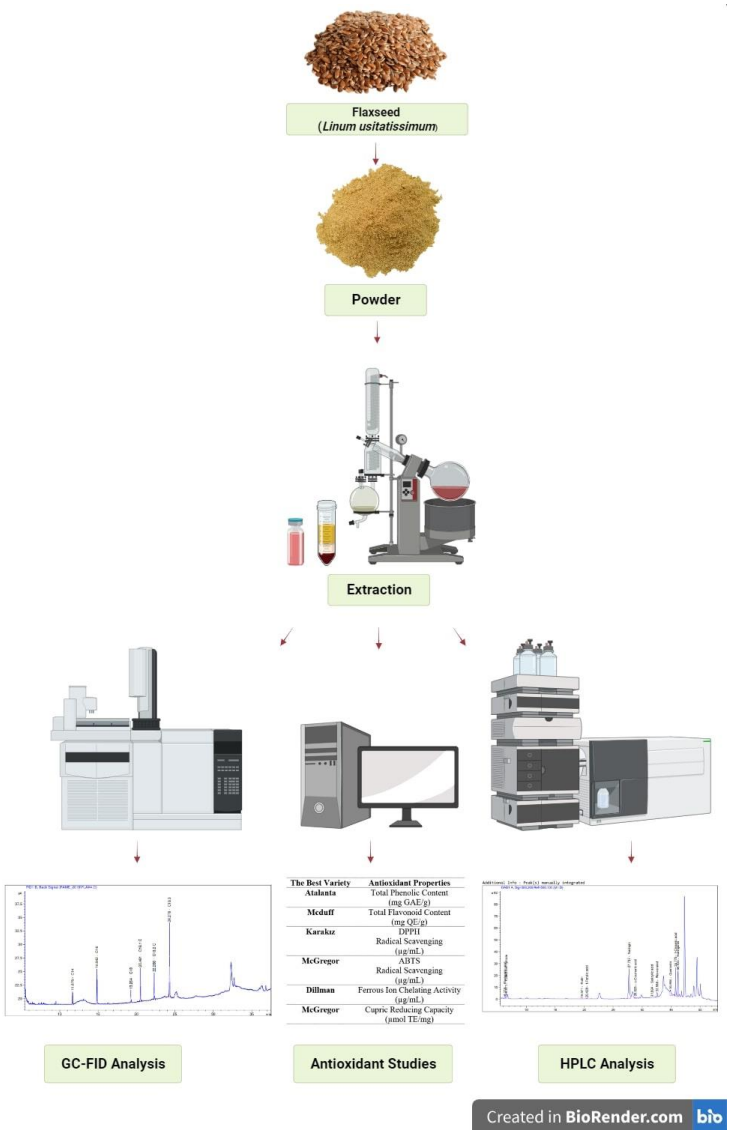

Figure 1. Workflow of the study.

2.2. Extraction Procedure

The extraction procedure was based on the report of Güller et al. [14]. Briefly, firstly, approximately 5 g of each of the available flaxseeds were taken and ground to a powder by a laboratory blender. Then, 2 g of each were mixed with 20 mL of 99.9% methanol and kept in a magnetic mixer at room temperature (25 °C ± 1) for 24 h. At the end of the process, the tubes were centrifuged at 5000 rpm for 30 min and the supernatant was removed and the solvent was removed using a rotary evaporator set at 36 °C. The flax powder obtained was stored at +4 °C for further analysis.

2.3. Total Phenolic Content Analysis (TPC)

The total phenolic contents in the ethanol extracts of the flaxseeds were determined by Folin Ciocalteu Reagent (FCR) method [16]. A standard graph was drawn by using gallic acid (GA). Firstly, 1 mg/mL concentration of GA in distilled water was prepared. Secondly, 10, 20, 40, 50, and 60 mL of GA from this stock solution was transferred into flasks and the volume was made up to 23 mL with distilled water. Thirdly, 0.5 mL of FCR and after 3 min 1.5 mL of 2% Na_2CO_3 were added to the flasks. The mixture was stirred at room temperature for 2 h; the absorbance of the samples was recorded at 760 nm against distilled water using a spectrophotometer ((San Francisco, CA, USA), Beckman Coulter DU730 UV-Vis Spectrophotometer). Absorbance values against µL gallic acid were plotted. The same processes were performed for extracts as well. The equation obtained from the standard graph was used to calculate the equivalent amount of gallic acid (GAE) corresponding to the absorbance readings of the samples.

2.4. Total Flavonoid Content (TFC) Analysis

The total amount of flavonoids in the ethanol extracts of flaxseeds was detected as reported by [17] and quercetin was used as standard antioxidant. A 1 mg/mL stock solution of quercetin was prepared. Then, 10, 20, 30, 40, and 50 µL quercetin solutions were transferred to test tubes. Finally, 4.3 mL of ethanol solution including 0.1 M CH_3COOK and 0.1 mL (10%) $Al(NO_3)_3$ was added into these tubes and mixed by a vortex. After incubation at room temperature for 40 min, absorbances at 415 nm were recorded against the ethanol. The same processes were performed for extracts as well. The total flavonoid concentration was estimated using the usual graph equation as microgram quercetin equivalent (QE).

2.5. Determination of Cu^{2+} Reduction Capacity

For determination of cupric ion (Cu^{2+}) reducing capacities in the ethanol extracts of flaxseed, 0.25 mL $CuCl_2$ (0.01 M) was added to the tubes. Then, 0.25 mL of 7.5×10^{-3} M ethanolic neocuprine solution and 1 M ammonium acetate buffer were added. Various concentrations (10–30 µg/mL) of flaxseed ethanol extracts or standards were added during the mixing of the solution. Absorbance at 450 nm was recorded during a half hour incubation [18].

2.6. Determination of Fe^{2+}-Chelating Activity

To determine the metal chelating activity of the flaxseed ethanol extracts, 2 mM 0.05 mL $FeCl_2$ and 0.35 mL distilled water were mixed and added to 0.2 mL solution containing various concentrations of flaxseed ethanol extracts. Then ethanol was added to a final volume of 4 mL. The reaction was started by adding 0.2 mL 5 mM ferrozine. The solution was vortexed and incubated for 10 min at room temperature. After incubation, the absorbance of the solution was recorded at 562 nm against distilled water [19]. Experiments were carried out as described, with 5 different extract concentrations ranging from 1–3 µg/µL. Ferrous chelation activities % of flaxseed extracts were calculated for each concentration and values of ferrous chelation activities % vs. µg extract amount were graphed. To compare with the literature, IC50 values, which are the amounts of substances that chelate 50% of ferrous, were calculated from the equation of the graph.

2.7. 1,1-diphenyl-2-picryl Hydrazyl (DPPH·) Radical Scavenging Activity

Flaxseed extracts were transferred to test tubes to form solutions at concentrations of 0.67, 1.33, 2, 2.68, and 3.35 µg/µL, and their total volume was made up to 3 mL with ethanol. Then, 1 mL of 1 mM DPPH was added to each sample tube. After incubation for 30 min at room temperature and in the dark, the absorbances of the remaining DPPH· at 517 nm were recorded against the ethanol. As a control, 3 mL of ethanol and 1 mL of DPPH were used. Radical scavenging % activities were calculated for each sample [14], and to discuss with the literature, the amounts of extract that scavenged 50% of DPPH (IC50 values) were calculated from the equation of the graphs. And also, DPPH scavenging % vs. Flaxseed extract concentrations (0.67–3.35 µg/µL) graphs were drawn. To discuss with the literature, the amounts of extract that scavenged 50% of DPPH (IC50 values) were calculated from the equation of the graphs.

2.8. 2,2′-azino-bis [3-ethylbenzothiazoline-6-sulphonic Acid] ($ABTS^+$) Radical Scavenging Activity

Firstly, 1 mL of $ABTS^+$ (7 mM) was mixed with 20 mM acetate buffer (pH 4.5) containing 2.45 mM potassium persulfate solution to obtain an absorbance of 0.700 ± 0.025 at 734 nm. Various concentrations of flaxseed extracts ranging from 0.2 to 1 µg/mL were taken and 2.970 mL of $ABTS^+$ was added. Then, a final volume was completed to 3 mL with ethanol. Solutions were incubated for 30 min and the absorbances were read at 737 nm against the buffer. Trolox was used as the standard. The $ABTS^+$ radical scavenging activities (%) were calculated for each sample [20], and experiments were carried out as described, with 5 different extract concentrations at 0.2, 0.4, 0.6, 0.8, and 1 µg/µL, and $ABTS^+$ scavenging % vs. Flaxseed extract concentrations (0.2–1 µg/µL) graphs were drawn. To discuss with the literature, the amounts of extract that scavenged 50% of $ABTS^+$ (IC50 values) were calculated from the equation of the graphs.

2.9. Analyzes of Fatty Acid and Phenolic Content

2.9.1. Extraction of Flaxseed Oils

The flaxseed oils' content was measured using the Soxhlet apparatus method [21]. Briefly, 5 g of the powdered samples were subjected to "Soxhlet Extraction" using 30 mL n-hexane for 5 h and then the solvents were evaporated at 40 °C using HEIDOLPH Hei-VAP Core (HL/ML) (Heidolph Instruments GmbH, (Darmstadt, Germany) apparatus. The oil present was evaluated as the total oil content of the linseed and each fatty acid as a percentage of the total fatty acids. Oil samples were stored at 4 °C until analysis [10].

2.9.2. Yield of Flaxseed Oil

The yield was calculated according to the formula [10]

$$Oil\ yield(\%) = \frac{Mass\ of\ the\ extracted\ oil}{Mass\ of\ flaxseed\ powder} \times 100\%$$

2.10. Gas Chromatography Flame Ionization Detector (GC–FID) Analysis for of Fatty Acid Composition

Flaxseed oil obtained from the seeds of 15 flax registered cultivars used in the present study was analyzed using the method used as the basis for the determination of fatty acid compositions in previous studies [10,22]. GC–FID graphics of fatty acid of flaxseed (*L. usitatissimum* L.) cultivars are given in Figures S2–S16.

2.11. High-Performance Liquid Chromatography (HPLC) Analysis for Phenolic Composition

The prepared flaxseed samples were filtered through 0.45 µm filter discs before High-Performance Liquid Chromatography (HPLC) analysis of the methanol extracts of the samples at Igdir University Research Laboratory Application and Research Center (ALUM). In addition, a (high-performance liquid chromatography (HPLC)) system (Agilent 1260;

Agilent, Santa Clara, CA, USA) equipped with a diode array detector was used. The mobile phases were as follows: (A) 0.1% phosphoric acid in water and (B) HPLC grade 100% acetonitrile. Phenolics were quantified by comparing the peaks recorded at 300 nm with the standard curves for each acid. Phenolic extraction of each flaxseed sample was performed as previously described [12,23]. In general, the expected phenolic compounds in flaxseed, chlorogenic acid, catechin hydrate, 4-hydroxy benzoic acid, vanillin, rutin, naringin, o-coumaric acid, salicylic acid, resveratrol, quercetin, t-cinnamic acid, and naringenin were monitored and quantified for each sample corresponding to the varieties [11,24]. HPLC graphics of phenolic compounds of flaxseed cultivars are given in Figures S17–S31.

2.12. Statistical Analysis

The experiments were conducted with three replicates. To compare the varieties analyzed, we used one-way ANOVA followed by the Duncan post-hoc test. The differences between individual averages were considered to be statistically significant at $p < 0.05$. The same data were subjected to correlation analysis (JASP). In addition, due to the high number of variables, heat-map clustering (ClustVis) and principal component analysis (PAST Software (PAleontological Statistics, 13 May 2001; Øyvind Hammer., David A. T. Harper., Paul D. Ryan.)) were performed after the normalization and transformation of the raw data.

3. Results

3.1. Total Phenolic/Flavonoid Content and DPPH/ABTS$^+$ Radical Scavenging Activities of Flaxseed Extracts

The analysis revealed significant differences in total phenolic (TPC) and total flavonoid (TFC) content among the varieties. The TPC values ranged from 613.6 mg GAE/g (Atalanta variety) to 3164.6 mg GAE/g (Michael variety), while the TFC values ranged from 176.25 mg QE/g (BonnyDoon) to 689.20 mg QE/g (Mcduff variety). All extracts tested showed higher radical scavenging activity than the standard antioxidants, ascorbic acid, and Trolox. In terms of IC50 values for radical scavenging activities, the Karakız variety was found to be the most potent variety with an IC50 value of 1.89 µg/mL for scavenging the DPPH radical. The McGregor variety exhibited the highest ABTS$^+$ scavenging activity, with an IC50 of 0.61 µg/mL, which is lower than that of ascorbic acid and Trolox. Furthermore, the extracts underwent additional assays to determine their ability to chelate and reduce. Similar to radical scavenging activities, all extracts were more effective than α-tocopherol, with the exception of the cupric reducing potential (Table 2).

Table 2. Results total phenolic/flavonoid content and DPPH$^{\cdot}$/ABTS$^{\cdot}$ analysis of flaxseed extracts.

Variety	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)	IC_{50} for DPPH Radical Scavenging (µg/mL)	IC_{50} for ABTS Radical Scavenging (µg/mL)	IC_{50} for Ferrous Ion Chelating Activity (µg/mL)	Cupric Reducing Capacity (µmol TE/mg)
Karakız	2117.4 ± 253.2 a–d	442.14 ± 71.37 a–d	1.89	1.88	1.56	10.04
BonnyDoon	761.9 ± 16.1 d	176.25 ± 19.17 a–d	4.75	3.21	1.91	10.35
Norman	768.7 ± 76.0 e	286.98 ± 9.59 a–c	3.91	2.19	1.59	2.23
McGregor	1808.2 ± 168.8 e	390.17 ± 87.35 d	2.24	0.61	1.92	11.78
Dillman	1127.1 ± 203.5 f	217.93 ± 48.98 a–d	3.35	2.15	0.92	8.82
Michael	613.6 ± 108.2 bc	187.55 ± 9.59 ab	4.41	2.24	2.01	6.34
Konya Beyazı	616.2 ± 100.9 d	230.49 ± 44.74 a–c	5.9	2.21	2.25	8.22
Florinda	702.6 ± 85.6 cd	326.9 ± 74.56 a–c	4.55	2.04	3.84	10.17
Lirina	689.4 ± 79.8 b	338.20 ± 39.41 a	3.78	1.68	2.74	7.99
Nareum	1303.2 ± 191.7 f	586.01 ± 90.96 a–d	2.29	1.10	3.71	11.12
Barbara	1802.1 ± 209.7 f	396.20 ± 90.40 cd	3.82	1.49	1.04	9.85
Atalanta	3164.6 ± 459.6 f	372.09 ± 61.78 d	3.11	1.69	2.3	10.00
Milas	2227.6 ± 398.5 f	641.00 ± 90.72 d	4.01	1.43	3.72	8.38
Zoltan	2045.6 ± 206.2 f	595.05 ± 92.74 b–d	6.03	1.30	2.3	10.25
Mcduff	2421.5 ± 189.2 a	689.20 ± 98.21 a–d	2.13	1.41	3.78	11.23
Ascorbic acid	-	-	15.51	20.49	-	-
α-tocopherol	-	-	-	-	12.46	-
Trolox	-	-	15.00	2.04	3.22	-

GAE: Gallic acid equivalent, QE: Quercetin equivalent, TE: Trolox equivalent. The means in the same column followed by the same letters were not significantly different according to Duncan's test ($p < 0.05$).

As shown in Figure 2, our extracts demonstrated greater efficiency in scavenging DPPH than the standard antioxidants (ascorbic acid and Trolox) as well as flaxseed from Hisar and Rajmahal hills, as reported by [25]. The authors [25] reported a DPPH scavenging activity of approximately 70% at a concentration of 0.5 mg/mL for both varieties. Furthermore, the activity of chelating ferrous ions was further examined. The activity of chelating ferrous ions was determined by assessing the competition between the ferrosine reagent, a potent iron chelator, and the metal-binding compounds found in flaxseed extracts for the binding of Fe^{+2} ions. As with the previously mentioned activities, all extracts displayed higher activity levels than α-tocopherol. The ferrous ion chelating activities' IC50 values ranged from 0.92 µg/mL (Dillman variety) to 3.84 µg/mL (Florinda variety). The percentage of chelated metal ion was determined and is presented in Figure S5a. Additionally, the extracts were evaluated for their cupric reducing capacity in Trolox equivalents, with a capacity range of 2.23–11.78 µmol Trolox/mg.

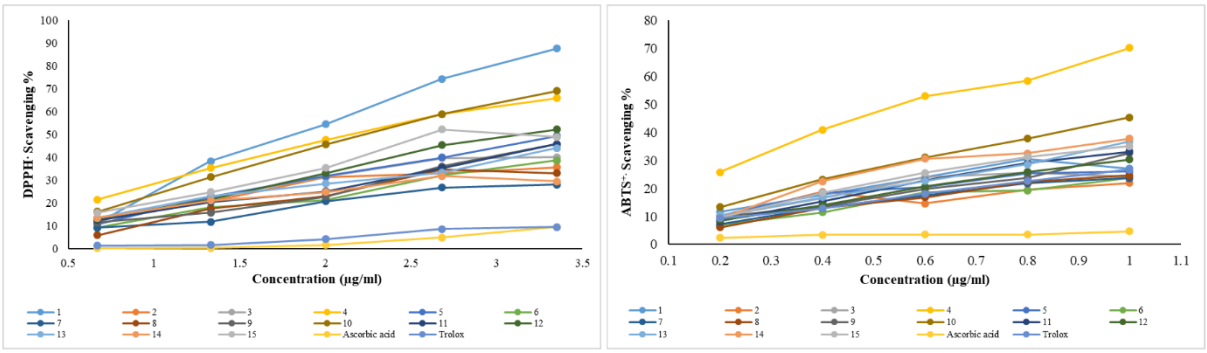

Figure 2. DPPH$^{\cdot}$ and ABTS^{+} radical scavenging activities of flaxseeds and standards.

3.2. Fatty Acid Composition in Flaxseed Extract and Correlation Analysis

Oil yield, oil content, and composition are important attributes of flaxseed. Firstly, we estimated the crude oil yield and content. Next, we analyzed the fatty acid composition of the oil using GC–FID. All estimated parameters were statistically different between the studied varieties ($p < 0.05$). The yield and oil content varied significantly among the varieties, with the Michael variety having the lowest yield of 0.82 g and the McGregor variety having the highest yield of 2.14 g. The oil content ranged from 17.4% in the Milas variety to 42.6% in the McGregor variety (Table 3). The GC–FID analysis revealed that flaxseed oils contained high levels of α-linolenic acid, linoleic acid, oleic acid, palmitic acid, and stearic acid, as shown in Table 3. The analysis of variance revealed significant differences ($p < 0.05$) in the amount of fatty acids among the analyzed varieties. In terms of the identified compounds, the Atalanta variety had the highest α-linolenic acid content at 54.20%, while the McGregor variety had the lowest at 39.21%. The BonnyDoon variety had the highest linoleic acid content at 17.89%, while the Barbara variety had the lowest at 13.23%. The oleic acid content ranged from 18.12% (McGregor) to 26.17% (Milas), while the palmitic acid content ranged from 5.73% (Nareum) to 17.59% (McGregor). The stearic acid content ranged from 3.71% (Nareum) to 7.31% (Milas).

In addition to the one-way variance analysis, we conducted a correlation analysis, principal component analysis (PCA) (refer to Figure S2a), and heat-map clustering analysis (refer to Figure S6a). The correlation analysis shows that crude oil yield is positively associated with oil content (r = 0.925; $p < 0.05$). Additionally, palmitic acid (r = 0.609; $p < 0.05$) is positively associated with crude oil yield (refer to Figure S1a). PCA was used to explain the variability, as shown in Figure S2a. For the analysis, two components (PC1 and PC2) with Eigenvalues greater than 1 were considered. The Eigenvalues for PC1 and PC2 were 3.22 and 1.98, respectively. PC1 and PC2 accounted for 46.02% and 28.22% of the variance, respectively, indicating that a total of 74.24% of the variation was explained. This explained ratio could be a clear indicator or predictor for discriminating between varieties based on oil yield, oil content, and fatty acid composition. Regarding the discrimination

of the varieties based on their components, PC1 showed a positive association with crude oil yield, oil content, palmitic acid, and stearic acid. Conversely, it exhibited a negative correlation with oleic acid, linoleic acid, and α-linolenic acid, as evidenced by the correlation coefficients. Furthermore, the HCA analysis revealed two distinct clusters for both the independent variables (varieties) and dependent variables (estimated parameters). In terms of the dependent variables, stearic acid and oleic acid were distinctly separated from the other variables. Among the varieties, McGregor and Barbara were distinctly differentiated from the others. This clear distinction may prompt the researcher to concentrate on these varieties for their oil profile. As explained in the following section, McGregor exhibits great potential as an antioxidant, as shown in Table 2.

Table 3. Oil contents and fatty acid compositions of flaxseed cultivars (%).

Variety	Crude Oil Yield (g/100 g)	Oil Content (%)	Palmitic Acid (C16.0)	Stearic Acid (C18.0)	Oleic Acid (C18.1)	Linoleic Acid (C18.2)	α-Linolenic Acid (C18.3)
Karakız	1.12 b	19.6 ef	11.65 a	7.12 a	24.75 ab	14.83 c–e	39.49 e
BonnyDoon	2.11 a	36.8 b	6.93 d–f	5.59 a–d	21.61 c–e	17.89 a	47.95 cd
Norman	0.89 b	18.4 ef	6.84 d–f	5.62 a–d	23.36 bc	17.65 ab	46.51 cd
McGregor	2.14 a	42.6 a	17.59 a	6.57 ab	18.12 fg	13.61 de	39.21 e
Dillman	0.91 b	19.8 ef	8.02 c–e	5.33 b–e	20.89 d–f	17.25 ab	47.87 cd
Michael	0.82 b	17.4 f	6.55 d–f	5.72 a–d	20.27 d–g	15.24 cd	52.18 ab
Konya Beyazı	0.93 b	19.3 ef	8.96 c	4.73 c–e	20.81 d–f	17.44 ab	46.62 cd
Florinda	1.03 b	20.6 e	8.35 cd	6.07 a–d	18.74 fg	16.22 a–c	49.19 bc
Lirina	1.81 a	27.8 c	5.76 f	4.57 de	20.55 d–f	16.02 bc	53.08 a
Nareum	0.98 b	19.7 ef	5.73 f	3.71 e	19.88 f–g	16.53 a–c	54.13 a
Barbara	1.94 a	37.6 b	11.04 a	6.77 ab	21.37 c–e	13.23 e	45.35 d
Atalanta	0.96 b	24.8 d	6.34 ef	5.34 b–d	20.8 d–f	13.3 e	54.2 a
Milas	0.97 b	17.4 f	6.55 d–f	7.31 a	26.17 a	15.24 cd	44.71 d
Zoltan	1.05 b	19.4 ef	6.2 ef	6.42 a–c	23.91 b	16.5 a–c	46.94 cd
Mcduff	1.48 a	21.3 e	6.26 ef	6.97 ab	22.64 b–d	16.29 a–c	47.81 cd
p-values	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

The means in the same column followed by the same letters were not significantly different according to Duncan's test ($p < 0.05$).

3.3. Phenolic Compounds Profile in Flaxseed Extract and Correlation Analysis

Table 4 displays the profile and quantitative contents of phenolic compounds in flaxseed samples. The main phenolic components of flaxseed are quercetin, vanillin, rutin, resveratrol, catechin hydrate, chlorogenic acid, and 4-hydroxybenzoic acid. The study found significant differences in the amount of compounds among the varieties considered ($p < 0.05$) through one-way analysis of variance. The levels of quercetin ranged from 1.29 µg/L (Zoltan) to 4.56 µg/L (Konya Beyazı), while the levels of catechin hydrate ranged from 0.48 µg/L (Florinda) to 10.23 µg/L (Atalanta). The hydroxybenzoic acid levels in the different varieties ranged from 1.33 µg/L (Florinda) to 7.09 µg/L (Norman), while the resveratrol content varied from 1.01 µg/L (Zoltan) to 8.03 µg/L (Konya Beyazı). Moreover, the recorded content of the different flaxseed varieties ranged from 1.26 µg/L (Norman) to 24.46 µg/L (Konya Beyazı) (Table 4). In addition, the chlorogenic acid content of flaxseed varieties ranged from 1.13 µg/L (Mcduff) to 12.08 µg/L (BonnyDoon). Furthermore, the Konya Beyazı variety exhibited higher levels of rutin, resveratrol, and quercetin compared to other flaxseed varieties. As with oil-related characteristics, there are numerous variables associated with phenolic compounds and varieties.

For this reason, in addition to the analysis of variance, a number of statistical analyses including correlation analysis, principal component analysis (PCA) (Figure S4a), and heat-map clustering (HCA) (Figure S7a) were used to associate and cluster the results. Accordingly, the correlation analysis showed that vanillin was positively associated with chlorogenic acid (r = 0.578; $p < 0.05$). Resveratrol (r = 0.838; $p < 0.05$) was positively associated with rutin. Quercetin was positively associated with o-coumaric acid (r = 0.888; $p < 0.05$) (Figure S3a). Again, the two first components (PC1 and PC2) with a higher Eigenvalue than 1 were considered for the analysis. Eigenvalues were found to be 3.81 and

2.18 for PC1 and PC2, respectively (Figure S4a). The PC1 and PC2 explained 29.30% and 16.77% of the variance, respectively, suggesting that a total of 46.06% of the variation was explained. The total explained variation is insufficient to distinguish between varieties based on their phenolic profiles when compared to the variation explained for oil-related attributes. This can be attributed to the disparity between secondary metabolites (phenolics) and primary metabolites (lipids). The differences between them can be explained by variations in their chemical diversity, functions, biosynthetic pathways, genetic regulations, or analytical methods used for extraction. However, such comments or assumptions will need to be substantiated in any future work. Considering the variables included in the components, PC1 was positively associated with rutin, naringin, coumaric acid, salicylic acid, resveratrol, quercetin, and naringenin. However, the component is negatively associated with chlorogenic acid, catechine hydrate, hydroxl benzoic acid, and vanillin. Concerning HCA for phenolic compounds, two major clusters were observed. The first cluster comprised catechin hydrate, salicylic acid, and t-cinnamic acid, while the second major cluster comprised resveratrol, chlorogenic acid, vanillin, rutin, quercetin, 4-hydroxy benzoic acid, naringin, o-coumaric acid, and naringenin. Regarding varieties considered for the analysis, Nareum, Barbara, Norman, Karakız, and Florinda were clearly separated from other varieties of flaxseed.

Table 4. Phenolic compounds profile of flaxseed cultivars (µg/L).

Variety	Chlorogenic Acid	Catechine Hydrate	4-Hydroxy Benzoic Acid	Vanillin	Rutin	Naringin	o-Coumaric Acid	Salicylic Acid	Resveratrol	Quercetin	t-Cinamic Acid	Naringenin
Karakız	1.88 de	5.83 b	0.00 d	0.00 d	0.00 f	67.39 a	3.11 a	0.00 e	1.79 c	3.99 b	9.51 a	11.40 a
BonnyDoon	12.08 a	3.97 c	1.38 c	5.63 b	4.09 b	6.78 b	0.00 d	0.00 e	0.00 e	0.00 e	7.79 bc	4.01 b
Norman	2.20 d	3.62 cd	7.09 a	5.73 b	1.26 e	0.00 e	0.00 d	0.00 e	1.94 bc	0.00 e	10.49 a	9.45 a
McGregor	7.56 a	6.48 a	0.00 d	2.02 c	0.00 f	0.00 e	0.00 d	0.00 e	1.02 d	0.00 e	8.66 a	4.83 a
Dillman	8.82 a	9.33 a	0.00 d	7.09 a	0.00 f	0.00 e	0.00 d	0.00 e	0.00 e	0.00 e	8.25 ab	4.29 ab
Michael	1.14 f	10.21 a	0.00 d	0.00 d	0.00 f	6.83 b	0.00 d	6.37 b	1.29 d	1.57 d	6.81 de	2.57 c
Konya Beyazı	6.87 a	0.00 f	0.00 d	0.00 d	24.46 a	1.66 c	2.65 b	4.11 c	8.03 a	4.56 a	6.88 de	6.88 a
Florinda	2.06 de	1.48 e	1.33 c	0.00 d	0.00 f	0.00 e	0.00 d	0.00 e	0.00 e	1.36 d	6.47 e	8.89 a
Lirina	3.22 c	6.93 a	1.45 c	0.00 d	3.46 c	0.00 e	0.00 d	0.00 e	0.00 e	1.43 d	8.95 a	1.56 de
Nareum	1.92 de	3.60 cd	0.00 d	0.00 d	0.00 f	0.00 e	0.00 d	0.00 e	1.06 d	0.00 e	7.35 cd	2.08 cd
Barbara	2.39 d	3.24 d	0.00 d	0.00 d	0.00 f	0.00 e	0.00 d	0.00 e	1.70 c	0.00 e	7.96 bc	2.50 c
Atalanta	5.05 b	10.23 a	0.00 d	5.80 b	1.86 d	1.07 d	0.00 d	8.98 a	2.24 b	0.00 e	12.52 a	1.35 e
Milas	1.54 ef	3.58 cd	0.00 d	0.00 d	0.00 f	0.00 e	0.00 d	0.00 e	0.00 e	1.32 d	6.21 e	0.00 f
Zoltan	6.62 a	6.60 a	1.78 b	0.00 d	0.00 f	6.56 b	0.00 d	1.04 d	1.01 d	1.29 d	5.20 f	4.89 a
Mcduff	1.13 f	8.12 a	0.00 d	0.00 d	8.65 a	1.94 c	1.53 c	0.00 e	1.03 d	2.27 c	10.28 a	0.00 f
p-values	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

The means in the same column followed by the same letters were not significantly different according to Duncan's test ($p < 0.05$).

4. Discussion

Flaxseed is a promising functional food due to its richness in physiologically active phenolic and flavonoids, as well as minerals found in the seeds. An array of critical functions including anti-inflammatory, anti-oxidative, and anti-carcinogenic activities has been attributed to the phenolic compounds. The balance between oxidants and antioxidants is crucial for biological activities. According to numerous sources [26,27], enzymatic and non-enzymatic defense components buffer oxidants. The orchestrated functions of antioxidants are of great interest in combating oxidants. A basic search on SCOPUS using "antioxidant" in the title-abstract-keywords yielded over half a million documents in various disciplines. Such a high number of documents related to antioxidant status is a clear indicator of the importance of this topic. As also clearly proven, the antioxidant status of a plant is associated with the metabolites available. Among the metabolites, phenolic compounds are of particular interest to researchers. The biosynthesis and accumulation of the relevant compounds are controlled by both genetic and environmental factors [28,29]. For these reasons, plant species are often investigated for their metabolite profile and biological efficacy. Factors such as harvest time, plant maturity, extraction method, and sampling location are important predictors [30]. Reports discussing the phytochemicals of flaxseed have been widely disseminated and studied, as evidenced by numerous sources [31–34]. Due to their remarkable uses in a wide spectrum of fields, the flaxseed species deserves to be investigated for ascertaining the promising properties of different the varieties. In the

frame of the present study, it was hypothesized that varieties of the flaxseed collected from different geographical origins would manifest themselves on the profile of the phenolics and fatty acid composition of the varieties and such critical differences would be translated into the variations in the radical scavenging and oxidant reducing potential of the varieties considered in the present study. To test these hypotheses, we conducted chemical analyses and antioxidant assays on fifteen varieties from different origins as materials.

4.1. Total Phenolic and Flavonoid Content

Accordingly, total phenolic content of the samples varied between 613.6 and 3164 mg GAE/100, as given in Table 2. Our results are higher than species reported by [35], which reported that TPC of ethanolic and methanolic extracts of flaxseed from Bahawalpur, Pakistan were 18.75 and 22.30 mg GAE/g, respectively. Furthermore, prior research has shown that flaxseeds contain 20.20 mg GAE/g in 80% methanolic extract and 32.60 mg GAE/g in 80% ethanolic extract. On the other hand, TPC in flaxseed was found to be 16.70 mg GAE/g in another investigation, which is lower than the results of this study [36]. Flavonoids are one of the most common types of phenolic chemicals found in plants and they are key secondary metabolites in the prevention of chronic diseases like hyperlipidemia and cancer [37]. TFC of flaxseed ethanol extracts were found between 176.25 and 689.20 mg QE/g. These results are higher than water extract (2.1 mg QE/g) and acetone extract (5.11 mg QE/g) of flaxseeds from Lahore, Pakistan [38]. Besides, TFC in the ethanolic extracts of the local or foreign registered varieties of flaxseed we studied were found to be higher than in the ethanol extract of flaxseed (Hisar) (62.67 mg/g) and flaxseed (Rajmahal hills) (185.00 mg/g) [25].

4.2. Individual Phenolic Compounds

Phenolic compounds are secondary metabolites in plants that contribute to reproduction, growth, and coloration of flowers and fruits, as well as defense against diseases caused by pathogens, parasites, and herbivores [39]. The one-way ANOVA analysis revealed differences in the amounts of phenolic compounds among the analyzed flax varieties, depending on the cultivar's origin. Furthermore, these variations may be attributed to the genetic composition of the seeds, the specific variety, agronomic, and climatic conditions, and the timing of the harvest [33]. The primary phenolic compounds found in flax seeds, namely 4-hydroxybenzoic acid, quercetin, rutin, resveratrol, and catechin hydrate, have been identified. Previous research [1] shows that resveratrol 5.72 mg/100 g, cinnamic acid 1.87 mg/100 g, and rutin 10.90 mg/100 g are the main phenolic compounds they obtained in their study with the Atalanta variety among five flaxseed varieties. In this context, when compared to our Atalanta variety, these values were found to be 2.24 μg/L for resveratrol, 12.52 μg/L for cinnamic acid, and 1.86 μg/L for rutin, which were lower than previous studies. This difference may be attributed to variations in the ecological conditions during seed growth and storage duration. In previous studies, the values of resveratrol were reported to be, respectively, 5.72 mg/100 g and 25.66 mg/100 g [1,40]. Of the major compounds, hydroxybenzoic acid was previously reported as 4.24% in one study [31] and as 0.84 mg g^{-1} in another [41]. Compared to our results, it was found to be higher. Differences in chemical composition of the plants are generally attributed to the origin and genetic makeup of the plants and environmental conditions [42]. Flaxseed typically contains phenylpropanoids, including p-coumaric acid, o-coumaric acid, ferulic acid, 4-hydroxybenzoic acid, vanillic acid, sinapic acid, secoisolariciresinol, resveratrol, rutin, chlorogenic acid, enterolactone, and enterodiol [43]. Comparing and characterizing flaxseed variants from other nations can aid in the development of new flaxseed breeding resources by providing quality criteria and potential commercial values.

4.3. Antioxidant Activities

To determine the antioxidant activities of the extracts, a variety of assays were used. It should be noted that there is no universal assay to accurately and precisely determine

the antioxidant potential of an extract. Therefore, we employed radical scavenging and reducing capability related assays. The findings indicate that extracts from the varieties examined for their antioxidant potential showed higher activity levels than standard antioxidants. This suggests alternative approaches for industrial use. It is widely accepted that activities are dependent on the available phytochemicals, whether in terms of diversity or quantity. Therefore, a simple correlation analysis was conducted to investigate potential relationships between the total amount of polyphenols and individual phenolics identified. The correlation analysis revealed a significant positive correlation between the IC50 values of DPPH scavenging activities and the total phenolic and flavonoid content. Additionally, a negative correlation was observed for t-cinnamic acid. However, the identified compounds were significantly associated with the activities. Significant correlations were found between the total content of phenolics and flavonoids and the IC50 values for ABTS. However, no significant relations were observed with the individual compounds identified. For the IC50 values of ferrous ions, the activity was not associated with either the total content of the polyphenols or individual compounds. Based on the cupric reducing values, it appears that 4-hydroxy benzoic acid has a negative association with cupric reducing activities. However, as with the discrimination analysis, no clear or significant correlations were found. These results suggest that further research is necessary.

4.4. Oil Yield and Content as Well as Individual Fatty Acids

In addition to the phenolic compounds, this study investigated oil-related attributes. It has been reported in numerous documents that the chemical compositions of plants exhibit remarkable flexibility depending on their spatial distribution and relevant climatic conditions [44]. Among the compounds identified in this study, the fatty acids found in flaxseed are primarily classified as monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Due to their effectiveness in treating serious illnesses, fatty acid profiling has become a major focus of researchers from various disciplines [45]. As anticipated, the fatty acid composition of flaxseeds varied depending on the cultivar used in the present study. Furthermore, the primary fatty acid found in flaxseed oil samples was α-linolenic acid. The fatty acid components of flaxseed oils are composed of 39.35–60.11% α-Linolenic acid, 12.09–17.45% linoleic acid, 15.89–26.21% oleic acid, 6.19–11.64% palmitic acid, and 4.67–7.68% stearic acid [4,40], according to research. Furthermore, it has been reported that flaxseed has a higher ratio of α-linolenic acid in its fatty acid profile, as shown in the dominant results [46]. In a similar study, [40] found that the rate of α-linolenic acid was 58.23%. These findings are consistent with previous reports.

4.5. Highlights and Limitations of the Present Study

This study report compares fifteen varieties of flaxseed for their phytochemicals and antioxidant potential. To our knowledge, this study is one of the first comprehensive works on these varieties. McGregor was found to be superior to other varieties. It is important to note that the phenolic profiling is based on the available standards. The identified compounds were used for correlations and subsequent discussions. Unidentified compounds were excluded from this analysis.

5. Conclusions

Fifteen varieties of flaxseeds underwent phytochemical analysis and antioxidant assays in this study. The analysis revealed significant differences in total phenolic and total flavonoid content among the varieties considered. The TPC values ranged from 613.6 mg GAE/g (Atalanta variety) to 3164.6 mg GAE/g (Michael variety), while the TFC values ranged from 176.25 mg QE/g (BonnyDoon) to 689.20 mg QE/g (Mcduff variety). All varieties' extracts showed higher antioxidant activity compared to standard antioxidants. In terms of oil content (g) and crude oil yield (%), McGregor had the highest oil content (2.14 g; 42.6%) while Michael had the lowest (0.82 g). The lowest crude oil yield (%) was observed in Milas (17.4%). Among the varieties, McGregor and Barbara were distinctly

separated from the others based on oil-related attributes. However, discrimination between the varieties was not clear based on the phenolic profile, and no significant correlations were observed in general between the activity and the identified compounds.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr12040689/s1.

Funding: This research received no external funding.

Data Availability Statement: Data are contained within the article and Supplementary Materials.

Acknowledgments: We extend our special thanks to Muhittin Kulak and M. Güven Kaysim for his criticism in improving the manuscript. In addition, I would also like to thank my dear wife Semra Koçak for her patience during my studies.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Zare, S.; Mirlohi, A.; Sabzalian, M.R.; Saeidi, G.; Koçak, M.Z.; Hano, C. Water Stress and Seed Color Interacting to Impact Seed and Oil Yield, Protein, Mucilage, and Secoisolariciresinol Diglucoside Content in Cultivated Flax (*Linum usitatissimum* L.). *Plants* **2023**, *12*, 1632. [CrossRef] [PubMed]
2. Özcan, M.M.; Uslu, N. Investigation of changes in some chemical properties, bioactive compounds, antioxidant activity, phenolic and fatty acid profiles of flaxseed and oils. *J. Food Process. Preserv.* **2022**, *46*, e17091. [CrossRef]
3. Goudenhooft, C.; Bourmaud, A.; Baley, C. Conventional or greenhouse cultivation of flax: What influence on the number and quality of flax fibers? *Ind. Crops Prod.* **2018**, *123*, 111–117. [CrossRef]
4. Koçak, M.Z.; Göre, M.; Kurt, O. The effect of different salinity levels on germination development of some flax (*Linum usitatissimum* L.). *Varieties TURJAF* **2022**, *10*, 657–662.
5. Moawia, R.M.; Nasef, M.M.; Mohamed, N.H.; Ripin, A.; Farag, H. Production of biodiesel from cottonseed oil over aminated flax fibres catalyst: Kinetic and thermodynamic behaviour and biodiesel properties. *Adv. Chem. Eng. Sci.* **2019**, *9*, 281–298. [CrossRef]
6. Pisupati, A.; Willaert, L.; Goethals, F.; Uyttendaele, W.; Park, C.H. Variety and growing condition effect on the yield and tensile strength of flax fibers. *Ind. Crops Prod.* **2021**, *170*, 113736. [CrossRef]
7. Parikh, M.; Pierce, G.N. Dietary flaxseed: What we know and don't know about its effects on cardiovascular disease. *Can. J. Physiol. Pharmacol.* **2019**, *97*, 75–81. [CrossRef] [PubMed]
8. Buckner, A.L.; Buckner, C.A.; Montaut, S.; Lafrenie, R.M. Treatment with flaxseed oil induces apoptosis in cultured malignant cells. *Heliyon* **2019**, *5*, e02251. [CrossRef] [PubMed]
9. Barre, D.E.; Mizier-Barre, K.A.; Griscti, O.; Hafez, K. High dose flaxseed oil supplementation may affect fasting blood serum glucose management in human type 2 diabetics. *J. Oleo Sci.* **2008**, *57*, 269–273. [CrossRef]
10. Wang, S.; Zhang, Z.S.; Zhang, T.F.; Xue, D.W. Extraction and characterization of flaxseed oil obtained with subcritical n-butane. *J. Oleo Sci.* **2020**, *69*, 1011–1020. [CrossRef]
11. Akl, E.M.; Mohamed, S.S.; Hashem, A.I.; Taha, F.S. Biological activities of phenolic compounds extracted from flaxseed meal. *Bull. Natl. Res. Cent.* **2020**, *44*, 27. [CrossRef]
12. Wang, H.; Qiu, C.; Abbasi, A.M.; Chen, G.; You, L.; Li, T.; Liu, R.H. Effect of germination on vitamin C, phenolic compounds and antioxidant activity in flaxseed (*Linum usitatissimum* L.). *Int. J. Food Sci. Technol.* **2015**, *50*, 2545–2553. [CrossRef]
13. Beema, N.; Mukkamula, N.; Mothuku, S.; Thumu, R.; Azmeera, T.; Biman, K.K. Comparative analysis of physico-chemical properties and fatty acid composition of linseed (*Linum usitatissimum* L.) oils of Indian accessions. *J. Appl. Biol. Biotechnol.* **2022**, *11*, 80–87. [CrossRef]
14. Güller, U.; Güller, P.; Çiftci, M. Radical Scavenging and Antiacetylcholinesterase Activities of Ethanolic Extracts of Carob, Clove, and Linden. *Altern. Ther. Health Med.* **2021**, *27*, 33–37.
15. Cirak, C.; Radusiene, J. Factors affecting the variation of bioactive compounds in Hypericum species. *Biol. Futur.* **2019**, *70*, 198–209. [CrossRef] [PubMed]
16. Singleton, V.L.; Orthofer, R.; Lamuela-Raventŏs, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu Reagent. *Meth. Enzymol.* **1999**, *299*, 152–178.
17. Park, Y.K.; Koo, M.H.; Ikegaki, M.; Contado, J.L. Comparison of the flavonoid aglycone contents of Apis mellifera propolis from various regions of Brazil. *Arq Bio. Tech.* **1997**, *40*, 97–106.
18. Köksal, E.; Gulcin, I. Antioxidant activity of cauliflower (*Brassica oleracea* L.). *Turk. J. Agric. For.* **2008**, *32*, 65–78.
19. Dinis, T.C.P.; Madeira, V.M.C.; Almeida, L.M. Action of phenolic derivates (acetoaminophen, salycilate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys.* **1994**, *315*, 161–169. [CrossRef]
20. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]

21. AOCS. *Official and Tentative Methods of the American Oil Chemists' Society*; American Oil Chemists' Society: Urbana, IL, USA, 1997; Volume 35.
22. Mubiru, E.; Shrestha, K.; Papastergiadis, A.; De Meulenaer, B. Improved gas chromatography-flame ionization detector analytical method for the analysis of epoxy fatty acids. *J. Chromatogr. A* **2013**, *1318*, 217–225. [CrossRef] [PubMed]
23. Corbin, C.; Fidel, T.; Leclerc, E.A.; Barakzoy, E.; Sagot, N.; Falguiéres, A.; Hano, C. Development and validation of an efficient ultrasound assisted extraction of phenolic compounds from flax (*Linum usitatissimum* L.) seeds. *Ultrason. Sonochem.* **2015**, *26*, 176–185. [CrossRef]
24. Czemplik, M.; Korzun-Chłopicka, U.; Szatkowski, M.; Działo, M.; Szopa, J.; Kulma, A. Optimization of phenolic compounds extraction from flax shives and their effect on human fibroblasts. *eCAM* **2017**, 3526392. [CrossRef] [PubMed]
25. Punia, J.; Deen, M.K. Comparative evaluation of phenols, flavonoids and antioxidant activity of flax seed from two locations. *Asian J. Chem.* **2016**, *28*, 2038. [CrossRef]
26. Mirończuk-Chodakowska, I.; Witkowska, A.M.; Zujko, M.E. Endogenous non-enzymatic antioxidants in the human body. *Adv. Med. Sci.* **2018**, *63*, 68–78. [CrossRef] [PubMed]
27. Belinskaia, D.A.; Voronina, P.A.; Shmurak, V.I.; Vovk, M.A.; Batalova, A.A.; Jenkins, R.O.; Goncharov, N.V. The universal soldier: Enzymatic and non-enzymatic antioxidant functions of serum albumin. *Antioxidants* **2020**, *9*, 966. [CrossRef] [PubMed]
28. Bourgaud, F.; Gravot, A.; Milesi, S.; Gontier, E. Production of plant secondary metabolites: A historical perspective. *Plant Sci.* **2001**, *161*, 839–851. [CrossRef]
29. Yang, L.; Wen, K.S.; Ruan, X.; Zhao, Y.X.; Wei, F.; Wang, Q. Response of plant secondary metabolites to environmental factors. *Molecules* **2018**, *23*, 762. [CrossRef] [PubMed]
30. Cetinkaya, H.; Kulak, M.; Karaman, M.; Karaman, H.S.; Kocer, F. Flavonoid accumulation behavior in response to the abiotic stress: Can a uniform mechanism be illustrated for all plants. *Flavonoids-Biosynth. Hum. Health* **2017**, 151–165. [CrossRef]
31. Alu'datt, M.H.; Rababah, T.; Ereifej, K.; Alli, I. Distribution, antioxidant and characterization of phenolic compounds in soybeans, flaxseed and olives. *Food Chem.* **2013**, *139*, 93–99. [CrossRef]
32. Oomah, B.D.; Kenaschuk, E.O.; Mazza, G. Phenolic acids in flaxseed. *J. Agric. Food Chem.* **1995**, *43*, 2016–2019. [CrossRef]
33. Herchi, W.; Sakouhi, F.; Arráez-Román, D.; Segura-Carretero, A.; Boukhchina, S.; Kallel, H.; Fernández-Gutierrez, A. Changes in the content of phenolic compounds in flaxseed oil during development. *J. Am. Oil Chem. Soc.* **2011**, *88*, 1135–1142. [CrossRef]
34. Noreen, S.; Taufail, T.; Ain, H.B.U.; Khalid, W.; Hanif, A.; Ali, B.; Abeed, A.H. Assessment of Antioxidant Activities of Flaxseed (*Linum usitatisimum* L.) and Fennel Seed (Foeniculum vulgare Mill.) Extracts. *Pol. J. Environ. Stud.* **2024**, *33*, 1–8. [CrossRef]
35. Tasneem, R.; Khan, H.M.S.; Rasool, F.; Khan, K.U.R.; Umair, M.; Esatbeyoglu, T.; Korma, S.A. Development of Phytocosmeceutical microemulgel containing flaxseed extract and its in vitro and in vivo characterization. *Pharmaceutics* **2022**, *14*, 1656. [CrossRef] [PubMed]
36. Anwar, F.; Przybylski, R. Effect of solvents extraction on total phenolics and antioxidant activity of extracts from flaxseed (*Linum usitatissimum* L.). *Acta Sci. Pol. Technol. Aliment.* **2012**, *11*, 293–302.
37. Chen, G.; Wang, H.; Zhang, X.; Yang, S.T. Nutraceuticals and functional foods in the management of hyperlipidemia. *Crit. Rev. Food Sci. Nutr.* **2014**, *54*, 1180–1201. [CrossRef] [PubMed]
38. Noreen, S.; Tufail, T.; Bader, U.L.; Ain, H.; Ali, A.; Aadil, R.M.; Nemat, A.; Manzoor, M.F. Antioxidant activity and phytochemical analysis of fennel seeds and flaxseed. *Food Sci. Nutr.* **2023**, *11*, 1309–1317. [CrossRef]
39. Kumar, S.; Abedin, M.M.; Singh, A.K.; Das, S. Role of phenolic compounds in plant-defensive mechanisms. *J. Plant Growth Regul.* **2020**, *1*, 517–532.
40. Bertoni, C.; Abodi, M.; D'Oria, V.; Milani, G.P.; Agostoni, C.; Mazzocchi, A. Alpha-Linolenic Acid and Cardiovascular Events: A Narrative Review. *Int. J. Mol. Sci.* **2023**, *24*, 14319. [CrossRef]
41. Waszkowiak, K.; Mikołajczak, B.; Gliszczyńska-Świgło, A.; Niedźwiedzińska, K. Effect of thermal pre-treatment on the phenolic and protein profiles and oil oxidation dynamics of golden flaxseeds. *Int. J. Food Sci. Technol.* **2020**, *55*, 1272–1280. [CrossRef]
42. Gai, F.; Janiak, M.A.; Sulewska, K.; Peiretti, P.G.; Karamać, M. Phenolic compound profile and antioxidant capacity of flax (*Linum usitatissimum* L.) harvested at different growth stages. *Molecules* **2023**, *28*, 1807. [PubMed]
43. Rocchetti, G.; Gregorio, R.P.; Lorenzo, J.M.; Barba, F.J.; Oliveira, P.G.; Prieto, M.A.; Lucini, L. Functional implications of bound phenolic compounds and phenolics–food interaction: A review. *Compr. Rev. Food Sci.* **2022**, *21*, 811–842. [CrossRef] [PubMed]
44. Qiu, C.; Wang, H.; Guo, Y.; Long, S.; Wang, Y.; Abbasi, A.M.; Jarvis, D.I. Comparison of fatty acid composition, phytochemical profile and antioxidant activity in four flax (*Linum usitatissimum* L.) varieties. *Oil Crop Sci.* **2020**, *5*, 136–141. [CrossRef]
45. Sokoła-Wysoczańska, E.; Wysoczański, T.; Wagner, J.; Czyż, K.; Bodkowski, R.; Lochyński, S.; Patkowska-Sokoła, B. Polyunsaturated fatty acids and their potential therapeutic role in cardiovascular system disorders—A review. *Nutrients* **2018**, *10*, 1561. [CrossRef]
46. Rodriguez-Leyva, D.; Bassett, C.M.; McCullough, R.; Pierce, G.N. The cardiovascular effects of flaxseed and its omega-3 fatty acid, alpha-linolenic acid. *Can. J. Cardiol.* **2010**, *26*, 489–496. [CrossRef]